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Dairy Animals Research Results

Clinical, epidemiological and therapeutic studies on Bovine Papillomatosis in Adami Tulu Agricultural Research Center

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Abstract

A study was conducted on bovine papillomatosis with the objectives of identifying better and field-based applicable treatments against the disease. Herd-specific autogenous vaccine and autohemotherapy were applied in the study. The study showed that autogenous vaccine resulted in 100% regression of papilloma while autohemotherapy showed only 88.9%. In both cases, regression of papilloma started in the third week of treatment. There was an association between the regression of papilloma and hematological parameters such as total leucocyte count and total lymphocyte count. There was neither recurrence nor new case of bovine papillomatosis on the treated animals and animals in the herd, respectively.

Key words: *Bovine, papilloma, autogenous vaccine, autohaemotherapy, therapeutic, adami tulu*

Introduction

Bovine papillomatosis is a disease caused by host, site and lesion specific papilloma viruses. The disease spreads by direct contact with infected animals and enters animal skin by cutaneous abrasions, fomites and possibly insects (Cynthia, 2005; Radostits *et al*., 2007; William, 2009).The growth of the disease varies with localization (abdominal and thoracic wall, udder, vulva, head, neck etc), age of the animal, sex (heifers are more frequently affected than steers), breed and season (Terziev *et al*., 2015).

Papillomatosis commonly occurs in young calves less than 2 years of age, and on teats and udder, and penis in mature cattle. In younger animals, the disease occurs on eyes, mouth, ears, sides of the neck, shoulders, back and abdomen.The extent and duration of the lesions of the disease depends on the type of virus, area affected, and degree of susceptibility. The course of the disease takes two months-a year after exposure for the appearance of the lesion.

Papillomatosis becomes a herd problem when a large group of young and susceptible animals become infected. Immunity against the disease usually develops three to four weeks after initial infection, but disease occasionally recurs due to loss of immunity. Bovine papillomatosis has economic importance in that it interferes with animal sales, and milking, it leads to secondary bacterial infection, it results in weight loss, stunted growth and anemia (Campo *et al*., 1992; Radostits *et al*., 2007; Roperto *et al*., 2008).

Majority of the clinical lession appears as epidermal proliferations though some appear as dermal fibroblasts and keratinocytes (papulonodule), which involves venereal regions leading to pain, disfigurement, infection of the penis of young bulls and dystocia when the vaginal mucosa of heifers is affected (Cynthia, 2005).

Various treatment and preventive measures have been used in the management of the Bovine papillomatosis. Host response to the disease depends on skin barrier protection, innate immunity, and acquired immunity. Isolation of an infected animals (takes 8-10 months) regression of 30% by its own (Messing and Epstein, 1963).

The use of host immune stimulants such as levamisole (Tizard, 2002) and Ivermectin (Borku *et al*., 2007), played a role in the regression of the disease through stimulation of T cells and response to antigens (Tizard, 2002). Under variable route of administration, number of doses, immunity of the host, intensity of papilloma per animal and nature of method for treatment and control of the disease, clinical recovery rate from the disease varies. For instance, recovery by using Levamisole is 82-88% (Jane, 2015) and it is in the range of 70%-100% when ivermectin is used (Jane, 2015; Abdi, 2018).

Autohaemotherapy is another treatment mothed that promotes nonspecific protein stimulation which leads to normal immune system that in turn allows maintenance of homeostasis (Santin & Brito, 2004). It alone resulted in 90% recovery of the disease, while its combination with levamisole resulted in 100% recovery after fourth administration (Jane, 2018). Treatment with autogenous vaccine showed a recovery of 93.5% (Turk *et al*., 2005) efficiency and it is varied with the type of vaccine diluent (Messing and Epstein, 1963). Autogenous vaccine provides recovery from clinical disease and prevent incidence of new cases in the herd (Turk *et al*., 2005) and therefore recommended to control the disease at the herd level. Vaccination before infection (at 4-6 weeks of age/calves and repeated at 8-12weeks of age), intradermally, is confirmed to provide advanced immunity and come in effect three to six months before its preventive value becomes evident.

A continued vaccination for one year after the wart disappears is recommended along with disinfection of stalls, stanchions, and other inert materials by fumigating with formaldehyde to remove contaminants from premises (Cynthia, 2005). The frequent observation of the disease in breeding herd at Adami Tulu Agricultural Research Center has got attension becaues it interferes with breeding program in terms of time of isolation of infected youngstock, unaesthetic value of animals for show and impeding in the distribution of heifers and bulls to the community.

Though various studies were conducted on either autohaemotherapy (general immunity stimulating method) or autogenous vaccine (specific immunity stimulating method) in other part of the world, such work was not done in the context of Ethiopian farm livestock farming condition. Therefore, it is important to evaluate treatment options for the disease in the research center. Thus, this study was hypothesized to evaluate the time saving and economic treatment and control options under our capacity with the following objectives:

Objectives

- To evaluate better treatment option of bovine papillomatosis
- To evaluate preventive role of various methods in the incidence of bovine papillomatosis
- To analyze regression time of bovine papillomatosis in dairy farm available management condition

Materials and Methods

Description of the Study Area

The study was conducted at Adami Tulu Agricultural Research Center which is located at 163 to the south of the capital of the country, Addis Ababa on the Addis Ababa- Hawassa Road. The center is used as a livestock research center where various livestock researches such as breed development and others are regularly undertaken.

Study Population: The study involved all cattle in the study herd. It involved both adults and young animals within the study farms.

Study Design: A randomized non-controlled experimental design was used during the study period.

Sample Size Determination

The sample size was determined conveniently based on the clinical presentation of warts on animals in the study population. Overall 18 animals which were clinically positive for bovine mastitis were selected for the study.

Sampling method

Purposive sampling was used during the study period. All cases with warts (papilloma) were purposively observed, recorded, sampled, and followed up.

Selection of Experimental Animals

After clinical observation of all animals in the study herd and identification of animals clinically positive for papilloma, from all age groups, the selection was undertaken based on the majority of age groups in which the disease was observed. Thus, because almost all the positive animals were calves less than 2 years, only calves were selected for the experimental work.

Material preparation, Treatment setting, and animal management

Autogenous vaccine

The autogenous vaccine was prepared according to a laboratory protocol reported previously by Hunt (1984). Approximately 5g of actively growing wart sample was excised with a portion of the skin from each animal and placed in a bottle with phosphate-buffered saline (PBS) in ice until processing. All wart samples were mixed together and cut up into thin sections using a scalpel blade and placed in a mortar. The sample was then grounded with a pestle to macerate the cells and release the virus particles. Phosphate buffered saline solution (PBS) was then added to the tissues and the maceration continued. The resultant fluid was then placed in 10ml bottles and centrifuged at 3,000 rpm for 5 minutes. The supernatant was placed into 10ml bottles and 0.5ml of 40% formaldehyde added into each bottle and mixed. The solution was then incubated at 37oC in a water bath for 2hrs after which it was stored at 4oC for 7days before use.

Autohaemotherapy Blood

Whole blood was aseptically drawn from the jugular vein of the experimental animals using a 20ml syringe. The collected blood (a total of 20ml at a time of collection) was then divided into two volumes: 10ml volume for injection to the same animal by intramuscular and 10ml volume for injection to the same animal by subcutaneous route.

Treatment Setup and Experimentation

The treatment was set into two categories Vis group that receives the autogenous vaccine and the group that receives autohemotherapy. The selected animals were randomly assigned to either group.

T1: Autogenous Vaccine: A prepared and sterile autogenous vaccine was injected intramuscularly at a 5ml dose every week for 4 weeks.

T2: Autohaemotherapy: By using an 18G hypodermic disposable syringe needle, blood was collected from jugular vein of the same animal and injected back into the animal every 7 days for 4 weeks.

Management of experimental animals and follows up

The calves were regularly supervised and examined for clinical parameters every two days during the study period 7 days before and 7 after treatments. Data was collected for body temperature, heart rate, and pulse rate, and rumen motility. Then after, the animals were supervised on week basis for any clinical change of the disease.

Data collection and management, and analysis

Using a data record sheet, any regression (disappearance) of the papilloma, recurrence of the disease on the study of animals during the study period, and occurrence of new papilloma on animals (in the study herd) other than those involved in the experiment and other possible parameters were recorded. All visible warts were described according to the location on the body and the total number of warts present within the follows up period and the number of warts per location of body.

The collected data was analyzed using descriptive statistics such as frequency and percent, number, and means. Additionally, inferential statistical analysis was undertaken by paired t-test to evaluate efficacy due to treatments. A p-value of ≤ 0.05 was considered significant.

Results and Discussion

Distribution of Bovine papillomatosis with respect to body parts and animal sex

This study reveals the distribution of the papilloma on the body of the animals. The majority of the papillomas (61.1%) were observed in the head and face region while the rest (38.9%) was observed on other parts of the body (neck, shoulder, and face). With regard to sex, out of the total positive animals 44.4% were in males calves and 55.6% were in females (Table 1).

Table 1: Distribution of Bovine papillomatosis with respect to body parts and animal sex

Parameters of epidemiological consideration	**Subcategory of the parameters**	**Descriptive results N (%)**
Location of papilloma-covered body parts	Head and face N (%)	11(61.1)
	Neck, shoulder, and face N (%)	7(38.9)
Sex	Male	8 (44.4)
	Female	10 (55.6)

Note: N- represents the number of animals

Burden of papilloma warts with respect to age of calves

The study showed that the number of papillomas per animal decreased with increase in the age of calves. The mean number of papilloma per animal is about 10 counts during ages 4-5 months, increased to more than 10 counts per animal at ages between 5 and 6 months, and decreased after 6 months age of calves (Figure 1).

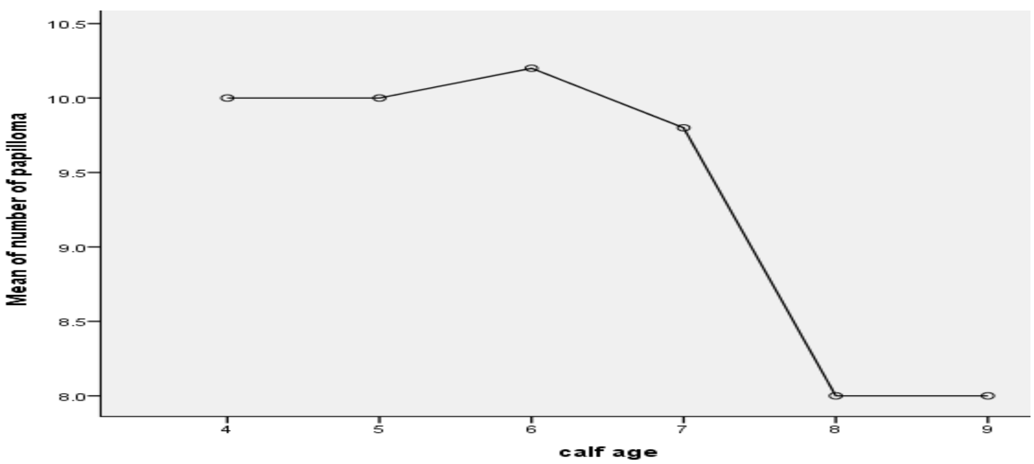

Figure 1: number of papilloma warts per animal with respect to age of calves

Clinical Incidence of papilloma in the study herd

Observation for the incidence of the disease was undertaken for 3 years (pre-treatment period to post-treatment period). The farm was visited regularly for any existence of papilloma, record was made for each experimental animals and any new case on non-experimental groups. The incidence of papilloma in the herd before and during experiment (2019/20 and 202020/21) was 2.0%. Papilloma was not observed after application of treatments in 2021/22 (Figure 2).

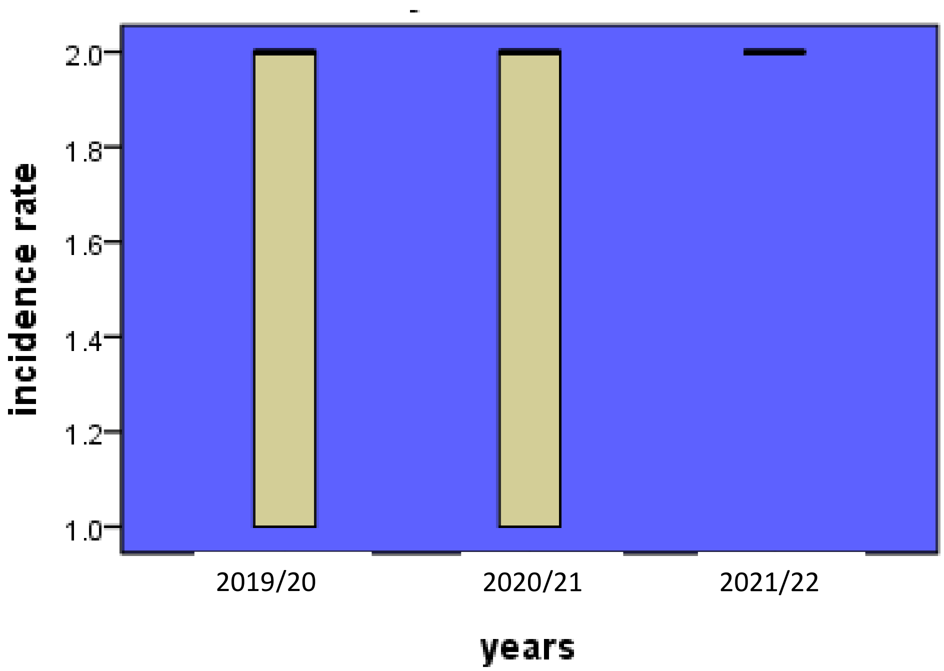

Figure 2: Incidence of bovine papillomatosis in the years 2012 to 2014

Effect of treatments on clinical regression, recurrence and new cases of papilloma

The study showed that papillomatosis was totally regressed (100%) in calves treated with an autogenous vaccine. This is higher than the report by Lesnik *et al*. (1999) who reported regression rate of 93.5% after 105 days post vaccination. The difference in efficiency of regression may be associated with inheritance, nutritional and hormonal imbalance, exposure to sunlight, and poor host immunity (Ranjan *et al*., 2013).

Out of the total calves treated with autohaemotherapy, papilloma was regressed in 88.9%. This agrees with other reports (Chand *et al*., 2018; Kale *et al*., 2019; Rothacker *et al*., 2015), but lower than some previous finding (Pattanayak, 2004) which showed complete regression of bovine papillomatosis. Autohaemotherapy is believed to stimulate the reticulo-endothelial system and to increase population of macrophages in circulating blood which might be responsible for enhancing regression of the papillomas (Ranjan *et al.*, 2013).

Majority of regression (≥75%) in both treatments (autogenous vaccine and autohaemotherapy) started regression after four (4) weeks of treatment (Table 2). No recurrence or new case of bovine papilloma was observed after 16 weeks of last treatment for both autohaemotherapy and autogenous vaccination. This agrees with previous reports (Turk *et al*., 2005; Nehru *et al*., 2017) which depicted that autogenous vaccine and autohaemotherapy prevent recurrence as well as spreed of the disease.

Table 1: Effect of treatment, time of regression of papilloma, recurrence and new case of papilloma

Treatment category	Recovery of papilloma N (%)	Chi-square	p-value	Time taken for total recovery (weeks)		Chi-square	p-value	Recurrence (after 16 weeks)	New case of papilloma after 16 weeks within the same herd
				3rd	**4th**				
Autohaemotherapy	8(88.9)	1.059	0.303	2(25%)	7(75%)	0.321	0.571	0(0.0%)	0(0.0%)
Autogenous vaccine	9(100.0)			2(22.2%)	7(77.8%)			0(0.0%)	0(0.0%)

2) Haematogical profile of experimental animals in association with treatment of Bovine papillomatosis

During the study period, haematological perameter particularly differential leucocyte count was undertaken to analyse cellular response as immunization against the disease. The result showed that the total leucocyte count and lymphocyte counts were significantly increased ($p<0.01$) after treatment when compared to the counts before treatment. Neutrophils and monocytes were slightly decreased after treatment. This agrees with Budianto *et al*, (2021) who reported an increase in the number of total leucocyte count and lymphocytes after treatment of the papilloma (table 2).

Table 2: Hematological profile of animals before and after treatment

Hematological parameter	Before treatment (Mean±sd)	After treatment (Mean±sd)	P- value
Total leucocyte	8222.22±427.793	14240.00±0.000	0.000
Lymphocyte	2240.00±0.000	3553.33±19.403	0.000
Neutrophil	4744.00±0.000	4000.00±0.000	ns
Monocyte	500.00±0.000	400.00±0.000	na
Eosinophil	400.00±0.000	402.22±6.468	na
Basophil	56±0.00	60±0.00	na

Note: *ns*- represents non-significant, *na*- represents not applicable

Conclusion and Recommendations

The study showed that both autogenous papilloma vaccine and autohemotherapy were effective for the treatment of bovine papillomatosis. Majority of regression took place after 4 weeks of treatment while in about 25% of cases regression took place in the third week of treatment. There was neither recurrence of the papilloma on the treated animals nor occurrence of a new case of papillomatosis during the study period. Based on the above conclusion the following recommendations are forwarded:

- Works should be done on awaring importance of cheap and simply practical methiods of treatment of papillomatosis such as autogenous vaccination and autohaemotherapy
- The use autogenous vaccine as well as autohaemotherapy in the herds prevents the spread of papillomatosis in the herds therefore due attention in its management at young stock is preferable
- Further work should be done on combination of autohaemotherapy and para immunity stimulators as such techniques are easy to be practiced at farmers' level
- Other works should be done. on papilloma size based, location of the body and others

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Assessment of the Status and Perception of Farmers on Estrous Synchronization and Mass Artificial Insemination in West Arsi and East Shewa Zones of Oromia Region, Ethiopia

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Abstract

Assessment of the status of Estrous synchronization and mass artificial insemination (OSMAI) program was conducted at East Shewa and West Arsi Zone of Oromia region, Ethiopia. The assessment was conducted using semi structured questionnaires. A total of 220 respondents were randomly selected from 12 Kebeles and 4 districts where the OSMAI project was initiated. Almost all 91.2% of respondents indicated that they get in estrous synchronization and mass artificial insemination service. Overall 92.4% of animals injected with PGF2α shows sign of heat and of which 87.9% of animals served by artificial insemination whereas the remaining 12.1% served by bull. There is no significant difference in conception rate animals across the study area in which overall rate of conception is 68.9%. Selection of the cattle included in the program was by and large based on the decision of the AI technicians. Majority of respondents (68.4%) don't know about the sire breeds with which their animals crossed. Al most all 99% of the breed and blood level of sire used for crossbreeding is decided by AITs across the study area. The result indicates that 36.5 of calves born was reach for reproduction age and 65.2%, 17.4% and 13% of calves were served by AI, crossbreed and indigenous bulls respectively. The result indicated that there was a significant difference in degree of satisfaction between respondents in which (50 %) of respondents were not satisfied with the service they got with OSMAI and also 51 % of respondents think that peoples living in and near their village were satisfied to some extent with OSMAI service given in their area. More than half (59.5%) the respondents indicated the program has to be continued and scaled up whereas 22.6% indicated the program has to be continued as such. On the other hand 17.9% of respondents indicated that the program success was failure and it should be abandoned. The implementation of breed improvement programs has to be participatory especially farmers has to be aware and engaged for success of the program. Farmers has to know and given the chance for selecting and deciding on breed and blood level of animals with which their animals crossbred.

Key words: ***Estrus mass synchronization and artificial insemination, Conception rate, Perception***

Introduction

Estrous synchronization is the manipulation of the Estrous cycle or induction of Estrous to bring a higher number of female animals into Estrous at a predetermined period (Paul, 2010). Under the Ethiopian scenario, it is also of paramount importance as there are seasonal shortages of feed and fodder and the demand for livestock products is largely dependent on the season (Tegegne *et al*., 1989). It can also minimize the challenges faced by AI services in the country (Solomon *et al*., 2016).

In Ethiopia studies on Estrous synchronization in dairy cattle was initiated in the late eighties (Azage *et al.*, 1989; Mukasa-Mugerwa *et al.*, 1989). The first large scale field trials were conducted under the IPMS project in Tigray and SNNPRS regions (Azage *et al.*, 2012). Following the field tests, the synchronization program was adopted and scaled up by the authorities of Ministry of Agriculture and regional Bureau of

Agriculture in collaboration with the authorities of international development partners (IPMS, LIVES projects (ILRI)) and the national research system. The first round of synchronization was initiated in the year 2012 in several locations in Oromia region and the scaling-out was carried out by the regional Livestock Development and Health Agency at some selected milk shed areas of the region (Tegegne *et al.,* 2016).

Studies have indicated that the rate of conception (among the cattle) varied between the regular development intervention and the action research with results varying across the regions and thus, the overall results of the scaled up project was inconsistent (Solomon *et al.,* 2016). However, information on calving rate, sex of calves born, effect on animals' fertility, contribution of the program on improving blood level and milk yield, perception of farmers on the program and others are not available in West Arsi and East Shewa Zones of Oromia region. Thus, this study was conducted with the objectives of assessing the status and perception of farmers on Estrous synchronization and Mass AI in West Arsi and East Shewa Zones.

Materials and Methods

Description of the study area

The study was conducted in East Shewa and West Arsi Zones of Oromia region. A total of 4 districts, 2 districts per each zones were selected based on previous exposure to estrous mass synchronization in the zones.

Sample Size Determination and Sampling procedures

Districts were purposively selected from areas where intervention in mass synchronization of cattle was undertaken. A total of 12 kebeles and 3 kebeles per district were randomly selected from those involved in estrous mass synchronization program. Furthermore respondents were randomly selected from each kebele based from lists on case book of mass synchronization with selection criteria based on experience of participating in mass synchronization program for at least in one round. Totally, 240 respondents (20 farmers from each Kebele) were interviewed by using pre-tested semi structured questionnaires.

Type of data and Data Collection Methods

Both qualitative and quantitative data were collected from primary and secondary data sources during the study period. The primary data were collected by using questionnaire survey while secondary data were collected from record books at various government offices (office of Agriculture at Zone and district level to assist the selection of Districts, Kebeles and respondents).

with regard to primary data household demographic characteristics, mating system, farmer's preference for breeding methods, artificial insemination service provision and its constraints, farmer's perception regarding estrous synchronization and mass artificial insemination, calving condition, sex of calves born and status of the calves were amongst the parameters of consideration.

Data Management and Statistical Analysis

The collected data were fed into Microsoft excel, coded and saved until analysis by statistical software. The qualitative were analyzed by using the Chi-square ($\chi2$) test procedure of SPSS Ver. 24.0. Quantitative data from survey were analyzed using the GLM (univariate). The variation between groups was considered significant when the $P \leq 0.05$.

Results and Discussion

Demographic characteristics of the Respondents

The result pertains to sex and major farming system of respondents are indicated in table1. The result indicates that majority of the respondents were males and this in agreement with Alemayehu *et al.* (2019) and Bainesagn *et al*. (2015). The result further indicates that there is a significant difference among major farming system with higher crop dominant mixed farming system in the study area (Table1).

Table 1. Sex and major farming systems of the respondents

Parameters	**Zone of respondents**		**Overall Mean**	**P value**
	East Shewa	**West Arsi**		
Sex of respondents				
Male	63.6	68.8	66.2	0.419
Female	36.4	31.2	33.8	
Major farming system of respondents				
Crop production	11.8	0	5.9	0.000
Livestock production	20	2.8	11.4	
Mixed with crop dominate	67.3	89	78.1	
Mixed with livestock dominate	0.9	8.3	4.6	

As indicated in figure 1 32.9% of respondents had educational background of grade 1 to 6 in East Shewa while more than 20% of the respondents attended the same school level in West Arsi.

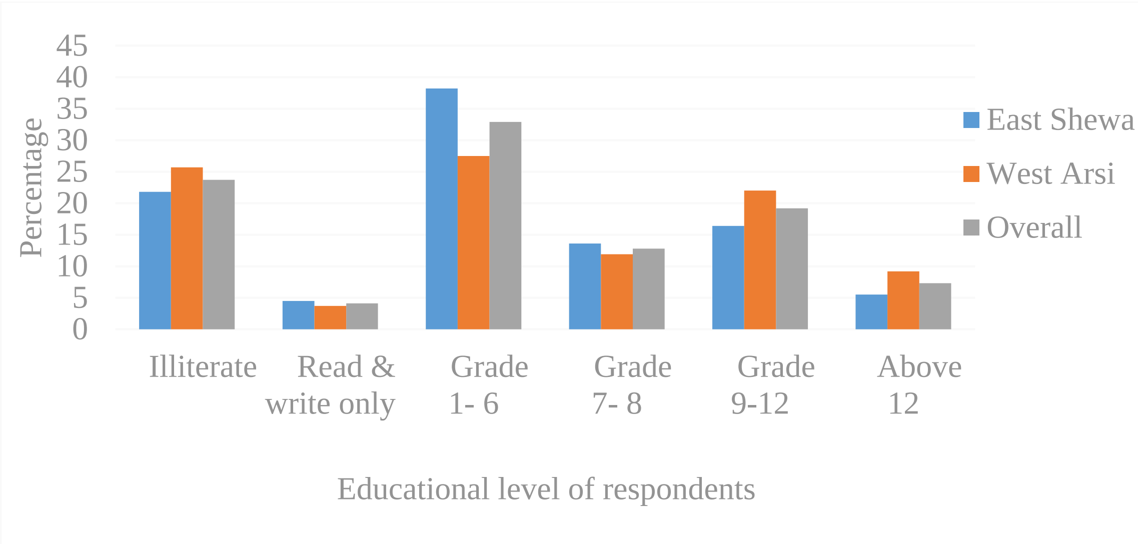

Figure 1. Educational level of respondents

Pertaining to the composition of the herd of the cattle in the study areas as shown in Table 2, it indicates that majority of livestock reared by the respondents were indigenous breeds. The result is in accordance with previous findings (Abera, 2016; Bainesagn, 2015) who reported that the numbers of crossbred cattle are quite low in the country and do not account for more than 5%.

Table 2. Livestock composition (Mean ± SD) and herd size (in TLU) of sampled respondents

Livestock type	**Breed**	**Kebele of respondents**		**Overall mean**
		East Shewa	**West Arsi**	
Cows	Indigenous	1.79±0.20	1.32 ± 0.12	1.56 ± 0.12
	Crossbred	0.95±0.15	0.92 ± 0.08	0.94 ± 0.08
Oxen	Indigenous	1.80 ±0.17	1.10 ± 0.11	1.45 ± 0.10
	Crossbred	0.10 ± 0.04	0.29 ±0.06	0.20 ± 0.04
Bulls	Indigenous	0.36 ± 0.08	0.53 ± 0.08	0.45 ± 0.06
	Crossbred	0.10 ± 0.03	0.19 ±0.05	0.14 ±0.03
Heifers	Indigenous	0.95 ± 0.16	0.51 ± 0.07	0.74 ± 0.09
	Crossbred	0.49 ± 0.10	0.54 ± 0.07	0.52 ± 0.06
Bull calves	Indigenous	0.65 ± 0.10	0.41 ± 0.07	0.53 ± 0.06
	Crossbred	0.26 ± 0.05	0.28 ± 0.04	0.247 ± 0.03
Cow calves	Indigenous	0.55 ± 0.09	0.28 ± 0.06	0.42 ±0.05
	Crossbred	0.24 ± 0.06	0.39 ± 0.05	0.31 ± 0.04
Sheep	Indigenous	1.77 ± 0.35	1.26 ± 0.28	1.51 ± 0.22
Goats	Indigenous	1.60 ± 0.34	0.75 ± 0.23	1.18 ± 0.21
Chicken	Indigenous	2.78 ± 0.45	1.11 ± 0.25	1.95 ± 0.26
	Crossbred	2.84 ± 0.75	0.96 ± 0.25	1.90 ± 0.40

Status and Perception of Farmers on estrous Mass Synchronization

Status of Estrous Mass Synchronization

Almost all (91.2%) of respondents said that they get in estrous synchronization and mass artificial insemination service. Out of the total cattle synchronized by PGF2α, 92.4% came to heat. Amongst the total 182 cows with sign of heat, 160 (87.9%) were inseminated by artificial insemination while the rest (12.1%) were served by bull. Overall, the conception rate in the study area was 68.9%. The current result is lower than the report by Tegegne *et al. (*2012). The difference might be due to the implementation differences as the later was done implemented by a group of experts in organized way. They have an experience of participating in the program with a range of once to three times with in the duration of 2008 to 2014 E.C.

Table 3. Summary of animals included in the program by respondents

No of animals		Zone of respondents		Total
		East Shewa	West Arsi	
Estrous synchronized	N	90	107	197
	%	45.7	54.3	100
Comes to heat	N	78	104	182
	%	86.7	97.2	92.4
Served by artificial insemination	N	69	91	160
	%	88.5	87.5	87.9
Served by Bull	N	9	13	22
	%	11.5	12.5	12.1
Conceived	N	44	80	124
	%	57.1	77.7	68.9

The result regarding number of animals injected with PGF2α, show sign of heat, served by AI, natural bull and conceived indicated in table 3. Overall 92.4% of animals injected with PGF2α shows sign of heat and of which 87.9% of animals served by artificial insemination whereas the remaining 12.1% served by bull. The overall rate of conception is 68.9% across the study area.

The result indicates that there is a significant difference in breed of calves born in which 75.6% of calves was crossbreed whereas the remaining 24.4% were local calves this might be due to the management and follow up of animals after first service and they indicate that 7.3 and 5.2% of respondents kept their animal with other animals without and with bull. The current result is in accordance with Alemayehu *et al*, 2019 whose report most of the calves born were of crossbred types with slight variation across the study areas. This might be due to the fact except few animals majority of animals comes to heat were inseminated by AI. Furthermore they indicated that 73.4% of their animals repeated after first service and 56.5% of them use natural bull service because they didn't get artificial insemination service and AITs were not in their area at a time when the animals were repeating since artificial insemination was provided in the form of campaign in their area this in turn has its own effect on the breed of calves to be born and affect the progress of genetic improvement in the area.

The survivability of the calves born (across both the genotypes) were 10.7% and were higher than the values reported by (Asseged and Birhanu, 2004, Alemayehu et al, 2019) from dairy farms in and around Addis Ababa and Adami Tulu Jido Kombolcha districts areas of the country, however, regular follow up has to be taken so as to ensure growth of the calves. The difference might be due to location of the area and management provided for calves just immediately after birth and onwards.

Animal Selection and Management

The results pertains that selection of the cattle for involvement in artificial insemination program was largely based on the decision of the AI technicians. The finding is in contrast with those of (Tegene and Zelalem, 2016) who reported that selection of animals was done by a group of experts and farmers. Such poor performance might be due to lack of participatory approach in the work.

About 41.6% of the respondents did not have any information on selection criteria of animals for mass synchronization service. The current result is in contrast with the report by Bainesagn, (2015) and Alemayehu *et al.(* 2019), who reported that farmers were aware about selection criteria of the animals. The difference might be due difference in the level of awareness of farmers about mass synchronization.

With regarding to animal management after hormonal injection, 87.5% of them kept their inseminated cows separate from other male animals and follows up for signs of heat, and took back for artificial insemination while 13.6% of them didn't take back their animals even though they show signs of heat for artificial insemination service. With respect to diagnosis of pregnancy, 61.9% of the respondents didn't experience bringing animals for pregnancy diagnosis. Overall the result indicates that there is a knowledge gap among respondents on management and follow up of animals in the study area.

Access to Breeding Service

The majority of respondents (87 %) indicated that they have accessed one of the breeding service during the last one year of data collection in which 71.9% get AI service while the remaining 14.6 % and 13.5 get both and improved bull service respectively.

Regarding the organizations providing the service during last one year of data collection the result reveals that 80.2%, 10.4%, 8.3% and 1% of service was provided by governmental organization, private, non-governmental organization and both respectively. In particular with AI service they indicated that 86.6% of respondents get service from governmental AITs whereas the remaining 6.7 % get service from private AITs and 6.7% from both.

Regarding the sire breeds used for crossbreeding in their dairy animals, the result indicates that the knowledge on sire breed which is used in the insemination of their animals is variable. Majority of the respondents (68.4%) don't know about the sire breeds with which their animals are crossed. Al most all 99% of the breed and blood level of sire used for crossbreeding is decided by AITs across the study area. The study showed that the respondents were not aware of the breed from which the semen for insemination collected. This is in contrast with previous observation by Bainesagn (2015) who reported that farmers were aware about sire breeds with which their animals are inseminated. This variation may be due to weak awareness creation at community level and poor implementation of extension approaches. This in turn results in failure in the success of adoption of the technology and weak initiation of farmers for utilization of the technology.

Breeds, decision on sire breed and blood level used for crossbreeding

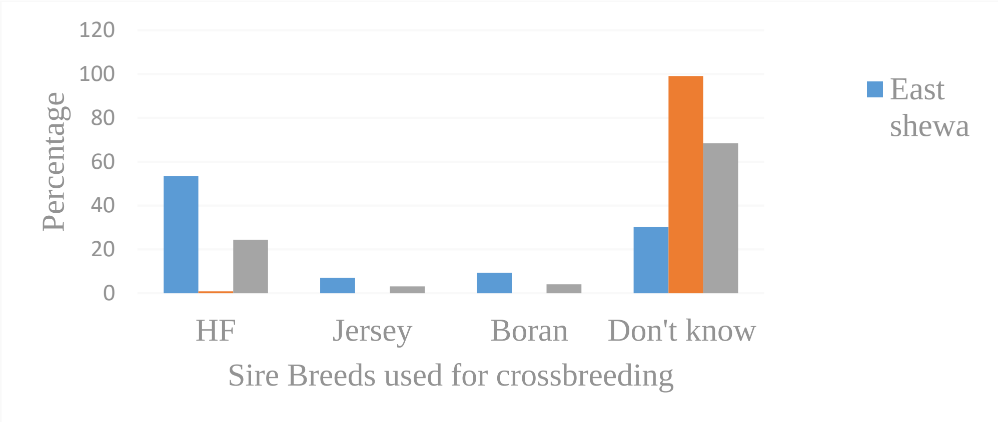

Figure 2. Sire breeds used for crossbreeding

The result revealed that there is significant difference among respondents in their preference for sire breeds they consider suitable for crossbreeding their area in which 72.7%, 9.1% and 4.0% prefer HF, Jersey and Boran breeds respectively whereas 14.1% of them doesn't know which breed to prefer.

Table 4. Decision maker and preference of sire breed and blood level used for crossbreeding

Parameters	**Zone of respondents**		**Overall mean**	**P value**
	East Shewa	**West Arsi**		
Decision maker of sire breed and blood levels used for crossbreeding (%)				
AITs	63.0	99.1	99.0	
Farmers	1.1	0	0.5	0.364
Both	0	0.9	0.5	
Preference of sire breeds by respondents				
Holstein Friesian	63.0	81.1	72.7	
Jersey	16.3	2.8	9.1	
Boran	8.7	0	4.0	0.000
Don't know	12.0	16	14.1	

Status of Calves Born

The result indicates that 36.5 of calves born was reach for reproduction age and 65.2%, 17.4% and 13% of calves were served by AI, crossbreed and indigenous bulls respectively. The reason for the lower number of calves which reached for reproduction age is that, the data from the year 2016 to 2020 was considered and most of the calves born were at younger age by then. On average they produce 7.56 ± 0.96 liter per day milk at first parity. Regarding the male calves born, 57.6% of respondents stated that they use them for ploughing whereas the remaining 39.41% and 3.6% sold and use for breeding purpose respectively.

Regarding sex preference of calves born 88.4% of respondents prefer female calves as they are important for dairy herd replacement whereas 7.2% and 4.4% prefer both sex and male calves.

Perception of Farmers

The current findings as indicated that the levels of satisfaction/dissatisfaction among the respondents with regard to estrous mass synchronization and artificial insemination is variable. Out of the respondents only 49.5% were satisfied while 50% were not. The satisfaction to the service by the people in and around the village was 26.5% followed satisfaction to some level (somewhat satisfaction) (51%) and dissatisfaction (22.4%). there is statistical evidence ($p<0.001$) that the level of satisfaction to the technology is variable (table 5). This is in agreement with previous finding (Bainesagn, 2015), who reported that in such mass insemination programs it is important to take into confidence the aspirations of the beneficiaries and the pros and cons of such project be intimated to them in advance.

The result reveals that the main reason for not satisfied with OSMAI service provided to them by respondents were cows having problem with conceiving and coming to heat in normal cycle. These observations are in close accordance with those of (Destalem, 2015) who also reported that cows which are provided with PGF2□ or their analogue had problems with conception in the subsequently. These observations were contradicted with the findings of (Azage *et al.,* 2012) who reported that there were no differences in subsequent conception among the cattle. The difference might be due to the implementation strategy followed during implementing the program since the later was done in action research form

Table 5. Satisfaction level of respondents and people in and around their village

Parameter	Zone of respondents		Total	P value
	East Shewa	West Arsi		
Level of satisfaction by respondents				
Satisfied	30.3	65.4	49.5	.000
Not satisfied	69.7	33.6	50	
Somewhat satisfied	-	1	0.5	
Level of satisfaction of people in and around their village				
Satisfied	18	33.6	26.5	.000
Not satisfied	42.7	5.6	22.4	
Somewhat satisfied	39.3	60.7	51	

Perception of farmers on future trends of estrous Mass Synchronization Program

More than half (59.5%) the respondents indicated the program has to be continued and scaled up whereas 22.6% indicated the program has to be continued as such. On the other hand 17.9% of respondents indicated that the program success was failure and it should be abandoned.

The farmers put their idea on the future trends in the utilization of mass synchronization technology. Some of the suggested supportive reasons for continuation of the program contribution of the technology in the improvement of genetic makeup of the animals (91.4%), the technology supports in the reduction of poverty (90.9%), and the technology helps in improving the social status of the owners (90.9%).

In contrary to support, some of the respondents claim that the technology should be discontinued due to Cows having problem in coming to estrous in the normal manner (66%), and Cows face problem with conceiving (72.9%) (Table 6). The current result is similar with report by Alemayehu *et al*. (2019) but contradicted with the findings of Azage *et al.* (2012), who reported that there was no differences in subsequent conception among the cattle.

Table 6. Reasons for the program scaling up and continuation and discontinued and abandoned

Parameter	Zone of respondents		Total	P value
	East shewa	West Arsi		
Reasons for scaling up and continuation				
1. Improve genetic makeup of animals				
Yes (%)	81	100	91.4	0.000
No (%)	19	-	8.6	
2. Help in poverty reduction				
Yes (%)	80	100	90.9	0.000
No (%)	20	-	9.1	
3. Help in improving the social status of the owners				
Yes (%)	80	100	90.9	0.000
No (%)	20	-	9.1	
Reason for discontinuation				
1. Synchronized Cows have problem in coming to estrous in the normal manner				
Yes (%)	58.3	90.9	66	0.046
No (%)	41.7	9.1	34	
2. Cows face problem with conceiving				
Yes (%)	64.9	100	72.9	0.021
No (%)	35.1	-	27.1	

Conclusion and recommendation

The status and perception of farmers on estrous Synchronization and Mass Artificial insemination in East Shewa and West Arsi Zone was assessed. Majority of animal owners doesn't know the sire breed and blood level with which their animals crossed and decision is done by AITs based the semen they have at hand write at that time. Conception rate of animals significantly differed across the study areas. Half of respondents were not satisfied with estrous Synchronization and Mass Artificial insemination service given in their area.

The implementation of breed improvement programs has to be participatory especially farmers has to be aware and engaged for success of the program. Farmers has to know and given the chance for selecting and deciding on breed and blood level of animals with which their animals crossbred. Estrous synchronization and mass AI service has to be implemented by group of experts and on job training, incentives, supply of inputs and logistics has to arranged for AITs to facilitate service delivery.

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Meat Animals Research Results

Effects of Substitution of Concentrate Mix with Vetch Hay on Feed Intake, Body Weight Gain & Carcass Characteristics of Arsi-Bale Sheep Fed a Basal Diet of Fodder Oat Hay.

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ABSTRACT

An experiment was conducted using thirty-six intact male Arsi-bale sheep to investigate the effects of different levels of vetch hay substitution to concentrate mix on feed intake, body weight gain and carcass characteristics of Arsi-bale sheep and to determine optimum level of vetch hay substitution to concentrate mix in the diet of Arsi-bale sheep. The sheep were blocked into Six blocks of Six animals based on their initial body weight and animals within each block were randomly assigned to one of the six treatment diets. The dietary treatments were fodder oat hay *ad libitum alone (T1)*; Fodder oat hay *ad libitum* + 350 g DM/day Concentrate mix (T2); Fodder oat hay *ad libitum* + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay (T3); Fodder oat hay *ad libitum* + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay (T4); Fodder oat hay *ad libitum* + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay (T5) and Fodder oat hay *ad libitum* + 350 g DM/day Vetch hay (T6). After the 21 days of quarantine period and 15 days of acclimatization to the experimental diets and pens the sheep were fed their respective treatments for 90 days. At the end of 90 days, the sheep were slaughtered for carcass evaluation. The sheep in T2 had higher ($P<0.001$) total dry matter and crude protein intakes than sheep in T1 and T6. Average daily gain (ADG) was higher ($P<0.001$) for T2 than T1, T4, T5 and T6. Among supplemented groups feed conversion efficiency (FCE) was higher ($P<0.001$) for T2 than T5 and T6. Slaughter body weight and hot carcass weight were also higher ($T<0.05$) for T2 than T6 and similar ($P>0.05$) for other treatments. The proportion of muscle is higher ($P<0.05$) for T4 than in T1 and T3. Partial budget analysis indicated that total variable cost decreased with increasing levels of vetch hay substitution for concentrate mix. However Gross return (GR) and total return (TR) were highest for T2. Therefore it can be concluded that substitution of concentrate mix as a whole (Energy + Protein source) at any level is not economical due to high cost of vetch production. However, biologically vetch hay can substitute 75% of the concentrate mix without significantly affecting body weight and carcass parameters of sheep. Furthermore investigation of the effect of substitution of only protein source with vetch hay is recommended.

Keywords: Arsi-bale sheep; weight gain; Carcass; Concentrate mix; substitution; Vetch Hay

INTRODUCTION

Supplementing concentrates to animals fed low-quality hay is known to improve intake and digestibility of roughages (Nurfeta, 2010) and enhances animal productivity. However, the use of such a supplement is limited under smallholder livestock production systems due to scarcity and high cost of concentrates. In order to mitigate the problems associated with the lack of protein supplement, there is a need to look for alternative protein sources that farmers can produce at their own farms. Supplementing poor quality roughage with leguminous forage results in the contribution of sources of both nitrogen and easily fermentable fiber, that can enhance microbial digestion in the rumen through increased attachment of microbes to the roughage feeds.

Among a number of native and introduced annual forage legumes, vetch is known to be well adapted to the highlands of Ethiopia (Getinet, 1999). Vetch hay has a very important nutritional value with high crude protein and minerals, but contains low tannins (Abbeddou *et al.*, 2011). It can be used as a disease break and is used to improve soil fertility for the subsequent cropping in cereal cropping rotations. Forage legumes like vetch have a significant role in enhancing soil carbon sequestration and reducing GHG emissions from the ruminant systems.

According to Berhanu *et al.* (2022) yearling Arsi-bale sheep supplemented with 350 g/day Gebisa vetch variety gained body weight of 152.5 g/day. It can be clearly observed that the average daily body weight gain reported by Berhanu *et al.* (2022) was very high compared to the results reported so far using concentrate mixture as a supplement. This suggests vetch hay may replace concentrate mixture and even may bring about higher performance than concentrate. However, the effect of substitution of concentrate mix with vetch hay was not investigated yet. Therefore, this experiment was conducted with the following objectives.

- ➢ To investigate the effect of different levels of vetch hay substitution to concentrate mix on feed intake, body weight gain and carcass characteristics of Arsi-bale sheep.
- ➢ To determine optimum level of vetch hay substitution to concentrate mix in the diet of Arsi-bale sheep.

MATERIALS AND METHODS

Description of the Experimental Area

The experiment was conducted in 2020/2021 at Sinana Agricultural Research Center (SARC), which is located in Bale Zone of Oromia National Regional State, South Eastern Ethiopia. The research center is situated 463 km south east of Addis Ababa at 07^0 07' N latitude and 40^0 10' E longitude and at an altitude of 2400 m above sea level. The average annual maximum and minimum temperature are 21^0C and 9^0C, respectively. The area is characterized by bimodal rainfall pattern with total annual precipitation ranging from 750 to 1000 mm. Two distinct cropping seasons are known in the study area, locally known as *Bona* (Meher) which extends from August to December and *Ganna* (Belg) extending from March to July. The farming system is mainly mixed crop livestock production and the major livestock raised in the study area are cattle, sheep, goats, equines and poultry. The common food crops produced in the study includes cereals (mainly wheat), highland pulses, highland oil crops, and horticultural crops, where as the major forage crop grown is fodder oat.

Preparation of Experimental Feeds

The experimental forages, vetch and fodder oat were sown according to their respective agronomic practices during the main rainy season (August-December) of the area. Vetch was harvested at 50% flowering, while fodder oat was harvested at heading stage during which they give optimum performance in terms of dry matter yield and nutritive value. The harvested fresh forages were field-cured and stored as hay under a roofed shelter to protect from rain and intense sunlight. Feeds enough for the entire experimental period were prepared before the commencement of the experiment. During the feeding period, the oat and vetch hays were chopped to about 3-5 cm in length to make uniform for sampling and easier to be seized by the animals. Concentrates (Wheat bran and Linseed cake) were bought from Goba town.

Experimental Animals and their Management

Thirty-six yearling intact male Arsi-Bale sheep with similar body weight were purchased from nearby market at Robe by a group of livestock researchers from Sinana Agricultural Research Center and others who had adequate experience in sheep rearing. The age of the sheep was estimated based on dentition and asking information from the owners of the sheep. The sheep were held in quarantine for 21 days and observed for any health problem. During this time, the sheep were vaccinated against ovine pasteurelosis, anthrax and sheep pox and dewormed against internal and external parasites. The sheep were ear tagged for identification and accommodated to an individual pens equipped with a bucket and a feeding trough in a well-ventilated concrete floor experimental barn. They were acclimatized for fifteen days to the dietary treatments to which they were allocated prior to the commencement of the actual experiment by offering them gradually. Pen cleaning was conducted twice a day at 6:00 AM and 6:00 PM.

Experimental Design and Treatments

Randomized Complete Block Design (RCBD) was used for the study. To minimize the error due to differences in initial body weight, the experimental sheep were blocked into six blocks of six animals each based on their initial body weight. Sheep within a block were assigned randomly to one of the six dietary treatments (Table 1). Sole fodder oat hay (T1) was used as a negative control treatment while T2 was used as a positive control. Fodder oat (Bona-bas) and vetch (Gebisa) varieties which were released from Sinana Agricultural Research Center in 2011 were used in the experiment. The treatments having supplementary diets were formulated on iso-nitrogenous bases to contain 18.6% CP. The proportion of wheat bran and linseed cake was determined by 'Pearson square' method of ration formulation. Accordingly wheat bran and linseed cake were mixed at a proportion of 64.4% and 35.6% respectively. The basal diet (fodder oat hay) was offered *ad libitum* to all experimental animals based on previous few days' intake at about 15% refusal while the supplementary feeds were offered in two equal meals at 8:00 AM and 4:00 PM in separate feeding troughs. Drinking water and common salt block were freely available to all experimental sheep throughout the experimental period.

Table 1.Dietary treatments

Treatments	**Feed offer per head (g DM/day)**		
	Fodder oat hay	**Concentrate mix**	**Vetch hay**
T1 (No supplement)	*Ad libitum*	0	0
T2 (100% CM)	*Ad libitum*	350	0
T3 (75% CM and 25% VH)	*Ad libitum*	262.5	87.5
T4 (50% CM and 50% VH)	*Ad libitum*	175	175
T5 (25% CM and 75% VH)	*Ad libitum*	87.5	262.5
T6 (100% VH)	*Ad libitum*	0	350

CM=Concentrate mix; DM=Dry matter; VH=Vetch hay

Feeding Trial

After an acclimatization period of 15 days to the experimental diets and pens, the feeding trial was conducted for 90 days. The daily feed offered and refusals were weighed and recorded for each sheep. Daily dry matter and nutrient intake were calculated as the difference between the feed offered and refused. Samples of feed offered were collected per batch while samples of refusal were taken from each sheep daily and pooled per animal individually over the experimental period and stored in plastic bags. Sub-samples of feed offered and refusal were taken after thorough mixing for nutrient composition determination, and the sub-samples taken were dried at 60^{o}C for 72 hours in a forced draft oven to make it ready for grinding and chemical analysis.

Body Weight Change

Body weight of the animals was taken at the beginning of the feeding trial and at 10 days interval during the 90 days of feeding trial. All animals were weighed in the morning hours before feed provision using weighing balance with a sensitivity of 100 grams. Average daily body weight gain was calculated as the difference between final live weight and initial live weight divided by the number of feeding days.

$$\textbf{Average daily body weight gain} = \frac{\textbf{Final body weight} - \textbf{Initial body weight}}{\textbf{Number of feeding days}}$$

Feed Conversion Efficiency

Feed conversion efficiency was determined by dividing the daily average body weight gain (ADG) by daily total DM intake of the animal. Similarly, Protein conversion efficiency was determined by dividing average daily body weight gain to crude protein intake.

$$\textbf{Feed conversion efficiency} = \frac{\textbf{Average daily body weight gain in gram}}{\textbf{Daily dry matter intake in gram}}$$

$$\textbf{Protein conversion efficiency} = \frac{\textbf{Average daily body weight gain in gram}}{\textbf{Daily crude protein intake in gram}}$$

Carcass Parameters

At the end of the experiment, feed was with held from all the sheep over night, they were weighed the next morning, and the weight was recorded as slaughter body weight (SBW). Once the slaughter body weight was taken the sheep were slaughtered immediately for carcass evaluation. The animals were killed by severing the jugular vein and the carotid artery with a knife. On slaughtering, blood was collected in a container, weighed and recorded. The animals were then suspended with head down. The head was detached from the body and weighed. Skin was carefully flayed to avoid attachment of fat and muscle tissues to the skin and then weighed without feet and the feet below fetlock joints were separately weighed and recorded. Empty body weight was calculated as the difference between slaughter body weight and gut fill. The hot carcass weight (HCW) was taken after removing the head, thorax, abdominal and pelvic cavity contents as well as legs below the hock and knee joints.

Offal components were categorized into edible and non-edible according to the culture of the people in the study area. Accordingly the edible offal components (EOC) namely, blood, liver, kidney, heart, tongue, stomach, small and large intestine, testicles and fats (kidney, heart, omental, scrotal and pelvic) were weighed and recorded individually. Total edible offal components (TEOC) were calculated as the total sum of the edible offal components. The non-edible offal components (NEOC), namely, head (without tongue), skin, lung plus trachea, pancreas, spleen, bladder, gall bladder, gut fill, genital organ and feet with hooves were weighed and recorded. Total non-edible offal components (TNEOC) was calculated as the total sum of the non-edible offal components.

The main carcass components were split down at the vertebral column having the two sides as symmetrically as possible and stored in a deep freeze at -4^{0}C for 24 hours for properly partitioning the carcass in to bone, muscle and fat. The frozen carcass was weighed and the weight recorded as chilled carcass weight (CCW). The right part of the frozen carcass was divided in to five main primal cuts carcass components namely: leg, loin, rack, breast and shank and shoulder and neck. The carcass was cut perpendicular to the backbone between the 12th and 13th ribs to measure the cross-sectional area of the rib eye (*longissimus dorsi*) muscle area (Purchas, 1978). The rib eye area was traced first on transparency paper then on a graph paper. The area was calculated by counting the squares on graph paper and multiplying with their area after the rib eye area was transferred to graph paper. The five main primal cut carcass components were partitioned to bone, muscle and fat and each part was weighed and recorded. The dressing percentage was calculated as the proportion of hot carcass weight to slaughter and empty body weight.

Chemical Analysis

The chemical analysis of the experimental feeds and refusals were carried out after taking the representative samples. Samples of feed offered and refusals were ground to pass a 1 mm sieve mesh. Analysis for DM, ash and N contents was done according to AOAC (2005). Dry matter and ash contents of representative samples of feed offered and refused were determined by oven drying at 105^{0}C overnight and by combusting in a muffle furnace at 550^{0}C for 3 h, respectively. Total nitrogen (N) content was determined by using Kjeldahl method and crude protein (CP) was calculated as N×6.25. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by using the procedures of Van Soest and Robertson (1985). Hemicelluloses (HC) and cellulose (Cell) contents were calculated as NDF minus ADF and ADF minus ADL, respectively.

Partial Budget Analysis

The partial budget analysis was undertaken to determine the potential profitability of substituting vetch hay for concentrate mix as a supplement to sheep fed a basal diet of fodder oat hay. The actual purchasing price of sheep was recorded while purchasing the sheep from the market and selling price of sheep was estimated by experienced dealers supplemented with market assessment at Robe town. The total quantity of basal diet and its cost of production as well as total quantity of supplement feeds and their cost of purchase were recorded and used as total variable cost (TVC) in the analysis. Since there was slight variation in price of sheep purchase it was also included in total variable cost. However, since other costs like labor and veterinary service were common for all treatments, it was not included in TVC. The selling price of sheep was considered as gross return (GR) and price differences between purchases and selling was considered as total return (TR). Then, net return of each treatment was computed as the difference between total return (TR) and total variable cost (TVC) and calculated as NR= TR-TVC. Change in net income is the most important criterion in deciding acceptance of different proportion of vetch hay and concentrate mix supplementation as profitable and to determine the best profiting treatment. Therefore, the change in net income (ΔNI) was computed as the difference between the change in total return (ΔTR) and the change in total variable cost (ΔTVC) and calculated as ΔNI= ΔTR-ΔTVC.

Data management and Statistical Analysis

Data on feed intake, body weight change and carcass parameters were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (SAS, 2004) version 9.1. When significant, Tukey's HSD test was employed to locate differences between the treatment means. Data was analyzed using the following model.

$$\mathbf{Y_{ij} = \mu + T_i + B_j + E_{ij}},$$

where: Y_{ij} = Response variable, μ = Overall mean, T_i = Treatment effect, B_j = Block effect, and E_{ij} = Random error

RESULTS AND DISCUSSION

Chemical Composition of Experimental Feeds and Refused Feed

The results of chemical analysis of the experimental feeds offered and refused are presented in Table 2. The 8.3% CP content of fodder oat hay used in this study was slightly lower than the 12.4% CP content of the same variety reported by Dawit and Teklu (2011). Although the CP content of fodder oat hay used in this study was lower than the previous reports, it was higher than the 7% CP required for microbial protein synthesis in the rumen that can support at least the maintenance requirement of ruminants (Van Soest, 1994). The CP content of Gebisa vetch variety used in this study was also lower than the 20.8% CP content of the same variety reported by Dawit *et al.* (2011). The lower CP content of experimental forages registered in this study might be due to losses of the leaf fractions containing high CP while curing the experimental forages in the field. The NDF and ADF contents of fodder oat hay used in this study were lower than the 62.6% NDF and 38.3% ADF reported by Dawit and Teklu (2011). The NDF and ADF content of Gebisa was comparable with the 40.8% NDF and 32.6% ADF of the same variety reported by Dawit *et al.* (2011).

The inconsistency in chemical composition of the experimental feeds used in this study with previous studies might be associated with growth stage of the plant, harvesting season, method of preparation and preservation. Soil fertility on which the forages were grown, plant morphological components mainly leaf to stem ratio and losses of nutrients during hay making which arises from the action of plant and microbial enzymes, chemical oxidation, leaching and mechanical damage are also among factors affecting the chemical composition of feeds (McDonald *et al.,* 2010) and causes for variability in nutrient content between the same species and variety of forage.

Singh and Oosting (1992) pointed out that roughage feeds containing NDF values of less than 45% to be classified as high quality, those with values ranging from 45 to 65% as medium quality and those with values higher than 65% as low quality. Accordingly, the vetch variety used in this experiment is categorized as high quality roughage.

Table 2.Chemical composition of experimental feeds and refused feed

Feed offered	**DM%**	Ash	OM	CP	NDF	ADF	ADL	HC	Cell
					%DM				
Fodder oat hay	89.0	10.7	89.3	8.3	57.8	32.0	2.9	25.8	29.1
Vetch	88.4	14.0	86.0	18.6	40.5	29.1	5.6	11.4	23.5
Wheat bran	84.9	4.4	95.6	14.4	41.5	10.9	1.8	30.6	9.1
Linseed cake	90.3	6.9	93.1	26.2	39.6	18.5	5.9	21.1	12.6
Fodder oat hay refusal									
T1	86.7	10.2	89.8	6.5	62.4	35.4	3.3	27	32.1
T2	86.3	10.2	89.8	6.7	60.5	34.9	3.2	25.6	31.7
T3	86.2	10.1	89.9	6.7	63.4	35.4	3.0	28	32.4
T4	85.4	10.4	89.6	6.6	63.6	34.7	3.4	28.9	31.3
T5	86.9	10.2	89.8	6.4	61.5	35.6	3.3	25.9	32.3
T6	86.8	10.3	89.7	6.6	62.4	35.8	3.1	26.6	32.7

ADF=Acid Detergent Fiber; ADL=Acid Detergent Lignin; Cell=Cellulose; CP=Crude Protein; DM=Dry Matter; HC=Hemicelluloses; NDF=Neutral Detergent Fiber; OM=Organic Matter; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay

The CP content of fodder oat hay refusals in this study was lower as compared to the CP content of the offered fodder oat hay. Fodder oat hay refusals in all treatments had comparatively higher contents of NDF, ADF and cellulose than the basal fodder oat hay offered indicating the selective nature of sheep in feeding more nutritious and palatable portion (leafy part) of the hay than the lignified parts.

Dry Matter and Nutrient Intake

The average daily dry matter and nutrient intake of Arsi-Bale sheep fed a basal diet of fodder oat hay and supplemented with different proportions of concentrate mix and vetch hay during growth trial are presented in Table 3. All sheep readily consumed the dietary supplement without any refusal across the experiment. The DM intake of fodder oat hay was significantly different ($P<0.05$) among treatments. The sheep in T1 (non-supplemented group) consumed higher ($p<0.05$) amount of fodder oat hay DM than sheep in T5 and T6. The higher intake of oat hay in the non-supplemented group could be due to an effort to satisfy their nutrient requirements. The sheep in T2 had higher ($P<0.001$) total DM intake than sheep in T1 and T6. This was attributed to higher oat hay intake by those sheep in T2 as the level of supplementation on DM basis was the same for all supplemented treatments. Likewise, crude protein intake was also significantly ($P<0.001$) different among treatments and is positively associated supplementation. With regard to the effect of supplementation on total DM intake, supplementation increased the total DM intake by 33.30%, 32.16%, 30.92%, 30.18% and 26.23% for T2, T3, T4, T5 and T6, respectively as compared to T1. This is in agreement with Berhanu *et al.* (2022), who reported that supplementation of 350g DM hay of Gebisa vetch variety increased total DM intake of Arsi-Bale sheep fed a basal diet of fodder oat hay, by 16.1%.

In general, the results of the current study and previous studies signify the positive effect of concentrate mix and vetch hay supplementation to sheep fed on low quality roughages. This might be due to the fact that addition of concentrate mix and vetch hay to a basal diet low in nitrogen content increases the nitrogen content of the total diet, which in turn is likely to increase feed intake and the rate of degradation of the basal diet in the rumen (Topps, 1997). The result of total DM intake in this study was higher than the DM intake previously reported for Arsi-Bale sheep by Teklu *et al.*, (2018) and lower than the results of Berhanu *et al.,* (2022).

Table 3. Average daily dry matter and nutrient intake of the Arsi-Bale sheep

Intake	Treatments						SEM	SL
	T1	T2	T3	T4	T5	T6		
OH DM (g/day)	610.9^{a}	565.9^{ab}	550.5^{abc}	534.4^{abc}	525.0^{bc}	478.1^{c}	12.27	*
WB DM (g/day)	-	225.4	169.05	112.7	56.35	-	-	-
LSC DM (g/ day)	-	124.6	93.45	62.3	31.15	-	-	-
VH DM (g/day)	-	-	87.5	175	262.5	350	-	-
Total DM (g/day)	610.9^{c}	915.9^{a}	900.5^{ab}	884.4^{ab}	875.0^{ab}	828.1^{b}	20.35	***
OM (g/day)	544.8^{c}	836.1^{a}	814.6^{a}	793.0^{ab}	776.7^{ab}	727.3^{b}	18.80	***
CP (g/day)	53.5^{c}	114.4^{a}	113.0^{ab}	111.8^{ab}	111.5^{ab}	107.6^{b}	3.77	***
NDF (g/day)	346.1^{c}	466.1^{a}	452.9^{ab}	443.2^{ab}	440.0^{ab}	410.6^{b}	9.0	***
ADF (g/day)	190.3^{c}	224.6^{b}	232.6^{ab}	242.0^{ab}	251.0^{a}	248.6^{ab}	4.82	***
ADL (g/day)	17.1^{e}	27.4^{d}	29.3^{cd}	30.3^{bc}	32.2^{ab}	33.1^{a}	0.95	***
HC (g/day)	155.8^{d}	241.5^{a}	220.4^{b}	201.2^{bc}	189.0^{c}	161.9^{d}	5.78	***

*$^{a, b, c, d, e}$ = Means with different superscripts in a row are significantly different; ***= (P<0.001); **= (P<0.01); *= (P<0.05); ADF=Acid Detergent Fiber; ADL=Acid Detergent Lignin; Cell=Cellulose; CP=Crude Protein; DM=Dry Matter; HC=Hemicelluloses; LSC=Linseed cake; NDF=Neutral Detergent Fiber; ns=non-significant; OH=Oat hay; OM=Organic Matter; SEM=Standard Error of the Mean; SL=Significance Level; VH= Vetch hay; WB= Wheat bran;*

Organic matter intake was significantly affected (P<0.001) by treatments. It was higher (P<0.001) for T2 than T1 and T6. Supplementation has increased CP intake by 53.2, 52.6, 52.1, 52, and 50.3% for T2, T3, T4, T5 and T6, respectively, as compared to T1. The CP intake was higher for T2 as compared to T1 and T6. This could be attributed to higher DM intake in T2. Overall CP intake in this study was higher than the CP intake previously reported for Arsi-Bale sheep by Teklu *et al.*, (2018) and lower than the reports of Berhanu *et al.,* (2022).

Neutral detergent fiber also varied among treatments (P<0.001) and it is positively associated with supplementation. The NDF intake of T2 was significantly higher (P<0.001) than T1 and T6. The lowest (P<0.001) ADF intake was recorded for non-supplemented group.

Body Weight Change and Feed Conversion Efficiency

The mean initial body weights of the experimental sheep were similar across all treatments due to blocking of sheep according to their initial body weight at the commencement of the experiment (Table 4). Final body weight (FBW), BWC, ADG and FCE significantly varied (P<0.05) among treatments and were positively affected by supplementation. Supplementation has increased the FBW of sheep by 26.8, 19.3, 18.0, 14.5, and 7.9% for T2, T3, T4, T5 and T6, respectively as compared to T1. Feed conversion efficiency was significantly varied (P<0.001) among treatments and was positively affected by supplementation. Supplementation improved FCE by 188.2, 126.5, 111.8, 91.2 and 38.2% for T2, T3, T4, T5 and T6, respectively. Among supplemented groups FCE was higher (P<0.001) for those sheep supplemented with 100% concentrate mixture (T2) than those sheep supplemented with 25% concentrate + 75% vetch hay (T5) and those sheep supplemented with 100% vetch hay (T6). Likewise, protein conversion efficiency (PCE) was significantly affected by treatments (P<0.01).

The improvement in body weight parameters and feed conversion efficiency observed in the supplemented groups over non-supplemented group could be associated with the concentrate mix and vetch hay supplementation which increased DM and nutrient intake. Chumpawadee *et al*. (2009) noted that when animals are fed relatively higher dietary protein, nutrient digestibility is improved leading to high nutrient uptake that promotes ADG. The FCE observed in this study was consistent with the trend of ADG. This is in agreement with Pond *et al.* (1995) who stated diets that promote high rates of gain usually result in a greater efficiency than diets that do not allow rapid gain. This is due to that fact animals with rapid body weight gain utilize less of the total feed intake for maintenance and more of it for body weight gain. Several previous findings also support that increasing protein and energy levels in the diet improved ADG and FCE of animals (Dawit and Solomon, 2009; Ermias *et al.*, 2013; Hunegnaw and Berhan, 2016; Teklu *et al.*, 2018; Berhanu *et al.*, 2022).

Table 4. Body weight change and feed conversion efficiency of the Arsi-Bale sheep

	Treatments							
Parameters	T1	T2	T3	T4	T5	T6	SEM	SL
IBW (kg)	20.9	20.9	21.0	21.1	20.9	21.1	0.30	ns
FBW (kg)	22.8^{c}	28.9^{a}	27.2^{ab}	26.9^{ab}	26.1^{abc}	24.6^{bc}	0.56	*
BWC (kg)	1.9^{d}	8.0^{a}	6.2^{ab}	5.8^{b}	5.2^{bc}	3.5^{cd}	0.42	***
ADG (g/day)	20.6^{d}	89.1^{a}	68.7^{ab}	64.1^{b}	57.6^{bc}	38.7^{cd}	4.71	***
FCE (g ADG/g TDMI)	0.034^{d}	0.098^{a}	0.077^{ab}	0.072^{abc}	0.065^{bc}	0.047^{cd}	0.005	***
PCE (g ADG/g TCPI)	0.389^{bc}	0.782^{a}	0.609^{ab}	0.569^{abc}	0.514^{bc}	0.361^{c}	0.371	**

$^{a, b, c, d}$ *= means with different superscripts in a row are significantly different; ***= (P<0.001); **= (p<0.01); ADG=Average Daily Gain; BWC=Body Weight Change; FBW=Final Body Weight; FCE=Feed Conversion Efficiency; IBW=Initial Body Weight; ns=not significant; PCE= Protein Conversion Efficiency; SEM=Standard Error of the Mean; SL=Significance Level; TDMI= Total Dry Matter Intake; TCPI= Total Crude Protein Intake; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

Carcass Characteristics

Main Carcass Parameters

Slaughter body weight (SBW), hot carcass weight (HCW), chilled carcass weight (CCW) and empty body weight (EBW) varied (P<0.05) among treatments and were positively affected by supplementation (Table 5). Among supplemented treatments, SBW and HCW were higher (T<0.05) for T2 than T6 and similar (P>0.05) for other treatments. Supplementation increased HCW by 46.9, 34.6, 44.4, 23.5 and 12.3% for T2, T3, T4, T5 and T6, respectively. Moreover, EBW was also higher (P<0.001) for those sheep supplemented with 100% concentrate mix than those sheep supplemented with 25% concentrate mix + 75% vetch hay and 100% vetch hay as well as for non supplemented group. Berhanu *et al.(*2022) reported a significant increase due to supplementation in carcass parameters of Arsi-bale sheep fed a basal diet of fodder oat hay and supplemented with different varieties of vetch hay. Other previous findings are in agreement with the current result in that there is a significant increase in carcass parameters of sheep as a result of high protein feed supplementation to low quality basal roughages (Ermias *et al.,* 2013; Mergia *et al.,* 2021). This may be due to increased dry matter and nutrient intake and dry matter and nutrient digestibility as a result of supplemental protein (Chumpawadee *et al*., 2009).

The slaughter body weight (SBW), HCW, CCW and EBW obtained by supplementation of different proportion of vetch hay and concentrate mix in the current study were higher than the values previously reported for Arsi-Bale sheep by Teklu *et al.* (2018) and lower than the result of Berhanu *et al.*(2022).This might be attributed to the differences in the feed offered to the animals and overall management practices.

Table 5. Main carcass parameters of the Arsi-Bale sheep

Parameters	Treatments						SEM	SL
	T1	T2	T3	T4	T5	T6		
SBW(kg)	21.9^{c}	27.9^{a}	26.2^{ab}	25.9^{ab}	25.1^{abc}	23.6^{bc}	0.55	*
HCW(kg)	8.1^{c}	11.9^{a}	10.9^{ab}	11.7^{ab}	10.0^{abc}	9.1^{bc}	0.40	*
CCW(kg)	7.4^{c}	11.1^{a}	10.2^{ab}	9.8^{abc}	9.3^{abc}	8.5^{bc}	0.36	*
EBW (Kg)	15.9^{d}	22.5^{a}	20.8^{ab}	20.3^{ab}	18.8^{bc}	17.3^{cd}	0.49	***
Dressing Percentage								
SBW basis	36.9	42.9	41.5	45.7	39.9	38.5	1.33	ns
EBW basis	50.9	52.9	52.4	57.8	52.9	52.4	1.50	ns
BFT (cm)	0.1^{c}	0.3^{a}	0.2^{b}	0.2^{b}	0.2^{b}	0.1^{c}	0.02	***
REMA (cm^2)	6.3	7.7	7.0	7.6	7.0	6.5	0.22	ns

^{a,b,c,d} *= means with different superscripts in a row are significantly different; *= (p<0.5); *** = (P<0.001); BFT= Back Fat Thickness; CCW=Chilled Carcass Weight; DP= Dressing percentage; EBW=Empty Body Weight; HCW=Hot Carcass Weight; ns=non-significant; REMA=Rib eye Muscle Area; SBW=Slaughter Body Weight; SEM=Standard Error of the Mean; SL=Significance Level; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

The dressing percentage both on slaughter and empty body weight basis were not significantly different (P>0.05) among the dietary treatments with the overall value of 40.9% and 53.2%, respectively. This is in agreement with previous findings (Berhanu *et al.,* 2022; Teklu *et al.,* 2018;) , who reported non-significant value of dressing percentages for Arsi- Bale sheep under different feeding regimes. The mean value of dressing percentage as ratio of slaughter body weight (40.9%) in this study was higher than the value of 31.5-37.3% for Arsi-Bale sheep fed different varieties of Faba bean straw with a concentrate mixture at a ratio of 70:30 (Teklu *et al.,* 2018). However, higher value of dressing percentage on slaughter weight basis (44.2%) was reported by Ermias *et al.(*2013) for Arsi-Bale sheep fed faba bean haulms as a basal diet and supplemented with 300g concentrate mixture of barley bran and linseed meal mixed at a ratio of 1:2 (33.3% barley bran and 66.7% linseed meal) and by Berhanu *et al.*(2022) for Arsi-bale sheep fed a basal diet of fodder oat hay and supplemented with 350 g of different varieties of vetch hay. Moreover, Hunegnaw and Berhan (2016) reported a comparable value of dressing percentage on slaughter weight basis (41.34%, 39.59% and 42.07%) for Wollo lambs fed natural pasture hay+200g wheat bran and supplemented with 243g DM pigeon pea, 260g DM cow pea and 225g DM lablab, respectively.

Edible Offal Components

Edible offal components of Arsi-Bale sheep fed a basal diet of fodder oat hay and supplemented with different proportions of vetch hay and concentrate mix are presented in Table 6. The weight of kidney is significantly affected by dietary treatments and positively affected by supplementation. Significantly higher weight of kidney was recorded for those sheep supplemented with 100% concentrate mix and for those sheep supplemented with 75% concentrate mix + 25% vetch hay than un supplemented group and those sheep supplemented with 100% vetch hay. Kidney size might have effect on removal of wastes and extra fluid and maintenance of healthy bones of the animals.

Table 6. Edible offal components of the Arsi-Bale sheep.

Parameters	Treatments							
	T1	T2	T3	T4	T5	T6	SEM	SL
Blood (g)	748.8	975.2	867.3	921.2	804.2	912.3	24.20	ns
Liver (g)	232.8^{c}	345.5^{a}	343.3^{ab}	303.0^{abc}	296.0^{abc}	270.5^{bc}	11.05	*
Kidney (g)	52.2^{c}	72.0^{a}	69.7^{a}	65.3^{ab}	66.7^{ab}	60.5^{b}	1.53	***
Heart (g)	82.3^{c}	112.0^{a}	104.7^{ab}	106.8^{a}	89.5^{bc}	83.5^{c}	2.87	***
Tongue (g)	59.8	73.0	62.0	80.5	66.8	65.5	2.75	ns
Stomach (g)	692.2	777.3	799.3	728.7	815.8	663.67	24.90	ns
SI (g)	551.5^{b}	8615^{a}	665.8^{b}	633.0^{b}	650.8^{b}	578.5^{b}	24.99	***
LI (g)	172.3	261.7	193.0	224.3	152.7	278.7	21.63	ns
Testicle (g)	212.7^{c}	360.7^{a}	357.7^{a}	296.5^{ab}	311.5^{ab}	267.0^{bc}	12.00	***
Tail (g)	257.2^{c}	693.8^{a}	695.2^{a}	560.2^{ab}	486.3^{abc}	403.8^{bc}	41.40	**
Kidney fat (g)	37.2^{d}	172.0^{a}	103.2^{b}	81.0^{bc}	75.8^{bc}	51.8^{cd}	8.62	***
Heart fat (g)	29.8	52.2	43.3	67.7	40.8	37.3	3.92	ns
Omental fat (g)	21.2^{c}	183.7^{a}	102.5^{b}	100.0^{b}	91.2^{b}	42.2^{bc}	11.97	***
Scrotal fat (g)	17.8^{c}	109.0^{a}	76.8^{ab}	77.8^{ab}	54.8^{bc}	30.2^{c}	7.00	***
Pelvic fat (g)	17.8^{c}	47.5^{a}	44.5^{a}	36.7^{ab}	30.8^{abc}	23.0^{bc}	2.73	***
TEOC (Kg)	3.2^{d}	5.1^{a}	4.5^{b}	4.3^{bc}	4.0^{bc}	3.8^{c}	0.12	***

[a,b,c,] = *means with different superscripts in a row are significantly different; *=(P<0.05); **=(P<0.01);***=(P<0.001); LI=Large Intestine; ns=non-significant; SEM=Standard Error of the Mean; SI=Small Intestine; SL=Significance Level; TEOC=Total Edible Offal Component; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

The weight of heart also significantly varied (P<0.001) among treatments and significantly affected by supplementation. Testicle weight was higher for those sheep supplemented with 100% concentrate mix and 75% concentrate mix + 25% vetch hay than un supplemented group and those sheep supplemented with 100% vetch hay. Testicular size might have effect on productive and reproductive performance of the ram lambs. The study by Mehari *et al.*(2009) also indicates that plane of nutrition influences testicular size which is significantly correlated with sperm production, output and quality. Total edible offal components (TEOC) was significantly higher (P<0.001) for those sheep supplemented with 100% concentrate mix. This might be associated with body weight of the animals as sheep in T2 had better SBW than sheep in all other treatments. However some of the edible offal components were not significantly varied among treatments. Riley *et al.* (1989) indicated that differences in internal organs are more influenced by age, breed and sex of the animals rather than plane of nutrition.

Non-edible offal components

Non-edible offal components of Arsi-Bale sheep fed a basal diet of fodder oat hay and supplemented with different proportions of concentrate mix and vetch hay are presented in Table 7. Most of the non-edible offal components were not significantly varied (P>0.05) across treatments showing that non-edible offal components were not affected by supplementation of different proportion of concentrate mix and vetch hay. This indicates that the treatment difference in this study was unable to bring differences in most of non-edible offal components. The result of the current study revealed that since the weight of offal components were less affected by plane of nutrition the major objective of supplementing sheep with high protein feeds should be to increase the weight of muscles rather than the weight of offal components.

Abraham (2015) indicated that animals that consumed higher weight of feed with low digestibility had higher gut fill weight at slaughter because of deterred ruminal digestibility of nutrients and increased ruminal retention time. However, in the current study the difference in the weight of gut fill was not significantly differed among treatments though there were significant differences in the DM and nutrient intake of dietary treatments. This might be due to the time elapsed (overnight fasting time prior to slaughter) until slaughtering that made to shrink their gut fill equally.

Table 7. Non-edible offal components of the Arsi-Bale sheep.

Parameters	Treatments						SEM	SL
	T1	T2	T3	T4	T5	T6		
Head without tongue (g)	1311.2^{d}	1956.5^{a}	1835.5^{ab}	1679.3^{bc}	1577.0^{c}	1537.3^{cd}	47.38	***
skin (g)	1503.5^{b}	2087.0^{a}	2079.5^{a}	2133.2^{a}	2004.2^{a}	1975.5^{a}	53.29	***
Lung with trachea (g)	241.7^{b}	316.3^{a}	321.0^{a}	308.7^{a}	309.3^{a}	261.0^{b}	7.21	***
Spleen (g)	27.3^{b}	39.7^{a}	42.3^{a}	39.3^{a}	32.7^{ab}	34.8^{ab}	1.52	*
Pancreas (g)	25.3	32.5	31.7	34.2	29.8	27.0	1.41	ns
Bladder (g)	9.5	9.3	8.2	12.8	9.8	7.7	0.81	ns
Gall bladder (g)	5.5	13.3	10.0	11.7	10.5	3.5	1.50	ns
Penis (g)	43.7	50.2	49.5	54.8	49.7	49.7	1.43	ns
Feet with hooves (g)	402.0^{b}	496.0^{a}	480.3^{a}	499.5^{a}	455.0^{a}	447.3^{ab}	8.42	**
Gut fill (g)	6021.5	5457.0	5465.7	5549.8	6286.5	6317.0	232.56	ns
TNEOC (kg)	9.6	10.5	10.3	10.3	10.8	10.7	0.27	ns

*^{a,b,c,} = means with different superscripts in a row are significantly different; *=(P<0.05); **=(P<0.01);***=(P<0.001); ns=non-significant; SL=Significance Level; SEM=Standard Error of the Mean; TNEOC=Total Non-edible Offal Component; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

Primal Cuts

Muscle from leg significantly differed (P<0.01) among treatments and was positively affected by supplementation (Table 8). Among supplemented treatments, the weight of leg muscle from dissected half carcass was lower for non-supplemented group (T1) than T2, T4 and T5. Similarly, muscle from loin, rack, breast and shank and shoulder and neck also significantly differed (P< 0.05) among treatments and positively affected by supplementation. However, bone from loin, rack and breast and shank did not significantly differ (P>0.05) among treatments. The better leg muscle of the primal cuts obtained from sheep supplemented with 100% concentrate mix might be associated with the better feed intake and body weight parameters of those sheep.

Regarding fat from the five primal cuts, it was significantly different (P<0.05) among treatments and positively affected by supplementation of different proportions of concentrate mix and vetch hay. Regarding totals of the five primal cuts, leg total significantly differed (P<0.001) among treatments and was positively affected by supplementation. Among supplemented treatments, the treatment supplemented with 100% concentrate mix (T2) and treatment supplemented with 50% concentrate mix + 50% vetch hay had higher (P<0.001) leg total from the dissected half carcass than non-supplemented group and those sheep supplemented with 100% vetch hay. Similarly, loin total was also significantly differed (P<0.001) among treatments. The loin total of those sheep supplemented with 100% concentrate mix (T2) was significantly highest (P<0.001) among other treatments. Moreover, rack total, breast and shank total and shoulder and neck total was significantly different among treatments (P<0.05).

Table 8. Primal cuts of dissected half carcass of the Arsi-Bale sheep.

Primal cuts (g)		Treatments							
		T1	T2	T3	T4	T5	T6	SEM	SL
Leg	Muscle	645.8^{b}	1131.5^{a}	887.5^{ab}	1151.7^{a}	1006.5^{a}	890.2^{ab}	43.24	**
	Bone	313.3^{c}	370.0^{ab}	369.2^{ab}	390.5^{a}	343.7^{bc}	359.2^{ab}	6.34	**
	Fat	62.2^{c}	219.0^{a}	190.8^{a}	165.7^{ab}	164.0^{ab}	96.3^{bc}	13.06	***
Leg total		1021.3^{c}	1720.5^{a}	1447.5^{ab}	1707.8^{a}	1514.2^{ab}	1345.7^{b}	54.48	***
Loin	Muscle	198.5^{c}	389.0^{a}	305.5^{b}	312.5^{b}	302.5^{b}	261.5^{bc}	13.79	***
	Bone	76.2	109.0	86.5	80.5	97.3	95.7	4.02	ns
	Fat	40.7^{b}	100.3^{a}	69.7^{ab}	58.2^{b}	59.0^{b}	33.7^{b}	5.87	**
Loin total		315.3^{c}	598.3^{a}	461.7^{b}	451.2^{b}	458.8^{b}	390.8^{bc}	20.22	***
Rack	Muscle	164.5^{b}	304.7^{a}	257.2^{a}	282.8^{a}	298.0^{a}	232.3^{ab}	12.72	**
	Bone	121.3	135.2	145.2	133.8	145.2	135.2	4.99	ns
	Fat	23.0^{c}	134.2^{a}	80.5^{b}	62.5^{bc}	69.5^{bc}	30.8^{bc}	8.59	***
Rack total		308.3^{c}	574.0^{a}	482.8^{ab}	479.2^{ab}	512.7^{ab}	398.3^{bc}	20.37	***
Breast and shank	Muscle	226.3^{b}	295.7^{a}	275.7^{ab}	317.2^{a}	259.3^{ab}	214.7^{b}	10.35	*
	Bone	142.2	173.8	153.7	149.2	158.3	158.3	4.50	ns
	Fat	42.5^{d}	188.0^{a}	120.5^{b}	100.3^{bc}	95.5^{bcd}	61.3^{cd}	10.49	***
Breast and shank total		411.0^{d}	657.5^{a}	549.8^{abc}	566.7^{ab}	513.2^{bcd}	434.3^{cd}	20.86	**
Shoulder and neck	Muscle	594.5^{c}	1132.0^{a}	1009.0^{ab}	1007.2^{ab}	941.5^{b}	856.0^{b}	34.04	***
	Bone	350.5	416.2	461.2	420.8	398.0	375.8	10.77	ns
	Fat	92.8^{bc}	206.2^{a}	177.3^{ab}	102.8^{bc}	123.8^{abc}	53.0^{c}	13.51	**
Shoulder and neck total		1037.0^{d}	1754.3^{a}	1647.5^{ab}	1530.8^{b}	1463.3^{bc}	1284.8^{c}	47.01	***

^{a,b,c,d} *= means with different superscripts in a row are significantly different; *=(P<0.05); **=(P<0.01); ***=(P<0.001); ns = not significant; SL = Significance Level; SEM = Standard Error of Means; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

Mean weight of muscle, bone and fat and their proportions in dissected half carcass of Arsi-Bale sheep fed a basal diet of fodder oat hay and supplemented with different proportions of concentrate mix and vetch hay are presented in Table 9. The sum weight of muscle from the dissected half carcass was significantly (P<0.001) affected by supplementation. Supplementation increased the amount of muscle by 77.8, 49.5, 67.9, 53.5 and 34.2 for T2, T3,T4,T5 and T6 respectively, compared to un-supplemented treatment. Among supplemented treatments, the amount of muscle from the dissected half carcass was higher (P<0.001) for T2 than T1,T3 and T6. This might be associated with the better feed intake and body weight parameters of sheep in that group which improved the carcass characteristics. Likewise, weight of bone and fat from dissected half carcass were also significantly different (P<0.05) among treatments. The mean weight of bone varied in the order of T2=T3=T4=T5>T1.The mean weight of fat for T2 was significantly highest (P<0.001) among other treatments. The weight of fat for T1 was similar (p>0.05) to that of T3,T4 and T5 but higher than that of T6.

The overall proportion of muscle of those sheep supplemented with different proportion of concentrate mix and vetch hay did not significantly differ (P>0.05) among treatments. However, the overall proportion of bone was significantly lower (P<0.05) in T2 than in T1, T3 and T6. According to Ameha (2008), the ideal carcass can be described as the one that has a minimum amount of bone, a maximum amount of muscle and an optimum amount of fat. Therefore, the higher proportion of muscle in T2 and bone in T1 suggested that supplementation enhanced carcass quality.

Table 3. Mean weight of muscle, bone and fat and their proportions of dissected half carcass of the sheep

Parameter		Treatments							
		T1	T2	T3	T4	T5	T6	SEM	SL
Muscle	g	1829.7^{d}	3252.8^{a}	2734.8^{bc}	3071.3^{ab}	2807.8^{abc}	2454.7^{c}	96.09	***
	%	58.9^{c}	61.1^{abc}	59.5^{bc}	64.8^{a}	63.0^{abc}	63.7^{ab}	0.65	*
Bone	g	1003.5^{b}	1204.2^{a}	1215.7^{a}	1174.8^{a}	1142.5^{a}	1124.2^{ab}	20.03	*
	%	33.4^{a}	22.8^{d}	26.7^{bc}	24.8^{cd}	25.6^{cd}	29.2^{b}	0.71	***
Fat	g	261.2^{b}	847.7^{a}	638.8^{b}	489.5^{b}	511.8^{b}	275.2^{c}	42.92	***
	%	7.7^{c}	16.1^{a}	13.8^{ab}	10.4^{bc}	11.4^{bc}	7.1^{c}	0.76	***

^{a,b,c,} *= means with different superscripts in a row are significantly different; *=(P<0.05); **=(P<0.01); ***=(P<0.001); ns = not significant; SL = Significance Level; SEM = Standard Error of Means; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay*

In general, muscle comprised the highest proportion (58.9-64.8%) followed by bone (22.8-33.4%) and fat (7.1-16.1%). The weight of muscle (1829.7-3252g) and fat (261.2-847.7g) from the dissected half carcass in this study was much lower than the report of Berhanu *et al.*, (2022) who reported 3.5-4.6 kg and 0.9-1.3 kg of muscle and fat, respectively, from the dissected half carcass of the same breed of sheep fed fodder oat hay as a basal diet and supplemented with different varieties of vetch hay. Unlike muscle and fat, the weight of bone reported by the same author (1.2-1.3 kg) was comparable with the current result (1003.5 -1174.8 g). The proportion of muscle in the current study (58.9-64.8%) was comparable with the value of 61.5-63.9% reported by Berhanu *et al.*, (2022) and within the range of 51.09-76.77% lean meat of Arsi-Bale goats with different age group and feeding regime (Mesfin, 2007) and slightly lower than 63-67.4% lean meat for Borana and Arsi-Bale goats under different durations of feedlot management (Hailu *et al.*, 2005). The proportion of bone in this study (22.8-33.4%) was higher than the earlier finding 18.5-21.0 %) reported by Berhanu *et al.*, (2022). As per the description of Ameha, (2008), the ideal carcass is the one that has a minimum amount of bone, a maximum amount of muscle and an optimum amount of fat. Similarly, Pinkerton (2009) noted that the most common carcass quality assessment in small ruminants is particularly focusing on muscling or meatiness, hence muscling not body fat is the most important measure of sheep and goat carcass quality. The result of this study showed that the carcass produced from Arsi-Bale sheep fed fodder oat and different proportion of concentrate mix and vetch hay is of maximum muscle, optimum fat and minimum bone. Therefore, it can be concluded that vetch hay can substitute concentrate mix not only in terms of producing high carcass yield, but also in terms of producing the ideal carcass as well.

Partial Budget Analysis

Partial budget analysis result of Arsi-Bale sheep fed a basal diet of fodder oat hay and supplemented with different proportions of concentrate mix and vetch hay is presented in Table 10. Partial budget analysis was done to evaluate the economic feasibility of substituting concentrate mix with different levels of vetch hay. The result of partial budget analysis showed that total variable cost decreased with increasing levels of vetch hay substitution for concentrate mix. Vetch hay substitution for concentrate mix decreased total variable cost by 1.9, 9.5, 12.9 and 18.8% for T3,T4,T5 and T6 respectively as compared to T2.

Gross return (GR) and total return (TR) were highest for those sheep supplemented with 100% concentrate mix (T2). The change in net income (ΔNI) show a benefit of 406.1, 221.3, 298.9, 244.8 and 117.7% for T2, T3, T4, T5 and T6 compared to T1. The highest net benefit (NR) and change in net profit (ΔNR) in T2 could be partly attributed to the numerically higher gain and good appearance of sheep which reflected on estimated selling price. Even though total variable cost decreased with increasing levels of vetch hay substitution for concentrate mix, most of body weight and carcass parameters as well as feed conversion efficiency decreased as the levels of vetch hay substitution for concentrate mix increased. Therefore, T2 is economically more feasible and more profitable because although total variable cost of T2 is highest total and net return is also highest for T2.

Table 10. Partial budget analysis of the Arsi-Bale sheep.

List of items/sheep ETB	T1	T2	T3	T4	T5	T6
Sheep Purchase	800.0	810.0	850.0	820.0	840.0	835.0
Feed purchase		364.1	273.1	182.1	91.0	0.0
Land rent for feed production	35.6	33.0	42.4	51.7	61.5	69.0
Feed production cost	53.4	49.4	66.6	83.8	101.5	115.9
Total variable cost	889.0	1256.5	1232.1	1137.5	1094.0	1019.9
Gross return	2090.9	4100.5	3373.6	3560.8	3319.6	2729.9
Total return	1290.9	3290.5	2523.6	2740.8	2479.6	1894.9
Net return	401.9	2034.0	1291.5	1603.3	1385.6	875.0
ΔNR	-	1632.0	889.6	1201.3	983.7	473.0
ΔTVC	-	367.6	343.1	248.6	205.0	131.0

ETB= Ethiopian birr; ΔTVC=change in total variable cost; ΔNR= change in net return; T1 = Fodder oat hay ad libitum; T2 = Fodder oat hay ad libitum + 350 g DM/day Concentrate mix; T3 = Fodder oat hay ad libitum + 262.5 g DM/day Concentrate mix + 87.5 g DM/day Vetch hay; T4 = Fodder oat hay ad libitum + 175 g DM/day Concentrate mix + 175 g DM/day Vetch hay; T5 = Fodder oat hay ad libitum + 87.5 g DM/day Concentrate mix + 262.5 g DM/day Vetch hay; T6= Fodder oat hay ad libitum + 350 g DM/day Vetch hay.

Conclusions and Recommendations

From the results of the current study it can be concluded that substitution of concentrate mix as a whole (Energy + Protein source) at any level is not economical due to high cost of vetch production. However, biologically vetch hay can substitute 75% of the concentrate mix without significantly affecting body weight and carcass parameters of sheep. Furthermore investigation of the effect substitution of only protein source with vetch hay is recommended.

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Prevalence of Trypanosoma Spp. on Cattle and Associated Risk Factors in Selected Districts of Borana Zone

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ABSTRACT

A cross-sectional study was carried out to determine the prevalence of bovine trypanosomosis and its associated risk factors, in purposively selected areas of Telttle and Elweya districts of Borana zone from August 2021 to April 2022. For this study a total of 768 blood samples were collected from cattle using systematic random sampling method considering different age, body condition and coat color; as well as both sex groups of cattle. The packed cell volume (PCV) of each sampled animal was measured using hematocrit reader after centrifugation at 12,000 rpm for five minutes. The overall prevalence of bovine trypanosomosis was found to be 5.08% (39/703). The most prevalent trypanosome species were Trypanosoma congolense (74.35%) followed by Trypanosoma vivax (15.38%) and Trypanosoma brucei (10.25%). The mean PCV recorded as 16.51 ± 6.05 in parasitaemic and 19.56 ± 6.22 aparasitaemic with results showed significant statistical difference (P<0.05) between the two groups of animals. From the risk factors; district, PCV, age groups and coat color of animals were significantly (P<0.05) associated with prevalence of bovine trypanosomosis. However, body condition and sex were insignificant (P>0.05). Therefore, the present study indicated that trypanosomiasis is important disease threat animals in the study area, thus it requires especial attention

Key words: *Prevalence, Trypanosomosis, Risk factors, Bovine, Borana*

INTRODUCTION

In Ethiopia, trypanosomosis is one of the major impediments to livestock development, and agricultural production contributes negatively to the overall development of agriculture, in general and food self-reliance efforts of the nation in particular (NTTICC, 2004). While tsetse-borne trypanosomosis excludes some 180 000 to 200 000 km of agriculturally suitable landing to the west and southwest of the country, 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines, and 1.8 million camels are at risk of contracting trypanosomosis at any 1 time (Dumesa and Demessie2015).

Trypanosomosis has been recognized as a massive constraint to animal husbandry, livestock production and mixed farming in vast areas of rural sub-Saharan Africa (Oluwafemi, 2014). Ethiopia is known for its large and diverse livestock resource. Livestock is primarily kept on smallholdings where they provide drought power for crop production, manure for soil fertility and fuels, serve as sources of family diet and cash income. Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constrains facing the livestock sub-sector (Bezabih and Michael2015). Since more than 90% of crop production in Ethiopia are dependent on animal, draught power mainly on ploughing oxen, many large fields lie fallow due to lack of these animals in trypanosomiasis-infested area (Haile *et al*., 2016).

Bovine trypanosomosis is one of the most economically important diseases that is caused by flagellated protozoal parasite belonging to the genus *Trypanosoma* which affects all domestic animals (Jember and Mitiku, 2013; Sharma *et al*., 2012; Sood *et al*., 2011; Singla and Sharma, 2009). The monitoring of impact assessment for trypanosimosis is a prime concern for the effectiveness of any control program, which has to meet at least three criteria (Slingenbersh, 1999). First, they should be economically sound and sustainable; secondly their direct and indirect effect on the environment should be minimal; and the third and most important is that they should fit into the rural development policy of a country. The impact of tsetse control can be best assessed by comparing changes before and after the implementation of a control intervention or alternatively by cross-sectional comparison of similar agricultural area under different level of trypanosomosis challenge or both (Van den Bossche and Rowlands, 2001).

According to annual report of Ethiopian Veterinary Association in 2008, most budgets allocated to rural development bureau is to buy drugs and spray to treat and control animal trypanosomosis. In Southern region of Ethiopia, the problem of tsetse is common where 75 districts are fully or partially infected. The infected area is about 48,000 km square. In the study area there was no information regarding the prevalence of the parasite and associated vectors. Based on this gap, the study was conducted with the objectives of determining infection rate of *Trypanosoma* spp. in cattle and determining predisposing factors that contribute to the occurrence of the disease in Borana zone.

MATERIALS AND METHODS

Study area

This study was conducted in Elweya and Teltele districts the Borana zone. Borana rangeland is characterized by a semiarid to arid climate (Kamara *et al*., 2005.). Geographically, the area is located between from 4 to 6° N latitude and from 36 to 42° E longitude with altitude ranging from 1000 to 1700 m above sea level (figure:1). The mean annual rainfall of the area ranges from 250 to 700 mm. The annual mean temperature varies from 19 to over 25 °C. Extensive pastoralism is the main means of livelihoods for the Borana people (Gelagay *et al*., 2007).

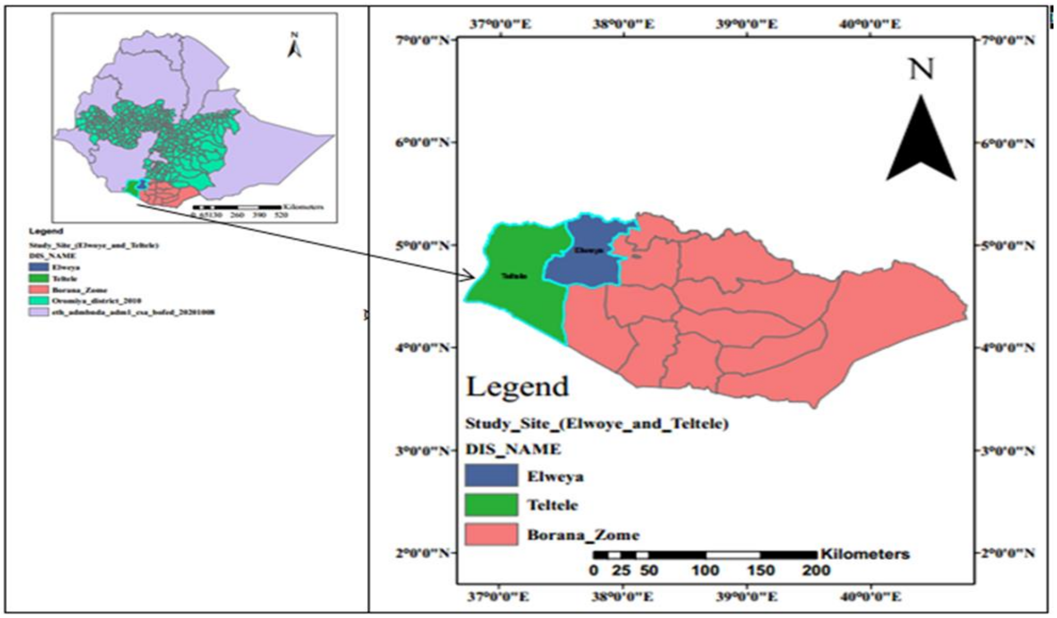

Fig1: Map of Borana zone showing the study areas

Study animals and study type

Indigenous borana breed of cattle with different age groups, body conditions and sex groups were included in the study. The study population was categorized into different age groups based on dentition technique (Johnson, 2003) as follows: young age class (than 2 years), medium age class (between 2 up to 4 years), and adult age class (greater than 4 years). The body condition score of cattle was determined according to Nicholson and Butterworth's method and classified as poor, medium and good.

Study design

A cross-sectional study design was employed to determine the current prevalence of bovine trypanosomosis and to estimate the potential risk factors associated with the epidemiology of the disease.

Determination of sample size

The sample size of required samples for estimation of the prevalence of the disease was calculated according to Thrusfield (2005). For this purpose, sample size calculation was based on 95% confidence level with 50% expected prevalence of the disease and desired precision of 5%.

$$N = \frac{1.96^2 P_{\exp}\left(1 - P_{\exp}\right)}{d^2}$$

Where N = required sample size, d = desired absolute precision, P = expected prevalence (50%). Based on the above calculation the minimum sample size was 384 cattle.

To adjust for intra-class correlation at herd, village, and district levels, a design effect of 2 was considered, and by using an epiinfo 7.2 the sample size was doubled and determined to be 768 (calculated with EpiInfo 7.2).

Laboratory analysis protocol of the samples

The prevalence of *Trypanosoma* infection was determined by buffy coat technique. Blood sample was collected by piercing the marginal ear vein with a sterile lancet and drawing the blood by a hematocrit capillary tube. Then one end (the heparinized end) of capillary tubes was sealed with crystal sealant, transported to laboratory and centrifuged at 12,000 rpm for five minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces. Then the Packed Cell Volume (PCV) was determined by PCV reader and recorded. The PCV values ≥25 and <25 was considered as non-anaemic and anaemic, respectively. The buffy coat was expelled onto microscopic slide and covered with a cover slip and then examined under 40X magnification power of microscope to identify and detect the presence of the parasites. All parasitological diagnostic tests and procedures were conducted as considered according to OIE (2017). Those positive samples were further processed using geimsa staining for identification into species based identification of the parasites under oil immersion using 100 x objectives.

Data management and statistical analysis

The collected data fed into Microsoft Excel spread sheet, sorted, coded and saved until analysis. After pretest, the data was transferred into Stata Version 13 for statistical analysis. Descriptive statistics, univariate logistic regression, and multivariate logistic regression were used during analysis of the data. A p-value of less than or equal to 0.05 was considered statistically significant for difference s between variables of consideration.

Results

Prevalence of Trypanosoma and Identified species

Out of the total768 sampled cattle, 5.08% (39/768) of them were infected with various type tyrpanosoma species. With consequent identification at species level by giemsa stain of blood samples, it was confirmed that *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* were the common pathogens in the study area. Amongst these species of trypanosoma, *Trypanosoma congolense* was the most prevalent (74.35%) followed by *Trypanosoma vivax* (15.38%) and *Trypanosoma brucei* (10.25%) (Figure: 2).

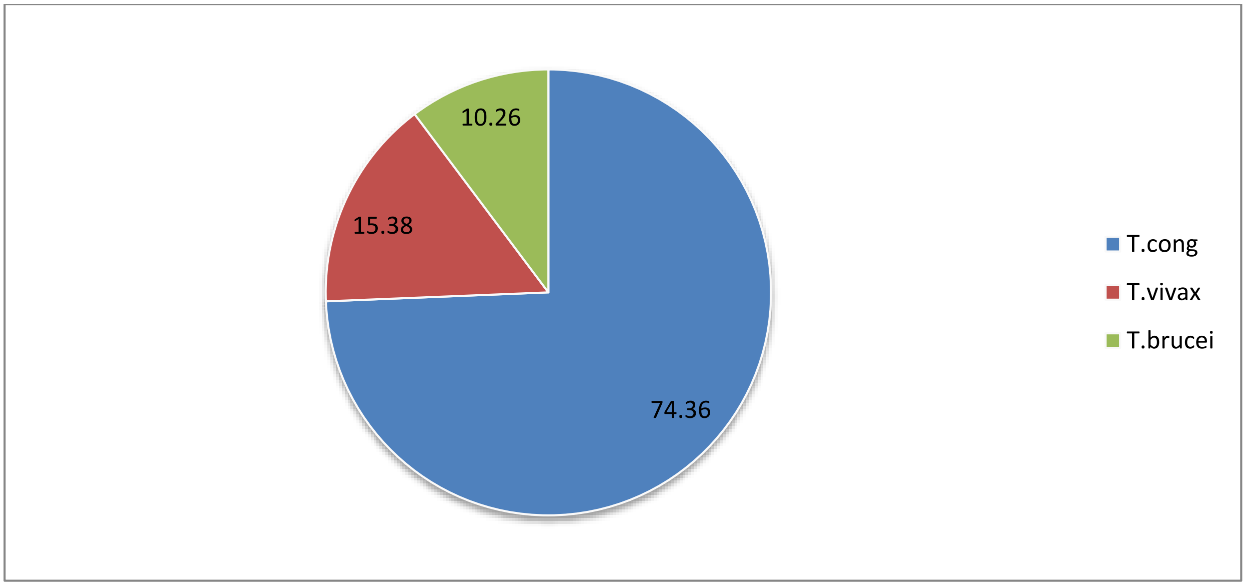

Figure2. The overall prevalence of trypanosoma spp. in the study areas

Risk factors Associated with trypanosoma prevalence

The prevalence of trypanosomosis was not similar between the study districts. (Table1) Accordingly, higher prevalence of bovine trypanosomosis ($P < 0.05$) was observed in Elawoye district (8.70%) than Taltalle district (2.52%). Similarly, a significant difference ($P < 0.05$) was observed between Anemic and non-Anemic animals and higher prevalence of trypanosome infection observed in anemic animals than their non-anemic counterparts. Moreover, animal age was found as one source of variation in the prevalence of the trypanosoma and older animals (age >6 years) were more susceptible than younger age groups ($P < 0.05$). Furthermore, body color of the study animals was significantly associated ($P < 0.05$) with trypanosome infection. Accordingly, trypanosome infection was higher in black-coated animals than red, brown, white and mixed-coated animals.

Table 1. Association of trypanosomosis prevalence with potential risk factors

Category	No. Examined	+ve	Prevalence (%)	χ^2	P-Value
Sex					
Male	559	33	5.90	1.31	0.251
Female	170	6	3.52		
Age Groups					
0 – 2 years	109	2	1.83	6.07	0.048
3 – 5 years	255	10	3.92		
>6 years	365	27	7.39		
Body condition score					
Poor	458	22	4.80	0.66	0.717
Medium	204	13	6.37		
Good	67	4	5.97		
Districts					
Elweya	333	29	8.70	12.22	0.000

Teltele	396	10	2.52		
PCV					
Anemic	523	35	5.55	6.03	0.014
Non anemic	206	4	1.94		
Coat Color of animals					
Black	43	14	32.55	50.18	0.000
Brown	220	6	2.72		
Mixed	93	6	6.45		
Red	174	6	3.45		
White	199	7	3.52		

Based on multivariate logistic regression analysis, district, hair coat color, age and anemic animal were significantly ($P < 0.05$) associated with trypanosomosis infection (Table 2).

Table 2. Multivariate logistic regression analysis of risk factors for trypanosomosis

Factors	Labels	Odds Ratio	Std. Err.	Z	P>|z|	[95% Conf. Interval]
District	Ref					
	Elawoye	0.1749	0.09	-3.38	0.001	0.0636 - 0.4811
Age group	Ref					
	3 – 5 years	4.0197	2.17	2.27	0.030	1.1775 - 23.5171
	>6 years	5.0198	1.15	2.30	0.003	6.4595 - 2.5176
Coat color	Ref					
	Black	0.0744	0.04	-4.09	0.000	0.0214 - 0.2584
	Mixed	0.4425	0.26	-1.37	0.050	0.1383 - 1.4160
	Red	0.0734	0.05	-3.73	0.000	0.0186 - 0.2899
	White	0.1184	0.06	-3.75	0.000	0.0388 - 0.3609
PCV	Ref					
	Anemic	0.0923	0.09	-2.28	0.022	0.0025 - 0.1103
Constant		0.1352	0.14	-1.85	0.064	0.0163 - 1.1219

Effect of trypanosomosis on packed cell volume (PCV)

The analysis of PCV results in the cattle revealed that the packed cell volume (PCV) of parasitaemic animals was 16.51 ± 6.05 which was lower than that of non-parasitaemic animals (19.56 ± 6.22) (Table 3). The mean PCV showed significant variation ($P < 0.05$) between the infected and non-infected animals.

Table 3. Analysis of the association of trypanosome infections with mean PCV (%) of cattle

Status	No. examined	Mean pcv ± sd	95% CI	T- value	P- value
Parasitaemic	39	16.51 ± 6.05	14.5488 - 18.4712	3.06	0.003
Non- Parasitaemic	729	19.56 ± 6.22	19.1077 - 20.0123		

Discussion

The overall prevalence of trypanosomosis in the cattle (5.08 %) is in line with previous study reports (Zelalem *et al.,* 2017; Hundessa *et al.*,2021; Alemayehu *et al.*,2012; Bekele *et al*.,2011; Biyazen *et al.*, 2014) in Gimbig district (4.9%), Sodo Zuriya District (5%), Kaffa zone (6.9%), Didessa district (5.47%), and Dale-Wembera district (2.86%), respectively. On the other hand, higher prevalence was reported from Benatsemay District (11.46%), Dugda Dawa district (13.8%), and Benishangul Gumuz region (13.3%) by Chanie *et al.*(2012) and Leta *et al.*(2016), respectively. Similarly, a higher prevalence of 17.2% in Metekel area by Afewerk *et al.*(2000) 23.0% in Deramallo in Gamo zone by Ayele *et al.(*2012) and 11.05 % in Gnangatom district in South Omo zone Tegegn *et al.,(*2021), were reported. Comparing to other tsetse-infested areas, the current study revealed a relatively lower prevalence of trypanosomosis. The dissimilarity observed might be due to several of reasons such as current investigation performed during dry season that confirmed to influence vector population leading to lower prevalence of trypanosomes infection. Moreover, the increase of veterinary services to pastoral areas, the accustomed migratory livestock husbandry practices of the area, frequent use of trypanocidal drugs and the attention given by government fly control program in the area contributed to the observed lower trypanosomes prevalence.

As per this study, district, PCV, age groups and Coat color of animals were significantly associated with prevalence of bovine trypanosomosis. The significant difference in trypanosomosis prevalence among the study distract might be associated with the difference in location of each study site and their proximity to suitable Glossina habitats in the district. Elawoye distract is located close to segen river which is the origin of the Glossina infestation in between konso and Borana zone. In line with the current study, Van den Bossche P. (2001) reported that prevalence of bovine trypanosomiasis and its impact on livestock productivity varies by location and heavily influenced by the level of interaction between vector (tsetse fly), domestic host animals, and game animals.

Variations may exist because of differences in vector infection rate, animal susceptibility and practice of trypanocidal drug use and fly control operations that may obviously influence on epidemiological situations of the disease (Reifenberg *et al*., 1997; Majekodunmi *et al*., 2013; Geiger *et al*., 2015).

PCV is one of the most fundamental quantitative measurements to estimate the anemic status of trypanosma infected animals. As per PCV result, animals with PCV less than 24% were considered anemic (Marcotty *et al*., 2008; OIE 2008; Van den Bossche and Rowlands, 2001). Anemia was considered an important clinical sign of trypanosomosis and reduces performance of infected animals (Radoostitis *et al*., 2007, Trail *et al*., 1993).

A significant difference was observed in mean PCV value between anemic and non-anemic animals (P <0 .05) and higher prevalence of trypanosoma infection was observed in anemic animals than in non-anemic animals. Simila reports were made in North Omo Zone by Muturi (1999) (16.7% in aneamic and 28.0% in non-anaemic); Alekaw (2004) found 21.60% in anaemic and 25.40% in anaemic in districts bordering Lake Tana in northwest Ethiopia; and Feyisa (2004) 21.65% in parasite infected and 25.54% in non-parasite infected animals in southwest Ethiopia. The significant decrease in mean PCV value of infected than non infected cattle might be due to the destructive nature of trypanosomes on erythrocytes in infected animals.

The prevalence of bovine trypanosomosis is higher and more prevalent in older animals (age >6 years) than in young age group (P <0.05). This result is consistent with previous findings those reported lower disease prevalence in young age groups than in older animals (Fesseha *et al*., 2022: Alemayehu *et al*., 2012; Gona *et al*., 2016; Tegegn *et al*., 2021). However, the current result disagrees with the report by others (Nigatu, 2004, Ayele et al., 2012; Tilahun, 2012) whose result indicated insignificant difference in infection rate of trypanosomosis among different age groups. This might be due to animal husbandry system (keeping calves at homestead in some areas while others release them to field with their dams), mobilization of pastoralists in case of borana area, persistency of the parasite in the area and others.

Body color of the study animals were significantly associated (P < 0.05) with trypanosome infection. Accordingly, trypanosome infection was higher in black-coated animals than in red, brown, white and mixed-coated animals. This might be associated with a color preference of Glossina vector to feed on black animals than other color types since the tsetse fly was more attracted by black color due to their shade-loving behavior during their flight from one area to another. Similar finding was reported by others (Ataro *et al*., 2015; Fuentes, 2017).

Conclusion and Recommendation

Trypanosomosis is a very important disease that causes economic loss in the livestock industry. Bovine trypanosomosis, which accounts for an overall prevalence of 5.08% is the major livestock constraint in the study area and affects their health, production and productivity. There was (P<0.05) a statistical significant difference in prevalence of bovine trypanosomosis in district, PCV, age groups and Coat color of animals.

Based on the aforementioned the following is recommended:

- Designing and implementation of control strategies of trypanosomosis focusing integrated approach (vector control and chemotherapy) should be continuing in the studied areas.
- The pastoralists in the area should be trained on how to control the vectors of the parasites and the disease properly.
- Expanding an appropriate tsetse control methods (Spot-on and insecticide impregnated targets) to reach tsetse infested area in a sustainable manner.

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Poultry Research Results

On-farm egg production performance evaluation of Koekoek chickens using locally formulated supplementary feed

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Abstract

*Three hundred (300) koekoek chickens pullet were distributed to Dodicha and Anano shisho kebele farmers with supplementary (commercial and locally formulated) feed to evaluate the production performance and survival of chicken supplemented under farmers condition. Based up on purposive sampling; twenty (20) farmers were selected because of their accessibility, interest to construct poultry house, their willingness to participate in research. For each farmer fifteen chickens with (12Female: 3Male ratio) of three months pullet and supplementary feed were distributed for evaluation. Commercial ration with 16 %CP and 2800 kilo cal ME/kg and locally formulated ration with 15.3%CP and 2700 kilo cal ME/kg were used for chicken feeding during evaluation periods. The experiment was done for four months. Both hen day production (HDP) % and hen housed production (HHP) % were not statistically different (P>0.05) between kebeles. There was a significant (P<0.05) different of HDP% and HHP% between treatment diets. Qualities of eggs were not affected (P>0.05) by kebels/ site. Egg weight, egg length, egg width and albumin weight of birds subjected to locally formulated diet is significantly (P<0.05) lower than the egg weight, egg length, egg width and albumin weight of birds subjected to commercial feed. Yolk color not affected (P>0.05) by both site and supplementary feed used. Higher growth return obtained from using commercial feed but its cost and transportation cost make higher variable cost for commercial feed used compared to the locally formulated feed. Within four months experimental period farmers obtained **101.67** and **114.84** net income in Ethiopian Birr from selling of koekoek chickens` eggs obtained from one bird by using commercial and locally formulated feed respectively.*

Key words: *Hen day production, Hen housed production, commercial feed, formulated feed, net income*

Introduction

Poultry production provides a major income-generation activity from sale of chickens and eggs. Poultry production also helps to meet the growing demand of animal source proteins. Production of both egg and chicken meat has certainly assisted in reducing the gap in the supplies of animal protein for human consumption (Taddele and Ogle, 2001; Dhuguma, 2009; Leta and Bekana, 2010). Fewer cultural and religious taboos associated with poultry products (Tadelle *et al.*,2003) and its contribution to balanced human diet have increased demand for poultry meat and eggs. In Ethiopia, indigenous poultry breeds produced eggs that used for income generation and consumption, but their eggs production potential are invariably small as compared with those of exotic breeds. The annual egg production potential of Ethiopian hen ranges from 30-60 (MOA.1997; Alemu, 2001) with a single egg weighing between 39 and 46g.

Poultry production needs little investment compared to other livestock production, hence land less laborers and people organized in micro enterprises are able to raise chicken with low inputs. In Ethiopia poultry sector is characterized by low production and productivity; the growth rate is much lower than that of fast growing populations (ILRI, 2004). With this potential and production system native chickens cannot meet the high demand of Ethiopian populations. Because of low performance of indigenous chickens breed koekoek chicken breed has been introduced and evaluated under Adami Tulu research center for 3-4 years.

Koekoek chicken is a composite of the White Leghorn, Black Australorp and Bared Plymouth Rock and bred for the production of brown eggs and for the attractive deep yellow colored carcass (Grobbelaar *et al.,* 2010). The breed matured at five months age, lays good numbers of brown eggs with excellent resistance to disease. Some time it can perform under back yard production system and it is good scavenger. The breed was introduced by Ethiopian Institute of Agricultural research because of its good production traits (fast growth rate and good egg layers).Under Adami Tulu Research center Koekoek chickens attained sexual maturity at five months and produced more than two hundred eggs/year (Tesfa *et al.,* 2018) using both the commercial and locally formulated diet.

Since it is a tropical dual purpose breed, good scavenger and pure bred (male & female) existed, it can be considered as suitable candidate breed for overcoming the problem of environmental stress. But under farmers condition using supplementary feed detail evaluation was not done prior this work in semi-arid area.

Therefore the activity was done with following objectives:

- To evaluate egg production performance and survival of koekoek chicken supplemented under farmers condition

- To evaluate egg quality characteristic of koekoek chickens reared under semi-scavenging conditions.

2. Material and Methods

2.1. Description of the study area

The experiment was conducted in Adami Tulu jido kombolcha district. The district is located in East shewa zone in the heart of central rift- valley of Ethiopia. The altitude of the district varies from 1500 to 2300 m a.s.l. It is located at 150 km from the capital city of Ethiopia, Addis Ababa. Minimum and maximum annual mean temperatures are 14 and 27 ^{0}C respectively. The woreda is characterized by bimodal pattern of rainfall; with short rainy season running from February to April and long rainy season from June to September. However, the pattern of rainfall is usually erratic with fluctuations in the start and end of the season. From the district Dodicha and Aannano shesho kepele were selected because of their accessibility and the experiment was conducted there.

2.2 .Housing and management of chickens before distribution

Experimental chickens were hatched and managed for three months under Adami Tulu Agricultural research center until the final vaccination given for them. Under Adami Tulu Agricultural research center the experimental birds housed in cleaned and disinfected and littered with properly dried tef *(Eragrostis tef*) straw house. Chickens were vaccinated against the most common chickens' diseases of the area namely Newcastle and Bursa (Gumboro), and fowl pox until three months. The chickens fed pullet ration formulated from the locally available feed (noug cake, maize, wheat bran, limestone and salt) under Adami Tulu research center. Clean water was provided each day morning and afternoon using ups down round plastic waters.

Koekoek chickens rearing under ATAR (before distributing)

2.3 .Poultry feed preparation

Recommended poultry ration was formulated from locally available feed resources. Feed resources like maize purchased from local market and grinded to be used under Adami Tulu Research center. Noug cake, wheat bran, limestone and salt purchased from local market and added to the ration. Finally layers ration with **15.3% CP and 2700 kilo cal ME/kg** was formulated to be used. Commercial ration with 16.5% CP and 2800 kilo cal/kg was purchased from Alema koudijs feed PLC to be used.

On-farm Koekoek chicken rearing

Table 1. The composition (g/100 g fresh basis) of the ingredients used in formulating ration

No	Feed ingredients	gram in ration
1	Maize	51.00
2	Noug cake	24.00
3	Wheat bran	25.00
4	Salt	1.00
5	Limestone	1.00

2.4. Site and farmers selection

The experiment was conducted in Dodicha and Aneno shisho kebeles. Training on exotic chicken management like feeding (ration formulation), watering, healthy care, hygiene and house construction was given for farmers, subject matter specialist (SMS) and Development agents (DAS) at farmer training center for two days. After training participant farmers were selected in collaboration with subject matter specialist (SMS), Development agents (DAs) and with Kebele leaders. Purposive sampling was used for farmer selection depending up on their accessibility, interest to construct poultry house, their willingness to participate in research. From the trained farmers ten (10) farmers were selected from each kebele purposively to participate in trial. Totally twenty (20) farmers were selected from two kebeles.

Selected farmers constructed separated poultry house from locally available materials according to the training given them. All the construction material and the bedding material used in poultry house supplied by farmers. All other feeders and watering equipments were also supplied by farmers themselves. Experimental chickens were multiplied by ATARC poultry team and managed for three months. Three hundred (300) Koekoek chickens with a uniform age of three (3) months were distributed for farmers. For each twenty farmers fifteen (15) koekoek chickens of three (3) months age were given with the ratio of (12F:3M).

Formulated feed from locally available and commercial layers feed used for chicken feeding during on-farm production performance evaluation of koekoek chickens supplied by ATARC poultry team during the four (4) months experimental period. Selected farmers from each kebele divided in to two groups. One group received formulated feed and the other group received the commercial feed. Ninety (90) gram /head /day were given for each koekoek chicken during the four months experimental period from each respective feed. From the daily given half of its given morning before it left for scavenging and half given after noon when it came back from scavenging.

2.5 Data collection

Production performance data

Egg laid collected ever day and stored until the numerator count and record on data recording book. After that the farmer used it either for selling or for feeding. Mortality and disease observed also recorded by numerator and researchers.

Egg quality parameters were determined from 120 eggs (60 eggs from each kebele) and six (6) eggs collected from each farmer. The collected eggs were fresh, clean eggs laid by the distributed chicken. Eggs were weighed using an electronic digital balance. The yolk weight was taken after gently separated the yolk from the albumen and the differences between [egg weight- (shell weight + yolk weight)] were considered as albumen weight. Egg length, egg width and eggshell thickness were measured using electronic digital caliper and Yolk color was determined by adjusting the score of yolk color on color fan from Roche (Vuilleumier, 1969).

The price of feeds was recorded based up on the market information of the price of each feed ingredient.

$$\textbf{\% Hen-day egg production (HDP)} = \frac{\textbf{Total eggs produced} \times 100}{\textbf{Number of days in laying} \times \textbf{number of birds alive}}$$

$$\textbf{\% Hen house production (HHP)} = \frac{\textbf{Total egg produced} \times 100}{\textbf{Number of birds initially housed} \times \textbf{number of days in lay}}$$

2.6 Data analysis

Analysis of variance of HDP%, HHP% and egg quality characteristic were analyzed according to the general linear model (GLM) procedure of the Statistical Analysis System (SAS, version 9). When the results were significant, mean com-parisons were made using Fisher`s LSD multiple range test procedure of the SAS package.

Models: $\mathbf{Yijk = \mu + Si + Fi + eijk}$

Where: Yijk = individual value of the dependent variables of chicken

μ = Overall mean;

Si = the effect of site (I = 1 to 2)

Fi-= the effect of feed (i=commercial, formulated)

eijk= random error

2.7. Partial budget analysis

Partial budget analysis was performed by considering variable costs (price of feed used), price of feed transportation and veterinary cost) and total revenue calculated from the price of egg collected from each bird during experimental period. Net income/net return obtained from the experiment was calculated as the difference of total revenue (total returns) and total variable costs according to the formula developed by CIMMT (1988); Ehui and Rey (1992).

$$NI = TR - TVC$$

Where, NI = net income, TR = Total return, TVC = Total variable cost

3. RESULTS AND DISCUSSION

3.1. Nutritional composition of the feed used

The nutritional composition of supplementary feed used was analyzed in Adami Tulu Agricultural research center in Animal nutrition laboratory and the nutritional composition of the commercial feed was taken from the company and shown in table below.

Table 2. Nutritional composition of supplementary feed used for on-farm koekoek chicken feeding

Feed type	**nutritional composition**				
	DM (%)	**CP (%)**	**CF%**	**Energy (Kcal/kg)**	**Price(EB)**
Commercial	90.00	16.5	7.00	2800	2715
Formulated	92.10	15.2	13.5	2700	2131

DM= dry matter, CP= crude protein, CF=Crude fiber, Kcal=Kilo calorie, kg=Kilogram, EB= Ethiopian Birr

Both HDP% and HHP% were not significantly ($P>0.05$) different between sites/ kebeles. This is because both villages existed in similar agro-ecology and resulted for similar scavenging feed resource and worms that chicken collected during scavenging. Management of the chickens (housing system), watering equipments and scavenging period were similar in both kebele that may also caused similar results. The current finding is lower than the previous results done at on-station using the same commercial and formulated feed because of the different management system (amount daily provided feed) and housing system. Poultry house constructed by farmer not well designed and covered to protect cool temperature, the ground also not properly covered with bedding materials like the type of poultry house used at on-station. In such case the feed intake used to maintain body temperature rather than converted to products (egg production). Also chicken expend more energy for scavenging rather than used for egg production.

The current on-farm egg production performance (%HDP and %HHP) of koekoek chicken is similar with the 187 eggs /year reported by Desalew Tadesse *et al.,* (2013) from on-farm study at Ada`a and Lume district. This is because of similar supplementary feed (maize and wheat) used, and frequencies of supplementation (three times per day) at Ada`a and Lume districts that make similar with the current on-farm finding. The current finding is higher than their egg production performance (164.8eggs/year) as reported by Tesfa (2020) at Dugda district. Since both works were done in similar agro-ecology the main reseasons were that the current work done using good quality supplementary feed and no external parasite observed on them compared to the work done at Dugda district.

Table 3. Least square mean of on-farm egg production of koekoek chicken

Main effect		**HDP%**	**HHP%**
		Mean	Mean
Site:	Annanoo	54.52	51.97
	Dodicha	47.71	46.1
Feed :	Commercial	55.57[a]	54.63[a]
	locally formulated	46.66[b]	43.45[b]
site X Feed:			
	Aannannoo *commercial	57.42	56.13
	Aannanoo * locaa	51.62	47.82
	Dodichaa* commercial	53.72	53.13
	Dodichaa *local	41.71	39.08
Site:	P	0.0898	0.1218
	LSD(P=0.05)	NS	NS
Feed type:	P	0.0275	0.0039
	LSD(P= 0.05)	7.89	7.46
Site X Feed type :	P	0.4357	0.4467
	LSD(P= 0.05)	NS	NS
	CV (%)	34	34

HDP= Hen day production, HHP= Hen housed production

The nutritional composition of the formulated feed is lower than the nutritional composition of commercial feed (table 1) and caused significantly(P<0.05) lower egg production performance of birds subjected to it as compared to birds subjected to commercial feed. Mono-gastric animals lack rumen micro-organizes that digested fiber but locally formulated feed contained higher fiber and that fiber combined with the fiber of the scavenged feed resources and caused less digestion and lower production performance of bird compared to the commercial feed used.

Qualities of eggs were not affected (P>.05) by site because both site located in similar agro-ecology and had similar scavenging feed resources. Egg weight, egg length, egg width and albumin weight of birds subjected to locally formulated diet is significantly (P<0.05) lower than the egg weight, egg length, egg width and albumin weight of birds subjected to commercial feed. This is because of higher protein content of the commercial feed used compared to the locally formulated feed. Egg weight of chicken subjected to the commercial and locally formulated feed were lower than the weight of chickens` eggs reported in previous work reported by Tesfa & Usman(2017) and Tesfa and Usman (2018) from on-station work. This is because the protein and energy content of the feed not fully used for production as the on-station work but also used for maintaining body temperature and used as energy source for walking there and here during scavenging and scratching the ground.

Egg weight, egg length, yolk color and albumin weight of the current work is similar with the Egg weight, egg length, yolk color and albumin weight of koekoek chicken reported in previous work done in Dugan district by Tesfa(2020). The current finding of egg weight is similar with the report of Desalew Tadesse *et al.,* (2013) because of similar supplementary feed used but the yolk color of the current work is lower than the report of Desalew Tadesse *et al.*, (2013) this is most probably due to different green feed used during scavenging because yolk color highly affected by green feed used.

Table 4. Least square mean of On-farm egg quality characteristic of koekoek chicken

Main effect		**Egg weight (g)**	**Egg length (mm)**	**Egg width (mm)**	**Shell thickness (mm)**	**Shell wt (g)**	**yolk color**	**Albumin Wt(g)**	**yolk Wt (g)**
Site:	Annannoo	46.50	52.71	39.88	0.60	5.15	4.42	25.83	15.5
	Dodicha	45.75	52.31	39.94	0.64	5.15	3.53	25.88	14.71
Feed type:	Commercial	48.82[a]	53.43[a]	40.58[a]	0.62	5.29	3.94	28.1[a]	15.44
	Local	43.01[b]	51.41[b]	39.17[b]	0.62	5.00	3.96	23.35[b]	14.66
site X feed type:									
Anannoo x commercial		49.12	53.84	40.36	0.62	5.29	4.50	27.65	16.18
Annano x local		43.08	51.23	39.26	0.57	5.00	4.30	23.46	14.62
Dodicha xcommercial		48.53	53.03	40.80	0.61	5.29	3.35	28.53	14.71
Dodicha x Local		42.94	51.59	39.26	0.66	5.00	3.71	23.24	14.71
Site : P		0.7232	0.6902	0.6866	0.4978	1.00	0.0154	0.7241	0.0846
LSD (P=0.05)		NS	NS	NS	NS	NS	NS	NS	NS
Feed type	P	<0.001	0.009	<0.0001	0.9116	0.1920	0.8546	<0.001	0.0518
	LSD(P=0.05)	2.0248	1.1491	0.6553	0.1018	0.4437	0.7098	1.8415	0.7832
site X feed type	P	0.8247	0.3137	0.3537	0.3305	1.00	0.4235	0.0513	0.0518
		NS	NS	NS	NS	NS	NS	NS	NS
LSD(P=0.05)									
CV (%)		8.76	4.36	3.28	32.74	17.17	35.84	14.21	10.37

g= gram, mm = millimeter, Wt= weight , P=probability, LSD = Least significant difference, CV = coefficient of variation

Investing on small scale semi-scavenging koekoek chicken production had a good economic return. Poultry litters that was used as organic fertilizer and for animal feeding was not included in total revenue calculation because poultry litters selling is not common among the farmers. Fixed costs such as feeders waters and poultry house were constructed by farmers and its cost were not included in calculation. The major cost that determined the profitability of on-farm poultry production is still feed cost as compared to the non-feed cost (Table 5). Higher growth return obtained from using commercial feed but its cost and transportation cost make higher variable cost for commercial feed used compared to the locally formulated feed. Within four months experimental period farmers obtained **101.67** and **114.84** net Ethiopian Birr from selling of koekoek chickens` eggs by using commercial and locally formulated feed respectively.

Table 5. Production cost and return of Koekoek chickens reared using supplementary feed under farmer's condition

Items	cost (EB)
Total cost of commercial feed consumed (EB/Bird)	293.22
Total cost `of formulated feed consumed (EB/Bird)	230.15
cost of medicament (EB/Bird)	16
cost of transportation of commercial feed (EB/Bird)	48.00
cost of transportation of local feed(EB/Bird)	4.00
Total variable cost of commercial feed consumed by chicken (EB/Bird	357.22
Total variable cost of formulated feed consumed by chicken(EB/Bird)	250.15
Gross return from commercial feed using (income from egg sell)(EB/Bird)	458.89
Gross return from formulated feed using (income from egg sell)(EB/Bird)	364.98
Net return from commercial feed using (EB/Bird)	**101.67**
Net return from formulated feed using (EB/Bird)	**114.83**

EB = Ethiopian Birr

4. Farmers feed back

Farmers got enough eggs for family consumption and for family income generation. It requires low input and in short term it generated income for the family. The production of chickens in both villages/kebels enhanced the nutritional security of the family. It created customers for them who purchased egg from the farmers' home and sold it at different market place. Experimental farmers at Dodicha kebele are near to each other and chicken owner especially women organized and involved in money saving ``ikub`` with each other. They used the money they got for purchasing small ruminants and others to purchase other material for their family. According to the farmers feedback the major challenge to poultry production was source of quality feed and disease. The characteristics of the chickens (their docile character), their fertile egg create high demand for the eggs according to farmers feedback. Non-experimental farmers around their vicinity purchased eggs from them and brood it using broody hens which are the indicators for the breed demand.

5. Conclusion and recommendations

Since the system is semi-scavenging system; farmers keep chicken as a side work which is a bottle neck problem to maximize income from it. In semi-scavenging system the number of improved chicken can be kept by farmer depends up on his/her dedication to provide improved management package for poultry production. Lack of improved management caused external parasite, disease and death of chickens. The current work created awareness on the value of improved managements like improved feeding, housing and bedding materials. Farmers can formulate a balanced poultry feed from the grain he produced and from the easily available agro-industrial by-products. But they have to remember to purchase and include premixes in to the formulated feed to maximize the production. Livestock experts should assist the ration formulation.

Those farmers who are not interested in ration formulation and lack skills of ration formulation can purchase the commercial poultry feed and can fetch income from it.

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Apiculture Research Results

Investigating the role of honeybee (*Apis mellifera* L.) pollination on seed yield of soybean (*Glycine max L. Merril*) at Adami Tulu Agricultural Research Center

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Abstract

Pollinator insects are essential in increasing the seed set of many flower and fruit crops. This experiment was carried out to evaluate the effect of honeybee pollination in seed yield and oil contents of Glycine max L. The plots were grouped into three treatments with four replications. The results of the study showed that the yield obtained from plots pollinated by honeybee was superior with the mean yield of 3945 kg/hectare followed by plots left open under natural conditions with the mean yield of 3754 kg/hectare. The lowest mean yield of 2483 kg/hectare was recorded for the plots excluding any pollinator. The results also revealed that honeybee pollination increases Glycine max seed yield by 38.9% over the natural pollination. The pod number in a plot caged with honeybees was 93.75 higher than in the one caged without honeybees. The average weight of 100 seeds was larger in both the plots caged with and without honeybees. The average oil content was 19.21%, 19.39% and 18.47% in un-caged plots, plots caged with honey bees and caged without any pollinator, respectively. The germination test was different (P<0.05) among the seeds in the different treatments. It was concluded that honeybee pollination in soybean increased seed production by 38%. Therefore, moving honey bee colonies to Glycine max farm during the flowering period is one of the most essential inputs to maximize Glycine max seed production.

Key words: *Apis Mellifera*, *Glycine max*, honey bees, pollination, seed yield

Introduction

Flowering plants and honeybees have a special relationship in which both are benefited from each other; where honey bees get nectar (as feed) and in turn facilitate the pollination process. According to (Guidry 1964; cited in McGregor, 1986) reported that half of the world's diet of fats and oils comes from oilseeds: coconuts, cotton, oil palm, olives, peanuts, rape, soybeans, and sunflower. These plants are dependent upon or benefited by insects pollination. Of these insects, honeybees are responsible for more than 80% of the total pollination of major crops and contribute a lot for the increased production. Inadequate pollination can result in reduced, delayed yield and a high percentage of inferior fruit/seed yields and with ample pollination, the plant can set fruit early and the grower may harvest quality and good crop a head of inclement weather (Belitz *et al*., 2009).

Soybean (*Glycine max*) is one of the most valuable crops in the world, due to its multiple uses as a source of livestock and aquaculture feed, human food, industrial purposes and more recently, as a source of bio-energy (Myaka *et al*., 2005). Soybean production in Ethiopia was 38,166.04 ha from which 81241.833 tons produced with productivity of 2.129 tons ha^{-1} and in Oromia region 14,626.78 ha was cultivated with production of 31,832.611 tons and a productivity of 2.176t ha^{-1} in 2015/2016 cropping season (CSA.2016). The current five-year plan, GTP II, has given due consideration for soybean production as industrial crop and its production is expected to increase from 0.72 million quintals in 2015 to 1.2 million quintals by the year 2020 to meet the demand of the market by creating a linkage with the industry and export market (GTP II, 2015). Since it is well adapted to lowland to mid altitude agro-ecologies of the country the entire low to mid altitude maize belt areas of the country are also suitable for soybean production. The realization of all these potentials and targets are expanding soybean production vertically and horizontally without adequate understanding of its production economics. The efficient pollination of honeybees is due to their great numbers, their physical and behavior of foraging on only one plant species at one time. Usually a honeybee can visit between 50-1000 flowers in one trip, which takes between 30 minutes to four hours. Honeybee is also believed to play a significant role in the economy of Ethiopia through pollination services. The previous studies on the value of honey bees as pollinators in Ethiopia indicated that honeybees can increase the yield of Niger (*Guizotia abyssinica*) by 43% (Admassu Addi and Nuru Adgaba, 1999) and *Allium cepa* by 84% (Admasu Addi *et al*., 2006).

According to (Issa *et al*. ,1984), the honeybee is an efficient pollinator for soybean varieties which resulted in an increase in the seed production by 95%. Moreti *et al*. (1998) also reported that soybean plants showed an increase in the number of pods 58.58% and seeds 82.31% when visited by the honeybees. Pollination studies of legume forages showed that in the culture of perennial soybean increase in the pod production 55.8% and seeds 44.7% in the presence of *A. mellifera* (Nogueira and Pereira, 1983; Nogueira-Couto *et al*., 1998). But no study has been conducted to see the effect of honeybees' pollination on soybean seed yield under Ethiopian conditions. This study was conducted with the objectives of investigating the effects of honey bee pollination on soybean seed yield and oil contents and to identify potential pollinators other than honeybees.

Materials and Methods

Description of the study area

The experiment was conducted at Adami Tulu Agricultural Research Center (ATARC) under rain-fed conditions. Adami Tulu Agricultural Research Center (ATARC) is located in the mid Rift Valley of Ethiopia 167 km South of Addis Ababa. It lies at latitude of 7° 9'N and longitude of 38° 7'E. and it has an altitude of 1650 m.a.s.l. It receives a bimodal unevenly distributed average annual rainfall of 760.9 mm per annum (ATARC, 1998). The rainfall extends from February to September with a dry period in May to June, which separates the preceding ''short'' rains from the following ``long'' rains. The long-term mean minimum and maximum temperatures are 12.6 0C and 27 0C, respectively. The soil is fine sandy loam in texture with the soil type of sand, clay and silt in proportion of 34%, 48% and 18% respectively and a pH of 7.88 (ATARC, 1998).

Experimental Management

The experimental land was prepared according to required standards. The plots were leveled manually and the sowing was done in June under rain fed conditions. The seeds were planted by hand at a specified spacing by placing two seeds per hill and thinning was done to one plant at each specific intra row spacing ten days after seedling emergence to achieve the desired plant density in each row. Recommended spacing was used with plot size of 4mx4m ($16m^2$). All the other agronomic practices were followed as per the recommendation for the crop.

Treatments and Experimental design

The experiment was laid out in RCBD with four replications per treatment. At 25% of the soybean blooming, bee colonies were transferred to the cage. Supplementary feed was given for bee colonies. After flowers shed, the bee colonies were taken out of the cages. Every pollinator recording was done between 06:00 and 18:00 hours at hourly interval.

Treatments

In the first treatment, plot was caged with honeybees at 50% flowering, in treatment two; the plot was excluded from any pollinators. The third treatment plot was left open for natural pollination.

Data collected

Seed yield, pod average weight, 100 seeds weight in (g), seed size, number of pods per plant, number of seeds per pod, germination capacity of seeds from different treatments, number of seed set, percentage of matured and aborted grains per different treatments, number of pollinators other than honeybees were collected.

Assessment of flower visit by the insects

The pollinators activity were observed for five consecutive days on the plot left open for any pollinators to assess which and how many insect species were visiting the soybean plants. Soybean plants that have approximately above 40 flowers were selected and number of honeybees was recorded for 15 minutes at 1 hourly interval from 6.00 to 18.00 hours. All insects encountered on flowers were recorded and the cumulated results expressed in the number of visits to determine the relative frequency of pollinators including honeybees.

Germination test

Germination test of the seeds from all the three treatments were also conducted. For this test 100 seeds of soybean were sprinkled on a 10cm diameter petri dish and covered with moist filter paper. Moisture was maintained by spraying water. The filter paper was removed after the germination was over and the number of seeds germinated out of the hundred was counted. The experiment was replicated four times. A germination success was conducted by considering the principle of maximum percentage germination, following the necessary steps used by the International Rules for Seed Testing (ISTA 2009). Finally germination percentage of the seeds was determined by using the following formula:

$$\textbf{Germination percentage} = (\frac{(n)}{N} X 100\%$$

Where: n= Total number of germinated seeds and N=Total number of seeds in the sample (Labouriau and Agudo, 1987)

100 seeds weight and seeds yield (g) per 24m^2

$$\textbf{Yield increment (\%)} = \frac{\text{yield from honey bee pollinated} - \text{yield from insect pollinated (100)}}{\text{Yield from open pollinated}}$$

Statistical analysis

All collected data were subjected to Analysis of Variance (ANOVA) using SAS software (The SAS system for windows 9.2) appropriate to the design of the experiment.

Results and Discussions

In this study the effect of honeybee pollination on seed yield and various parameters of *Glycine max were* compared to open pollination and caged without any pollinator.

Total seed yield (kg/ha)

The findings demonstrated that the yield obtained from plots pollinated by honeybees was superior with the mean yield of 3945 kg/hectare followed by plots left open under a natural condition with the mean yield of 3754kg/hectare. The lowest mean yield of 2483 kg/hectare was recorded from the plots from which insects and honey bees were excluded (Table 1). Honeybee pollination increased the yield of *Glycine max* by 38.9% over the treatment without any pollinators. This agrees with Issa *et al,*. (1984) who found that honey bee can increase *Glycine max* seed production by 9-81%. These results suggested that the pollination made by insects, in particular by A. *Mellifera* was the most important pollination for the increasing the productivity of *Glycine max*. Seed yield differences among the treatments indicated that the crop requires insect pollination particularly honeybees for seed production. The *A. mellifera* honey bees were efficient to accomplish the cross-pollination tasks in the soybean flower and their use in agriculture brings a considerable gain.

Average of seed weight and pod diameter

Seeds from honey bee pollinated *Glycine max* weighed more compared to those from the control (open pollination) treatment. The average seed weight of *Glycine max* in honey bee pollinated plots was 13.75gm as compared to the 12.25gm obtained from the open pollinated plots, revealing a 38.2% increment in seed weight as a result of honeybee pollination. The least (10.0gm) seed weight was recorded for plots caged without any pollinators (Table 1). Bradley (2015) also reported that *Glycine max* seed weights increased when honeybee colonies were included. Pod diameters of 6 and 5 cm were recorded in honeybee pollinated and open pollination plots, respectively. For the plots caged without honeybees, pod diameters of only 3.8cm were recorded (Table 1). Hossanian *et al*., (2018) also stated that lower pod diameter was found in pollination caged without honeybees. Statistical analysis of the pod diameter data showed significant difference between plots caged with honeybees and those caged without honeybees, but no statistically significant difference was observed between pollination treatment caged with bees and the open pollinated treatments.

Table 1. Effects of honeybee pollination on *Glycine max* seed yields 100 seeds weight, and pod diameter.

Treatments	**Seed yield kg/ha**	**weight of 100 seeds (mg)**	**Pod diameter (cm)**
Uncovered plot	3754 ± 13.74^{a}	12.25 ± 1.109^{b}	5 ± 0.354^{b}
Covered plot with honey bees	3945 ± 20.42^{b}	13.75 ± 0.854^{b}	6 ± 0.408^{b}
Covered plot without honey bees	2483 ± 38.39^{c}	10 ± 0.408^{a}	3.875 ± 0.239^{a}
CV (%)	1.62	12.8	13.36

a,b,c=Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Number of seeds and pods and oil content

The pod and seed yields from uncovered plots, plots covered with honeybees and those covered without honeybees were presented in Table 2. The average numbers of pods produced were 84, 93.75 and 68 for open pollination plots, plots caged with honeybees and those caged without any pollinator, respectively. The respective average numbers of seeds produced were 51, 63 and 45. (Table2). Seeds produced per *Glycine max* were found to be significantly different among the treatments.

Analysis of seeds sampled in the three treatments showed that there was no difference ($P>0.05$) in oil content among the treatments. Crude oil extracts were at the normal rate for the soybean grain. The average content of crude oil extract in the seeds were 19.21±0.112%, 19.39±0.052% and 18.47±0.192% for plots open for any pollinator, plots caged with honey bees and those caged without any pollinator, respectively (Table2). Chiari, W. C. et al. (2012) also stated that there was no statistically significant difference among the open plots, the plots caged with honeybee and those caged without any pollinators.

Table 2. Average number of pods, oil content and seeds counted from samples of plants of soybean Glycine max.

Treatments	**Number of pods**	**No. of seeds**	**Oil content**
Uncovered area	84 ± 1.080^{b}	51 ± 6.721^{b}	19.21 ± 0.112^{a}
Covered area with honey bees	93.75 ± 1.315^{a}	63 ± 9.1^{a}	19.39 ± 0.052^{a}
Covered area without honey bees	68 ± 1.080^{c}	45 ± 3.559^{c}	18.47 ± 0.192^{a}
CV (%)	2.95	22.24	1.26

a,b,c=Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Germination potential

There were statistically significant differences ($p<0.05$) among the three treatments in germination percentages. Statistically, the highest germination percentage (27.25%) was recorded for plots caged with honeybees followed by the plot left open (21.7%). The least germination percentage (16.5%) was recorded for plots caged without any pollinators (Table 3). The increase in germination rate for plots caged with honeybees indicates a superior pollinating efficiency of honeybees. Similarly Yücel and Duman (2005) reported that the germination rate was greater on average by 12% in onions with honey bees. The normal plant number was superior ($P<0.05$) for treatments covered with honeybees, in relation to the uncovered and that covered without honeybees (Table 3).

Table 3. The Percentage of Glycine max seeds germination, normal, and abnormal plants.

Treatments	**Germination (%)**	**Normal plants (%)**	**Abnormal plants (%)**
Uncovered area	21.25 ± 1.315^{b}	40.25 ± 1.702^{b}	9 ± 0.707^{b}
Covered area with honey bees	27.7 ± 0.854^{a}	45.5 ± 3.32^{a}	6.75 ± 0.629^{a}
Covered area without honey bees	16.5 ± 1.37^{c}	36.75 ± 1.93^{c}	10.5 ± 0.654^{b}
CV (%)	11.8	10.62	14.75

a,b,c=Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Insect visitors on *Glycine max* flowers

Various insects visited *Glycine max* flowers during its flowering time. These include honeybees, butterflies, flies, and moths. Among the insect visitors recorded on *Glycine max* flowers during the study period, honeybees were the major visitors (60.9%) in the open pollination, while moth was the least visitors (fig 1).

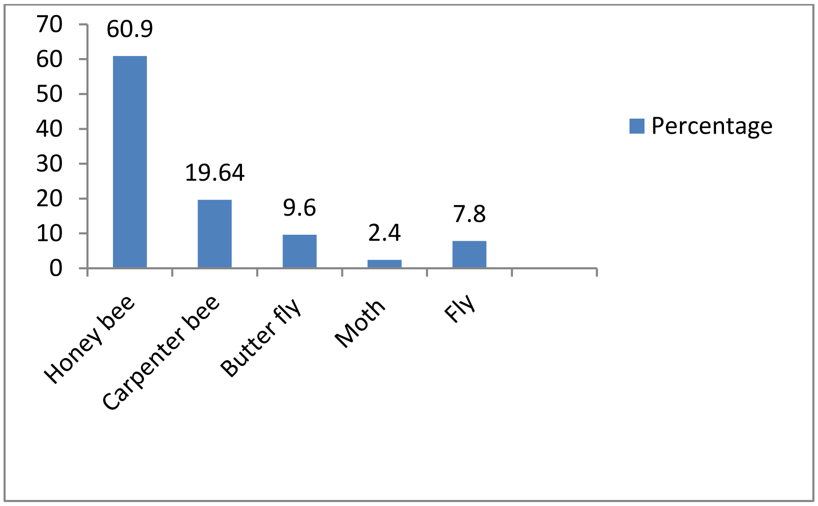

Figure 1 insect visitors on Soybean flowers

Conclusions and Recommendation

The study revealed that soybean (*Glycine max* L.) is largely dependent on intensive honeybee pollination. The results of this study revealed that honeybee pollination increases Glycine max seed yield by 38.9% over natural pollination. Therefore, moving honeybee colonies to *Glycine max* farm during the flowering period is one of the most essential inputs to maximize *Glycine max seed* production. It is important also to create awareness on the value of crop pollination in boosting crop yield and to solve the challenges they are facing.

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Diagnostic survey of honeybee diseases, pests and predators in selected districts of East Shewa and West Arsi Zones of Oromia, Ethiopia

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Abstract

The study was conducted in East Shewa and West Arsi Zones of Oromia Regional Satet to determine the occurrence and prevalence of honeybee diseases, pests and predators and to determine the effect of honeybee pests and predators on honeybee colonies and products. Questionnaire survey, field observation and laboratory diagnostic methods were used for the study. In the questionnaire survey, 115 beekeepers were included. For laboratory work, 80 honeybee colonies in 26 apiary sites were examined for the presence of honeybee external parasites and pathogenic diseases. The collected data were analyzed using descriptive statistics and chi-square test with SPSS version 23. Musa et al. (2006) rank index calculation was also employed to put the order of importance of pests and predators, which challenged honey production in the study areas. The major honeybee pests and predators identified were ants (27.4%), wax moth (21.9%), honey badger (14.4%), bee-eater birds (10.8%), small hive beetles (8.9%), lizards (6.8%), spiders (4.9%), death head hawks moth (3.2%) and wasps (1.6%). The total honeybee colonies absconded due to pests and predators was estimated to be 526 (58.6%). About 14%, 13.7%, 13%, 11.3% and 5.9% of the colonies absconded were due to ants, wax moth, honey badge, bee-eater birds and small hive beetle, respectively. Beekeepers in the study area have their own indigenous knowledge to prevent honeybee pests and predators. This includes cleaning apiary, putting ash and burned fuel around hive stands, strengthen the colony, frequent inspection, removing unoccupied suppers and combs, fixing of smooth iron sheet on hive stands and trees on which traditional hives are hanged, chasing with dogs, fencing with thorny plants and hanging hives by rope on long trees.Varroa mite, Nosema apis, Amoeba (Malpighamoeba mellificae), bee lice and small hive beetle were confirmed during the study period while Tracheal mite, Stone brood, American and Europian foul brood, Chalk brood did not confirmed during the study period. Out of 80 samples, the overall prevalence of Varroa mite, Nosema apis, Amoeba (Malpighamoeba mellificae), Bee lice and Small hive beetle was 82.5%, 60% and 83.8%, 43.8% and 33.8%, respectively. The study indicated that honey production in the study area is affected by several bee enemies and diseases. Thus, good extension service should be given and awareness creation to empower beekeepers for controlling honeybee diseases and enemies by coordinating their indegenous knowledge with scientific methods.

Key words: Disease, honeybee, pests and predators, prevalence

Introduction

Ethiopia has high potential for beekeeping as a climate is favorable for growing different vegetation and crops, which are good sources of nectars and pollen for honeybees.The ideal climatic conditions and diversity of floral resources allow the country to sustain around 10 million honeybee colonies,of which 7 million are kept in local beehives by farmers and the remaining exist in the forest as wild colonies (Workeneh, 2007). Having the highest number of bee colonies and surplus honey sources of flora, Ethiopia is a leading honey producer in Africa and one of the ten largest honey producing countries in the world (Rivera *et al*., 2007). Ethiopia has a share of around 23.58% and 2.13% of the total Africa and world honey production respectively (Ayalew, 1990). Despite of having the highest bee density and being the leading honey producer as well as one of the largest beeswax exporting countries in Africa,the share of the sub-sector in the GDP has never been matched with the huge number honeybee colonies and and the country potentialityfor beekeeping. Among the major challenges of beekeeping in Ethiopia, ecological degradation,recurrent drought, farm land expansion, recently emerging bee pests and diseases, indiscriminate use of agro-chemicals, low technology input, poor pre and post-harvest management,inadequate extension services and poor marketing (Desalegn Begne,2014). Ethiopia has the potential to produce about 500,000 tonnes of honey per year and 50,000 tonnes of beeswax per annual, but currently production is limited to 53,000 tonnes (CSA, 2014)

Beekeeping is an environmental friendly and non-farm business activity that has an enomous contribution to the economic segments of the society and to a national economy as a whole. Beekeeping is economically important activity that generates income for millions of people in Ethiopia and the world at large. It provides substantial benefit to address household's food security and poverty alleviation through income diversification for beekeepers in potential areas (FAO, 2009). It also assists to increase crop production through honeybee pollination (Amulen *et al*., 2019). However, the essential and valuable contributions of honeybees depend upon the healthy population of honeybees (FAO, 2012). The health of honeybees has been one of the most important topics in apiculture research in recent years (Genersch, 2010). This is mainly associated with the recent emergence of high honeybee colony losses in many parts of the world (Vanengelsdorp *et al*., 2008; Genersch, 2010) and the vulnerability of honeybees to parasitic mites, fungi, viruses and bacteria (Bradbear, 2009). These diseases and pests of honeybees are known to cause the death of honeybees and subsequent reduction of honeybee products and moreover have negative impact on domestic and international marketing of honeybee products (vanEngelsdorp and Meixner, 2010).

Currently the loss of honeybees has increased because of different reasons among which the interrelated factors were pathogens such as viruses, bacteria, fungi and metazoan parasites (Potts *et al*., 2010). The decline is characterized by sudden loss of worker bees from colonies without signs of dead or diseased bees, despite the presence of abundant breeding cells, pollen and honey (Le Conte *et al*., 2010). Some of pests and diseases are quite common while others are rarely encountered. In United States the average honeybee loss per beekeeping operation was 25.5% (Spleen *et al.*, 2013). Similarly, 16% honeybee colony reduction has been reported in Europe (Hendrikx *et al*., 2010: Potts *et al*., 2010). It has been reported that several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality (Genersch, 2010; Vanengelsdorp and Meixner, 2010). This is a threat and an alarm for governments, conservationists and the private sector engaged in the subsector in different parts of the world as bee diseases and pests do not respect borders.

The agro-ecology of Ethiopia is not only favorable for honeybees but also for different kinds of honeybee pest and predators that are interacting with the life of honeybees (Desalegn Begna, 2001). In Ethiopia several surveys have been conducted on the existence of honeybee diseases and pests and predators. The most commonly known honeybee diseases reported to exist in Ethiopia are *Nosema apis and Melpighamoeba mellificae* (Amssalu Bezabeh and Desalegn Begna, 2006); Chalk brood (Desalegn Begna , 2006); Some major types of honeybee pests and predators (Desalegn Begna ,2001;Desalegn Begna and Amssalu Bezabeh,2001); Small hive beetle (*Aethina tumida Murray; Coleoptera: Nitidulidae*) (Desalegn

Begna and Amssalu Bezabh ,2006); different ants (*Dorylus fulvus*) (Desalegn Begna ,2007); the bee lice (*Braula coeca*) (Adeday Gidey *et al*., 2012; Gemechu Gizachew *et al.,* 2013). The occurrence of the above mentioned diseases and pests and non existence of bacterial and viral diseases were reported before eight years. As a rule, regular monitoring of honeybee diseases and pests must be conducted at least in five years interval unless outbreaks are reported (Vanenglesdorp *et al*., 2012). On the other hand, several reports (both formal and informal) indicated the deaths and declines of honeybee colonies due to unknown factors. The identification and severity of each economically important honeybee pests, predators and disease have not been well documented in the study area, despite the little information available. To fully exploit the opportunities in beekeeping sector, addressing the constraints and detecting the occurrence and distributions of honeybee's health problems and their harmful effects is a key step towards prevention Therefore, the objectives of this study were:

- To determine the occurrence and prevalence of honeybee diseases, pests and predators
- To determine the effect of honeybee disease, pests and predators on honeybee colonies and their products in the study areas

Materials and Methods

Description of the study areas

The study was conducted in the selected districts of East Shewa and West Arsi Zones of Oromia Regional State namely: Dugda, Adami Tulu, Wondo and Dodola districts. The districts were selected based on their potential for beekeeping, agro-ecological representativeness and accessibility to transport facility.Dodola district represented highland whereas Wondo district represented midland, Dugda and Adami Tulu represented lowland agro-ecology.

Dugda Woreda is located in East Shewa Zone of Oromia Regional State at a distance of 138 km from Addis Ababa (the capital of the country) and 88 km West of Adama(the capital of East Shewa Zone). Geographically, the district is located between 8° 02′59″N latitude and 38° 43′59″'E longitude and the altitude of the areas ranges from 1576 to 1750 m above sea level. According to the climatic data collected in 2019 from Adami Tulu Agricultural Researcher Center, the total annual rainfall of the area is 795.4 mm and the mimimum and maximum temperature is 13.6°C and 29.2°C

Adami Tulu Agricultural Research Center is located in the Mid rift valley (MRV), 167 km south of Addis Ababa on Hawasa road at an altitude of 1650 m above sea level. It lies at 7°9`N latitude and 38° 7`E longitude. The mean annual rain falls is 760mm. The mean minimum and maximum temperatures are 12.6 and 27°C , respectively. The soil type is fine, sandy loam with sand: silt: clay in the ratio of 34: 38: 18, respectively. The average pH of the area is 7.88 (Adami Tulu Agricultural Research Center profile, 1998).

Wondo district is located in West Arsi zone of Oromia Regional State.The district is located at a distance of 271 km from Addis Ababa the capital city of Ethiopia and 19 km from Shashaemene, the zone of town. The altitude of the area ranges from 1700 to 2300 m above sea level. It is generally characterized by warm climate with a mean annual maximum temperature of 19°C and a mean annual minimum temperature of 17°C.

Dodola district is situated at a distance of 320 km from Addis Ababa and 75 km from the zone's capital city, Shashemene. Dodola is situated in southeastern Ethiopia. Located in the West Arsi Zone of the Oromia Region, this town has a latitude and longitude of 06°59′N 39°11′E, with an elevation ranging from 2362 to 2493 meters above sea level (Wikipedia, 2021). The mean annual rain fall of the area is from 800 to1200 mm with the annual mean temperature is 13 to 26°C.

Study design

A crossectional study was carried out from September 2020 to November 2021 in the four districts of East Shewa and West Arsi Zones on honeybee colonies managed under transitional and modern beekeeping methods to investigate the prevalence of major honeybee diseases and pests which are causes of significant economic loss in honeybees by observing and collecting samples from the colonies. Then, isolation and identification of bee diseases causing pathogens were conducted. A diagnosis was confirmed by integrating both clinical and parasitological studies. Honeybee colonies were inspected internally and externally to collect data on the health status and samples of adult honeybee were collected for further laboratory diagnosis. Records on the history and status of the colony, clinical symptoms of diseases and pests were taken. In order to examine the prevalence/distribution and infection/infestation rates of the onset of diseases and pests according to the activity periods of honeybees, samples were collected. Finally, prevalence at apiary level and infestation/infection at colony level were calculated using (Vanenglesdorp *et al*., 2013) protocols:

$$\text{Prevalence} = \frac{\text{Number of positive cases}}{\text{Total number of sampled population}} \times 100$$

$$\text{Infestation/infectious level} = \frac{\text{Number of positive bee}}{\text{Total number of sampled bees}} \times 100$$

Sampling techniques and sample size determination

A multistage stage sampling procedure was employed to select districts, beekeepers and honeybee colonies. At the first stage, two administrative zones were selected using purposive sampling based on their potential for beekeeping and accessibility. In the second stage, four administrative districts were selected purposively based on their relative beekeeping potential and representativeness to highland, midland and lowland agro ecologies. In the third stage, three represetative kebeles/PAs were selected from each district proportional to the agro-ecological variation using purposive sampling techniques based on beekeeping potential of the kebeles and transport accessibility. In the fourth stage, honeybee colonies and beekeepers were sampled from all rural kebeles using simple random sampling technique. The sample size required for the study was determined based on sample size determination in a random sampling methods using 50% expected prevalence with 95% confidence interval at 5% absolute precision, according to Thrusfield (2005) as follows:

$$n = \frac{1.96^2 * P_{exp} * (1-P_{exp})}{d^2}$$

Where: n=required sample size, P_{exp} = Expected prevalence (50%) d= desired absolute precision (5%).

Both adult and brood bee samples were randomly collected from each bee colony and examined in laboratory following the standard methods for Varroa research by Dietemann *et al*. (2013).

Data sources and collection techniques

Both primary and secondary data were used to achieve the objectives of the study. Primary data were collected from sample household beekeepers, field observation and laboratory diagnosis techniques of adult worker honeybees and broods whereas secondary data were obtained from the reports of districts Agriculture and Rural Development Office, Zonal Agricultural Department Office, NGOs and other published and unpublished materials.

Types of data collected

This study required a wide range of information with reference to honeybee pests, predators and diseases. Both qualitative and quantitative data were collected using conventional survey and laboratory diagnostic methods. Data collected by the survey include sex, educational level, family size, experience of beekeeping, number of hives owned, frequency of hive inspection, trend of honeybee colony and hive products, number of honeybee colony absconded, major honeybee pests and predators, prevalence and infection rates of honeybee disease and parasites.

Laboratory Examination Procedures

Field observation and diagnosis were conducted through colony inspections for major honeybee diseases including Nosema, Amoeba, Chalk brood, American foul brood, European foul brood, Varroa mite and Tracheal mite (where there is suspected clinical symptoms). Besides field observations, laboratory testing for each type of diseases examination were conducted following the standard procedures for each honeybee diseases.

Examination of Varro mites

The study follows the standard methods for Varroa research by Dietemann *et al*. (2013). Samples of adult bees were collected from bee colonies hived in improved movable frame hives and transitional beehives. From each bee colony, 250 to 350 adult honeybees were brushed off from the brood comb directly into a wide mouth plastic container. The collected adult bees were killed using 70 % ethyl alcohol and placed in 10 ml of 1% detergent-water solution (10 ml detergent in 1000 ml water) and vigorously shake for 1 minute to dislodge mites. The mites were collected filtering the solution through a ladle (8- to 12-mesh) that hold the bees back and let out the mites with the solutions. Then, wire gauze was used to hold the mites back and discharge the solutions. The wire gauze was turned down to white paper on which the presence/absence of the mite was examined and counted. Furthermore, brood examinations were done by cutting off 5 X 5 cm brood comb areas from drone and worker pupae broods. About 100 pupae were removed from their cells using forceps and checked for the presence of Varroa mites on the worker and/ or drone pupae. At the end, number of Varroa mites per diagnosised sample was recorded.

Examination of Tracheal mite

According to Sammataro *et al*., (2013), samples of 20-30 adult honeybees were collected at random. The sample bees were preserved by adding 70% alcohol. The head and first pair of legs of honeybees were removed using scissor. Transverse-section thoracic disks were sliced and placed directly in a small dish containing 10-percent potassium hydroxide (KOH). The sliced thoracic disks in KOH were heated and stirred gently near to boiling point for approximately 10 minutes until the soft internal tissues dissolved to expose trachea rings. The trachea ring sections were retrieved through filtration and washed with tap water. The disktrachea suspension was examined for infested part and *Acrapis woodi* under a dissecting microscope at 10 magnification power Sammataro *et al*., (2013).

Examination of Nosema and Amoeba diseases

As these two diseases are initiated by protozoan agent that affects the abdominal contents of adult honeybees, their sampling and diagnostic techniques are almost the same. Following the Fries *et al*. (2013) procedure, a sample of 30-60 worker adult honeybees were collected from the hive entrance. The sample bees were collected in 70% alcohol until laboratory analysis. The abdomen of honeybees from each sample was cut using scissors. The cut abdomens were placed and grounded in mortar containing 5-10 ml distilled water until an even suspension is formed using pestle. The mortar and pestle were thoroughly cleaned before being used again. A loop of suspension were placed on microscopic slid using the sterilized loop and covered with cover slid. The suspensions were examined under light microscope using 40-magnification power for the presence of Nosema spores and Amoeba cysts.

Examination of Chalk brood disease

Both external and internal inspections were conducted for the presence of Chalk brood disease clinical symptoms. Dry scales with white to dark colour moulds and Chalk brood mummies were carefully observed in the comb cells and on the bottom boards of the hives. Mummies were moistened with distilled water and the supernants were placed on microscope slid, covered with cover slid and and examined under light microscope for spores and/or spore balls and cysts of *Ascosphera apis* (Jenssen *et al*., 2012).

Examination of American Foulbrood and European Foulbrood

Field diagnostic procedures for AFB and EFB were used based on the OIE (2008) procedure. In randomly selected apiaries a minimum of three colonies were inspected internally for major clinical symptoms of bacterial diseases with emphasis to AFB and EFB. Typical clinical symptoms such as irregular brood arrangement, sunken and dark capping with puncture holes, dead and decayed larvae with dark “scales” and slight to pronounced odor were examined for the occurrence of AFB in the colonies. Similarly, twisted larvae with creamy-white guts visible through the body wall, melted and yellow white larvae with unpleasant sour odour and loosely-attached brown scales were directly observed for the infected colonies by EFB. Furthermore, match stick test (stretch test) was employed to observe the robby thread stretching for the typical clinical symptoms of bacterial diseases. From any suspected brood showing one of the above important clinical symptoms, brood smear samples were prepared on frosted end microscopic slide and legibly labeled for further laboratory diagnosis according to Primefact (2009). Both Nigrosine and

Holstic milk test approach were conducted according to the procedure of Shimanuki *et al*, (2000) for the presence and protolytic reaction of gram+ spores, respectively. Samples were examined under microscope for the presence of *Paenibacillus* larvae and *Melissoccocus pluton* in positive samples AFB and EFB, respectively using Zeiss AxioVert A.1 light microscope under oil immersion (magnification power of 100X).

Diagnosis for major honeybee pests and predators

Pests and predators of honeybees cause devastating damage on honeybee colonies and at most time cause swarming, abscond or colony collapse thus being a challenge for beekeeping in tropical and sub tropical countries. The occurrence and economic importance of major honeybee pests and predators, including *Wax moth, small hive beetle, ants, spiders, bee-eater birds, honey badger, bee lice, lizards, Dead hawks moth,* etc., in the all the study areas were determined through beekeepers interview using semi-structured questionnaires and internal and external hive inspections. Moreover, clinical symptoms and infested combs, adult and larvae of small hive beetles and wax moth and other decayed materials were observed in the hive through inspection of the beehives described by Neumann *et al.* (2013). The presence of small hive beetle infestation **(***Aethina tumida***)** was identified through its adult, larvae or pupae and colony examination methods as larvae of SHB have pairs of prominent brownish dorsal spines on each segments with3 pairs of anterior prologs only. Based on Ellis *et al*. (2013),the larvae of wax moth has no spines, but number of setae (hairs) on each segments with 8 pairs of prologs (3 pairs,4 pairs and 1 pairs on anterior, abdominal and last segments respectively).Unlike Small hive beetles, it produces silken galleries.

Data analysis

The collected survey data were analyzed by descriptive statistics using SPSS software version 23 and chi-square test. Musa *et al.* (2006) rank index calculation was also employed to put the order of importance of pests and predators,which challenges honey production in the study area.The rank index can be calculated as follow: Rank index=sum of (5 X number of household ranked first + 4 X number of household ranked second + 3 X number of household ranked third + 2 X number of household ranked fourth + 1 X number of household ranked fifth) for an individual reason divided by the sum of (5 X number of household ranked first + 4 X number of household ranked second + 3 X number of household ranked third + 2 X number of household ranked fourth + 1 X number of household ranked fifth) for overall reasons. A chi-square test **(χ2)** was used to assess the association of the risk factors with the prevalence of the major honeybee diseases and pests. Statistical significance was set at a *p* value of less than 0.05.

Results and discussion

Socio-economic characteristics of the respondents

Sex of the respondents

Out of the total 115 interviewed beekeepers, about 90.4% were male headed households and the rest 9.6% were female headedhouseholds. The study showed that very limited number of female were eganged in beekeeping activities.The main reason for low participation of female could be attributed to the forest beekeeping system of the area that culturally prohibit women involvement in beekeeping for the reason they cannot climb up the tree either to bait swarm bees or to udertake beekeeping by hanging on big trees. This was in line wit the reports of Workneh Abebe (2011) who noted the conventional placement of the hives in forest areas makes it impossible for women to operate them thus reducing women's participation. This very limited number of female participation also agrees with Alemu Tsegaye (2015) results of the study, the majority of sampled respondents 91.1% were male and the rest 8.9% were female.

Educational level of the respondents

Regarding the level of education, about 24.3% of those interviewed beekeepers did not receive any formal or informal education while the rest 27.8%, 20.9%, 16.5%,8.7% and 1.7% for read and write, had attended elementary education, primary education, junior education, high school and higher education completed,respectively (Table 1). This reveals that beekeeping is practiced by both groups (literate and illiterate). The study result in line with the findings of Tessega (2009) in which most of the respondents were capable of read and write in Burie district of Amhara region. Formal education is important to farmers to adopt modern inputs and technologies in beekeeping sector. Empowering beekeepers with knowledge and skills ensures availability of modern technologies Mujuni *et al*. (2012). Similarly, Workneh, (2011) reported that education increases the access to information and thereby possible knowledge of beekeepers regarding modern hive. Education status of beekeepers affects their chances of shifting from traditional beekeeping to modern technology beekeeping (Kidane Mollaw, 2014).

Experience in beekeeping

The survey result indicated that, about 40% of beekeepers in the current study had been involved in beekeeping for about over 15 years. The rest of the respondents, 33%, 17.4% and 9.6% had beekeeping experience for about 11 to 15 years, 6 to 10 years and 1 to 5 years, respectively (Table 1). This implies that the respondents have rich indigenous knowledge with regard to beekeeping activities.The present result is higher than the experience reported in Gomma district which is 5.66 years (Workneh Abebe, 2011). Beekeeping experience might be responsible to influence the attitude and adoption of new beekeeping technologies (Hussien *et al.*, 2015) and a good knowledge the beekeepers have on apiary management and honey usage habits (Kiros and Tsegaye, 2017).

Family size of the respondents

According to the survey result, higher percentages (53%) of beekeepers have family size ranging from 6 to 10 persons per household whereas lower percentages (17.4%) have family more than 10 persons per household. The household size of a family may imply the level of dependency in the household or the labor force in the household. This labor force has involved in different beekeeping activities like beehive and apiary inspection, honey harvesting and colony management. Workneh *et al*. (2011) stated that beekeepers with large family size have interest and potential for promotion of improved technologies to improve productivity and incomes. Also the study by Guesh Godifey (2015) states that labor is one of the important factors to their beekeeping practices. Adopting improved box beehives demands additional labor and therefore, households with larger family size are more able to meet these demands.

Table 1. Sex, educational level, experience in beekeeping and family size of respondents (N=115)

Variable	**Category**	**Frequency**	**Percentage**
Sex	Male	104	90.4
	Female	11	9.6
Educational level	Illiterate	28	24.3
	Read and write	32	27.8
	Primary education	24	20.9
	Junior education	19	16.5
	High school	10	8.7
	Higher education	2	1.7
Experience in beekeeping	1-5 years	11	9.6
	6-10 years	20	17.4
	11-15 years	38	33.0
	>15 years	46	40.0
Family size	1-5	34	29.6
	6-10	61	53.0
	> 10	20	17.4

Honeybee colony inspection practices in the study area

Colonies and apiary inspection was necessary to make sure that the honeybee colonies are safe from pests, predators and diseases. Sample respondents were interviewed to describe the frequency of inspection of their apiary. Both external and internal inspections of apiary site and honeybee colonies were practiced by beekeepers of the study area. Majority of the respodents (34%) inspect their honeybee colony and apiary site externally every day.The rest, 27.8%, 19.1%, 12.2% and 7% inspect externally every month, weekly, sometimes and rarely, respectively (Table 2). Results of this study are in line with the findings of Tessega (2009), who reported external colony insepection of every day, every month and weekly to be 33.3%, 20.8% and 16.7%, respectively. Internal honeybee inspection is practiced rarely (49.6%), sometimes (24.3%), every month (16.5%) and 9.6% weekly. According to Sisay *et al*. (2015) in Jigiga zone of Somali region, Ethiopia 13.3%, 14.02% and 23.45% of the respondents replied that they take a look internally into the hives, every week, every fifteen days and every month, respectively.

Table 2: Apiary site and honeybee colonies inspection practices in the study areas (N=115)

Inspection frequency	External inspection		Internal inspection	
	Frequency	Percentage	Frequency	Percentage
Every day	39	34.0	-	-
Weekly	22	19.1	11	9.6
Every month	32	27.8	19	16.5
Sometimes	14	12.2	28	24.3
Rarely	8	7.0	57	49.6

Trends of honeybee colonies and hive products in the study areas

Out of the total respondents, 62.6% beekeepers replied that honeybee colonies and hive products were decreasing from time to time in the study area. This result agrees with the results of Dabessa and Belay (2015) who reported that 94.24% of beekeepers mentioned decreasing trends in their honeybee colonies and products from time to time at Walmara district. This finding also agreed with the research finding of kerealem (2005), Tessega (2009) and Tsegaye (2015) who reported a decreasing trend of honeybee population and their products in Enebse and in Bure districts of South Wollo and Waghimra zones, respectively. Contrary to this, 27% of the respondents stated thatd their colony population and hive products are increasing whereas 10.4% stated thatd their colony population and hive products are constant (Table 3). These respondents assumed that the increased trend of honeybee colonies and honey products due to the adoption of improved beekeeping practices, availability of bee forages, aforestation programs and water conservations and attractive price for honey. The better integration of beekeeping with natural resources development programs in the study areas could also be the reason for the increasing production of honey and number of colonies.

Table 3: Trends of honeybee colonies and hive products in the study areas (N=115)

Catagories	**Frequency**	**Percentage**
Decreasing	72	62.6
Increasing	31	27.0
Constant	12	10.4

Reasons for decreasing trend of honeybee colonies and hive products

The respondents were asked to list the reasons for decreasing of their honeybee colony and hive products. According to the sample respondents, the possible reasons behind this trend could be shortage of bee forages and water, agro-chemicals application, pests and predators, drought, diseases and parasites, absconding, high cost of modern hives and equipments and shortage of honeybee colonies in order of their importance (Table 4). The result is in line with Kerealem Ejigu *et al*. (2009), who reported that shortage of bee forage is ranked first due to population pressure, lack of land use policy and the high demand for farmlands put pressures on mountainous areas to be used for crop production and livestock grazing. Deforestation was under way in some parts of the districts and this caused rainy season irregularity that reduced nectar and pollen sources for honeybee colonies. Shortage of bee forage causes the honeybee colony to abscond to areas where resources are available for their survival. Application of chemicals such as fungicides, pesticides and herbicides hinder the productivity and production of honeybee colonies.

Table 4: Reasons for decreasing of honeybee colonies and hive product in the study areas

Problems	**Rank by frequency**							**Index***	**Over all rank**
	1st	2nd	3rd	4th	5th	6th	7th		
Shortage of bee forages and water	55	23	8	3	1	2	0	0.149	1
Agro-chemicals application	37	20	16	17	8	0	0	0.142	2
Pests and predators	35	32	17	0	0	7	4	0.139	3
Drought	6	46	21	8	5	0	0	0.121	4
Diseases and parasites	4	38	31	6	8	0	0	0.118	5
Absconding	9	33	16	14	18	0	0	0.116	6
High cost of modern hives and equipments	5	40	20	8	4	1	0	0.108	7
Shortage of bee colony	0	37	16	12	15	6	0	0.105	8

***Index =** *sum of (7*ranked 1st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5 th+2* ranked 6th+1* ranked 7th) for individual and predators divided by the sum of (7*ranked 1 st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2*ranked 6th+1* ranked 7 th) for over all Constraints.*

Honeybee pests and predators

Respondents were asked to identify the major honeybee pests and predators in their respective areas. Accordingly, beekeepers indentified nine major honeybee pests and predators such as ants, wax moth, honey badger, bee-eater birds, small hive beetle, lizards, spiders, death head hawks moth and wasps (Table 5). Similarly, the presence of the pests and predators such as honey badger, ants, wax moth bee-eater birds, spiders and lizards in Kilte-Awlaelo district were reported by Adeday Gidey *et al*. (2012). These pests and predators were also reported from Atsbi-Womberta by Workneh Abebe (2007), Bure district by Tesega Belie (2009), Gomma district by Chala Kinati *et al.* (2013), Central zone of Tigray by Haftu Kelelew *et al*. (2015) and from Walmara district by Dabessa Jatema and Belay Abebe (2015). According to Kajobe *et al*. (2009), at least 12 pest and predators that attack honeybees and their hives in Uganda were documented. In Ethiopia, more than 15 honeybee pests were identified and recorded (Desalegn 2001, Desalegn and Amssalu, 2001, Desalegn and Yosef, 2005, Desalegn and Amssalu, 2006). According to these studies, ant, wax moth, mice, birds, honey badger, wasps, death's head hawks moth, bee lice (*braula coeca*), beetles , lizards, toads, prey-mantis, spiders, pseudo scorpions (*chelifer species*) were among the major honeybee pests registered locally. Similarly, honeybee pests such as ant, wax moth and spiders were found to be the major constraints for beekeeping industry in Nigeria (Akinwande *et al.*, 2013).

In the present study, ants were identified to be the first ranked pest in the study area which affected about 27.4% honeybee colonies in the study areas. The current finding was in line with the research finding of Dinaol Belina *et al*., (2016), who reported that 23% honeybee colonies to have been affected by ants in West Shewa zone of Oromia, Ethiopia. Similarly, out of the total honeybee colonies owned by the respondents, about 21.9% honeybee colonies were affected by wax moth next to ants in the study areas. The current finding was in line with the research finding of Segni Shimelis, (2017), who reported that about 18.2% honeybee colonies were affected by wax moth in Ejere District, West Shewa Zone, Oromia, Ethiopia. At the same time, 14.4% of honeybee colonies were affected by honey badger in the study areas. Segni Shimelis, (2017) also reported that 14.4% honeybee colonies were affected by honey badger in Ejere District, West Shewa Zone Oromia, Ethiopia. Furthermore**,** about 10.8% and 8.9% honeybee colonies were affected by bee-eater birds and small hive bettle, respectively in the study area. This result is in agreement with Bekele Tesfaye *et al*., (2017) who reported that 17.8% honeybee colonies were affected by bee-eater birds in Bale Zone. The study also showed that ants, wax moth, honey badger, bee-eater birds and spiders were frequently occuring pests and predators in the study areas whereas, lizards, small hive beetle, death's head moths and wasps were found to occur rarely in the study areas.

Table 5: Rank index for major honeybee pests and predators in the study area

Major pests and predators	**Rank by frequency**							**Index***	**Over all rank**
	1st	2nd	3rd	4th	5th	6th	7th		
Ants *(Dorylus fulvus)*	40	31	17	7	3	0	0	0.274	1
Wax moth *(Acheroea grisella)*	38	21	9	6	1	0	0	0.219	2
Honey badgers (*M. capensis*)	14	17	12	8	2	3	0	0.144	3
Bee-eater birds *(Meropidae)*	9	7	17	4	5	3	1	0.108	4
Small hive beetle (*Aethina tumida)*	0	5	15	12	7	8	0	0.089	5
Lizard *(Agama agam)*	0	10	7	5	8	2	1	0.068	6
Spiders (*Latrodectus mactan*)	5	2	9	1	3	0	0	0.049	7
Death's head moths (*Acherontia atropos*)	0	4	6	1	2	0	3	0.032	8
Wasps (*Polistes fuscatus*)	0	2	4	0	0	1	0	0.016	9

***Index =** *sum of (7*ranked 1st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2* ranked 6th+1* ranked 7th) for individual pests divided by the sum of (7*ranked 1st+ 6* ranked 2 nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2* ranked 6th+1* ranked 7th) for over all pests and predators*

Effect of pests and predators on honeybee colony and honey production in the study area

The interviewed beekeepers indicated that ant, wax moth, honey badger, bee-eater birds and small hive beetle and spiders were the most common problems in the study area. The respondents indicated that ants (*Dorylus fulvus*) are the top ranked pests that cause the dwindling of honeybee colony (70.4%), honey production loss (75.7%) and colony absconding (80.9%). Ant was one of the important honey bees' enemies and causing a serious problem in West and south western Shoa Zones shows so that that 44.2% of honeybee colonies are yearly attacked by ants of which 24% absconded and 4.2% died (Desalegn, 2007). Ants kill honey bees, rob their products and forces the bee colonies to leave their proper nest which results in reduction of honey production.

Similarly, based on the beekeepers responses, wax moth was found to cause dwindling of honeybee colonies (73%), honey production loss (78.3%) and absconding of honeybee colonies (74.8%). Wax moth, which is one of the most important pests of honeybee colonies with worldwide distributions, is also identified as one of the serious local honeybee pest in the country (Desalegn 2001). The study that was conducted in three zones of the country showed investigated wax moth prevalence variations from zone to zones with South west Shoa zone having high infestation level (26.66%) followed by West and East shoa zones 22.85% and 26.66%, respectively. Also, the same study indicated that about 56%-75% of the wax moth infected honeybee colonies absconded and the remaining dwindled (Amsalu Bezabeh and Desalegn Begna, 2012a). In addition to this, honey badger was also cause loss of honey production (62.6%) and absconding (58.3%). Similarly resported by Tesfaye K, and Tesfaye L,(2007) indicated that honey badgers (*Mellivora capensis*) as a series predator causing considerable amount of honey lost and causing absconding in Adami Tulu Jido Kombolcha district. Moreover, bee-eater birds was found to be cause dwindling of honeybee colony (63.5%), honey production loss (58.3%) and absconding (29.6%).The respondents also replied that small hive beetle cause for the dwindling of honeybee colony (56.5%),honey production loss (55.7%) and colony absconding (60.9%). Small hive beetle is native to Africa, where it is considered as a minor pest of honeybees. Both adults and larvae are able to be serious pests that weakened honeybee colonies or honey supers. The beetles multiply to vast numbers, their larvae tunnel through comb to eat brood, damage stored honey, and ultimately destroy infested colonies or cause them to abscond. In Ethiopia the small hive beetle was reported in the south and South-West parts of the country with prevalence rate of 10% (Amsalu and Desalegn, 2001). The small hive beetle was reported in the Oromia regional state; 60% Jimma and 1.1% in Horo Guduru walaga (Amsalu and Desalegn, 2008) likewise it was reported Southern parts of Ethiopia with prevalence rate ranges from 21% in Konso to 66% in Teltele (Amsalu and Desalegn, 2006). Respondents also reported spiders as a series pest causing dwindling of honeybee colony (48.7%), honey production loss (41.7%) and absconding (36.5%). Spider is one of the major honeybee pests causing considerable amount of damage by making a web to trap adult bees inside and outside of the hives.

Table 6. Percentage of respondents declared the effect of pests and predators on honeybee colony and honey production (N=115)

Pests and predators	**Colony dwindling**		**Honey production loss**		**Colony absconding**	
	Yes	No	Yes	No	Yes	No
Ants	81 (70.4%)	34(29.6%)	87 (75.7%)	28(24.3%)	93 (80.9%)	22(19.1%)
Wax moth	84(73%)	31 (27%)	90 (78.3%)	25(21.7%)	86 (74.8%)	29(25.2%)
Honey badger	-	-	72 (62.6%)	43(37.4%)	67 (58.3%)	48(41.7%)
Bee-eater birds	73 (63.5%)	42(36.5%)	67 (58.3%)	48(41.7%)	34 (29.6%)	81(70.4%)
Small hive beetle	65(56.5%)	50(43.5%)	64(55.7%)	51(44.3%)	70(60.9%)	45(39.1%)
Spiders	56(48.7%)	59(51.3%)	48(41.7%)	67 (58.3%)	42(36.5%)	73(63.5%)
Lizard	35 (30.4%)	80(69.6%)	52(45.2%)	63(54.8%)	-	-

Number of honeybee colonies absconded due to pests and predators

Out of the total 898 honeybee colonies owned by the surveyed beekeepers,526(58.6%) of them were absconded due to ants, wax moth, honey badger, bee-eater birds and small hive beetle (Table 7).From the total of 224 honeybee colonies infested by ants,126(14%) were absconded. The same effect but higher than the current result were reported by Desaleng Begna (2007) in West and South West Shewa zones of Oromia region, indicated that 44.2% honeybee colonies were yearly attacked by ants of which 24% absconded and 4.2% died which resulted in 29% of total honey yield lose which is estimated to or over 3,839,810 ETB. Ants feed on honey, brood, beeswax, pollen and lead to absconding of bees and destroying the entire bee colony (Tesfaye, 2015).

Similarly, out of the total 209 honeybee colonies infested by wax moth, 123(13.7%) were absconded due to wax moth. The current finding was in line with the research finding of Amssalu Bezabeh and Desalegn Begna (2007), who reported high infestation level (26.66%) in South West Shewa zone followed by West and East Shewa zones, respectively.This study indicated that about 56%-75% of the wax moth infected honeybee colonies absconded and the remaining dwindled. According to Kajobe *et al*.,(2009),wax moths are generally found wherever there are honeybee and particular warm conditions and cause destruction of honeybee colonies.Annually it is estimated that the wax moths causes almost more than 5 million dollars in the U.S.(MAAREC,2000). At the sme time, from the total of 190 honeybee colonies attacked by honey badger in the study areas, 117(13%) honeybee colonies were absconded (Table7).The main damage of this predator is disturbance of the life of honeybee colonies througth its capacity of opening and overturnuing beehive. Honey badger attack was a serious problem and stand out in the area causing disappearance of honeybee colonies. As a result of this predators attack, a considerable amount of honey and other hive products is lost and disappearance occurs. Out of the total 185 honeybee colonies attacked by bee-eater birds, 107(11.3%) honeybee colonies were absconded. Bee-eating birds are widely distributed and many beekeepers regard them as seriou pests (Mahmoud *et al.*,2012). Bee-eater birds feed on the bees especially those that forage for nectar. These birds strategically position themselves near the hives and capture the bees that are flying out or into the hive. The level of damage caused by honeybee-eater birds varies. An affected by a single bird or by a few group rarely constitutes a serious problem, but when a large flock descends upon a few colonies or an apiary, a substantial decline in the worker population in some or all the hives may be observed.

Simillarly, from the total of 109 honeybee colonies infested by small hive beetles in the study area, about 53(5.9%) honeybee colonies were absconded (Table7).Small hive beetles are one of the most economically detrimental pests of honeybees not only can they significantly reduce brood and honey production, in extreme cases they can cause total collapse of the colony (Ellis, 2003).

Table 7: Number of honeybee colonies absconded due to pests and predators in the years of 2017-2021

Variables	**Districts**				
	Dodola	**Wondo**	**ATARC***	**Dugda**	**Total**
Total colony owned by respondents	321	228	24	325	898
No of colony infested by ants	75(24.8%)	52(22.8%)	9(37.5%)	88(27.1%	224(24.9%)
No of colony absconded by ants	36(11.2%)	28(12.3%)	7(29.2%)	55(16.9%)	126(14%)
No of colony infested by wax moth	68(21.2%)	45(19. 7%)	10(41.7%)	86(26.5%)	209(23.3%)
No of colony absconded by wax moth	38(11.8%)	34(14.9%)	5(20.8%)	46(14.2%)	123(13.7%)
No of colony attacked by honey badger	65(20.2%)	46(20.2%)	-	79(24.3%)	190(21.2%)
No of colony absconded by honey badger	41(12.8%)	25(11%)	-	51(15.7%)	117(13%)
No of colony attacked by bee-eater birds	80(25%)	51(22.4%)	7(29.2%)	47(14.5%)	185(20.6%)
No of colony absconded by bee-eater birds	43(13.4%)	33(14.5%)	5(20.8%)	26(8%)	107(11.3%)
No of colony infested by small hive beetle	38(11.8%)	26(11.4%)	-	45(13.8%)	109(12.1%)
No of colony absconded by small hive beetle	18(5.6%)	12(5.3%)	-	23(7.1%)	53(5.9%)
Total colonies absconded	176(54.8%)	132(57.9%)	17(70.8%)	201(61.8%)	526(58.6%)

**ATARC=Adami Tulu Agricultural Research Center*

Traditional preventive measures of honeybee pests and predators

Beekeepers have developed different indigenous knowledge and practices in controlling some of the honeybee pests and predators. Some of the cultural practices are: placing ash under hive stands, fencing off the apiary, clean apiary, plastering the hive stand with plastic materials,fixing smooth iron sheet on the trunks of a tree where hives are hanged, hanging hives on long trees, protecting by dog, removing branches of tree near apiary,strengthen colonies fia feeding,removing unoccupied suppers and combs. Similar result also reported by Dabessa and Belay (2015) as beekeepers used different mechanisms to protect their honeybees from pests and predators in Walmara district of Oromia Region. Accordingly, the indigenous knowledge used by the beekeepers in the study areas are summarized in (Table 8).

Table 8: Major honeybee enemies and traditional preventive measures (N=115)

Pests and predators	**Respondent %**	**Indigenous preventive measures**
Ants	27.8	Place fresh ash under hive stands, clean apiary, frequent inspection, hang hives using wires, pour used engine oil around the hive stands, plastering hive stand with plastic, laying newly white eucalyptus leaves, destroying ants nests and fixing of smooth iron sheet on hive stands
Wax moth	18.3	Clean apiary,strengthen the colony, frequent inspection, removing unoccupied suppers and combs, plastering cracks using cow dung and use of new foundation sheets
Small hive beetles	10.4	Clean apiary,remove old combs,killing and seasonal colony management
Spiders	14.8	Clean apiary, removal of spider webs, strengthen the colony and frequent inspection
Lizards	5.2	Clean apiary and kill and strengthen the colony
Bee-eater birds	9.6	Putting cloths, festal similar symbol of man, removing branches of tree near apiary site,destroying the birds' nest, killing using 'Wonchif' and local traps and suspending dead birds near the apiary site
Honey badger	7.8	Fixing smooth iron sheet on hive stands and trunks of a tree, fencing off apiaries with thorny plants, putting barriers, use dog to keep at the night
Death head hawks moth	4.3	Clean apiary, lighting torch during night time and smoking cow dung
Wasps	1.7	Clean apiary and remove nests of wasps

Perception of beekeepers towards honeybee diseases

According to this study 34.8% of the respondents have observed honeybee diseases in their colony and the rest 65.2% of the respondents did not observe honeybee diseases in their colonies. Some respondents did not have any idea about honeybee diseases,but they have observed dead honeybee around hive entrance,bees fail to fly ,crown on the ground in front of hive. A situation called "Mushen" caused by "Abrik" recurs during summer season, this is justify that might be due to Nosema disease. Shortage or lack of technical training and lack of experience on honeybee diseases are believed to explain the rather low frequencies reported. This research result in line with Tesege Belie (2009) reported in Burie Woreda beekeepers didn't have an idea about honeybee diseases and their causes.Apparently, 37.4% of the respondents have awareness about honeybee diseases transmission from infected colonies to healthy ones and 62.6% of the respondents do not have awareness about honeybee diseases tramsmission: some even believe that honeybees do not get affected by diseases. Informed respondents beekeepers know different honeybee disease transmission mechanisms such as unclean materials (41.7%), through comman feed (27.8%), beekeepers (20.9%) and robbing (9.6%) (Table 9). Majority of the respondents (84.8%) indicated that weak colonies are the most victims for honeybee diseases and pests (Table 9). Respondents also reported that very aggressive behavior honeybee colonies (60%) confers more defensive capability to diseases and pests attack compared with aggressive (24.3%) and docile colonies (15.7%), justify that aggressive behavior colonies might have more hygienic behaviors of dead broods which leads to disease agents and attacking abilities of pests. About 84.8% of the respondents believed that weak colonies get infected by honeybee diseases and attacked by pests than medium and strong colonies (Table 9). This might be show that beekeepers knowledge and experiences is limited on honeybee diseases and pests effect on honeybee colony.

Table 9: Frequency of reported occurrence of honeybee diseases

Description	**Response variables**	**Frequency**	**Percentage**
Presence of honeybee diseases in your colony	Yes	40	34.8
	No	75	65.2
Awarenessa of honeybee diseases	Yes	43	37.4
	No	72	62.6
Diseases transmission mechanisms	Equipments	48	41.7
	Comman feed	32	27.8
	Beekeepers	24	20.9
	Robbing	11	9.6
Colony status infected by diseases and pests attack	Weak	86	84.8
	Moderate	29	25.2
	Strong	-	-
Defensive behavior of honeybee colony against diseases and pests attack	Agressive	28	24.3
	Very aggressive	69	60
	Docile	18	15.7

Prevalence of honeybee diseases and parasitic mites

The laboratory investigation has made possible of identification and confirmation of the major honeybee parasites varroa mites (bee lice and tracheal mites), adult honeybee diseases (Nosema and Amoeba) and brood diseases (Chalk brood, Stone brood, American Foul brood and European Foul brood).

Prevalence of Varroa mites (*Varroa destructor*)

From the total of 80 honeybee colonies examined for infestation of Varroa mites, 66 (82.5%) were positive to Varroa mites in adult bees. The present result was found to be slightly higher than previous research finding of varroa mite prevalence by Desalegn Begna (2015) of 82% in Tigray Region. However, lower than report of Tsegaye (2015), who found 85.9% Varroa mites prevalence in the Eastern parts of the Amhara Region. Different African countries reported different result on varroa mite prevalence: 78.6% in Nigeria (Akinwande *et al*., 2013), 100% in South Africa (Strauss *et al*., 2013), 83% in Kenya (Muli *et al.*, 2014) and 92% in Tanzania (Mumbi *et al*., 2014). The overall prevalence of Varroa mites in highland, midland and lowland was 92.3%, 70.8% and 83.3%, respectively. However, the difference in prevalence of Varroa mites was not statistically significant across agro-ecologies (P>0.05).Regarding the hive type varroa mite prevalence was higher in intermediate (90.6%) than movable hive (79.2%) but the differences was not statistically significant (P>0.05) (Table 10).This current research result was in line with the findings of Alemu Tsegaye (2015) who reported 94.2%, 84.8% and 79.85% in movable frame hive, intermediate and traditional hives, respectively, in the eastern parts of the Amhara Region. In contrast Guesh Gudifey (2015) reported in Tigray Region that varroa mite prevalence was high in traditional hives than in movable frame hives (68% versus 58.8%).

Table 10. Prevalence of Varroa mites by study areas and hive types

Variables	Category	Total colony examined	Prevalence (%)	χ2	P-value
Agro-ecology	Highland	26	24(92.3)	4.0093	0.135
	Midland	24	17(70.8)		
	Lowland	30	25(83.3)		
Hive types	Modern	48	38(79.2)	2.438	0.118
	Transitional	32	29(90.6)		
Overall prevalence		80	66(82.5)		

Prevalence of Nosema disease

The current investigation revealed that the overall prevalence of Nosema disease was 48 (60%) during the study period (Table 11). The overall prevalence level of Nosema disease in highland, midland and lowland was 76.9%, 54.2% and 50%, respectively (Table 11). There was significant difference among these agro-ecologies ($p < 0.01$). This might be due to the effect of temperature and humidity that affect the spread of Nosema disease. The current finding is in agreement with the finding of Nega *et al*. (2019) who stated that an increase in humidity and rainfall limit honeybees to fly out for cleansing, which in turn enhances the spread of the disease among the members and autoinfection. The differences in prevalence between the hive types was statistically significant ($\chi 2$=27.22; P=0.000) and it was higher in modern hive 83.3% than transitional hive 25%. This result is in agreement with the study result of Desalegn Begna and Yosef Kebede, (2005) who stated *Nosema apis* disease was more prevalent in the moden beekeeping system 72.2% than in the traditional 41.3% and transitional 35.3% systems. This variation might be associated with the difference in the management practices like the placement of hive and changing of the frame. The overall prevalence of Nosema disease was 60% greater than the previous reports in Ethiopia Diagnosis made on 152 honeybee colony at Addis Ababa reported prevalence of 53.3% Desalegn and Yosef. (2005). In Ethiopia Nosema was also reported from different regions with varying prevalence ranges such as 58% in Oromia, 60% in Benishangul-Gumuz and 47% in Amhara regions Aster *et al*., (2010) and other countries such as Nigeria (64.29%) by Akinwande *et al*. (2013), Kenya 83.3% by Fazier *et al*. (2010) and Turkey 65.25% by Aydin *et al*. (2001).In the central highlands of Ethiopia, Nosema infection rate was reached up to 82 % Amsalu, (2012).

Table 11. Prevalence of Nosema by study areas and hive types

Variables	Category	Total colony examined	Prevalence (%)	χ2	P-value
Agro-ecology	Highland	26	20(76.9)	4.693	0.096
	Midland	24	13(54.2)		
	Lowland	30	15(50.0)		
Hive types	Modern	48	40(83.3)	27.22	0.000
	Transitional	32	8(25)		
Overall prevalence		80	48(60)		

Prevalence of Amoeba disease

In this study, eighty (80) honeybee colonies were assessed for the presence of amoeba disease. The overall prevalence of amoeba disease was found to be 83.8% (n=80). This finding is higher than diagnosis made on honeybees in field and laboratory at Addis Ababa reported a prevalence rate of 73% of amoeba prevalence. The result of the current study is lower than those of previous studies in different parts of the country such as the Oromia region with a prevalence rate of 88% and the Amhara region with a prevalence rate of 95% (Aster *et al*., 2010). This might be due to seasonal variation, as the amoeba infection is highly influenced by seasonal change since the current study was conducted around September and October where these were seasons of reduced rainfall. The idea was supported by FERA, (2013) explaining that bees are unable to retain the large accumulation of water in their bowels, resulting in diarrhea. Fermented stores also stimulate dysentery, as will acid-inverted sucrose. The result also indicated that amoeba (*M. mellificae*) disease was more common in highland 92.35% than the midland 83.3% and lowland agroecology appeared to be affected the least 76.7% (Table 12). The current result is in agreement with the finding of Begna and Kebede (2005) who reported the prevalence of amoeba disease in highland, midland and lowland was 85%, 52.3% and 50%, respectively. This may be due to differences in weather conditions of different geographical locations. No significantly difference (P>0.05) was observed among these agro-ecologies and hive types. The highest prevalence of amoeba disease was observed in the transitional hive 87.5% than modern hive 81.3%. The current result disagrees with the finding of Begna and Kebede (2005) who reported the prevalence of amoeba (*M. mellificae*) was high in the modern beekeeping system (88.9%) than in the traditional (61.9%) and transitional (47.1%) system. This may be due to differences in management practices, for instance changing old combs, handling of equipment and use of traditional control methods.

Table 12. Prevalence of Amoeba by study areas and hive types

Variables	Category	Total colony examined	Prevalence (%)	χ2	P-value
Agro-ecology	Highland	26	24(92.35)	2.508	0.285
	Midland	24	20(83.3)		
	Lowland	30	23(76.7)		
Hive types	Modern	48	39(81.3)	0.551	0.458
	Transitional	32	28(87.5)		
Overall prevalence		80	67(83.8)		

Prevalence of small hive beetle

According to the result obtained by the current study, the prevalence of small hive beetles was found to be 43.8%. This is in agreement with the study conducted on 427 bee colonies located in 16 districts of south and South West parts of the country, which revealed six districts and 43 bee colonies were positive to small hive beetles with the incidence ranging from 21% to 66% (Desalegn Begna and Amssalu Bezabh, 2006). In this study, 65.6% of the transitional hive was infested with small hive beetles than modern hive 29.2%. There is a significant difference between hive type (p< 0.01).For the prevalence of small hive beetles, there is also a significant difference regarding agroecology (p< 0.01). Lowland is found to be affected more 56.7% followed by midland 45.8% and highland 28.9% (Table 13). The causes of variation in prevalence among the studied districts may be attributed to different factors such as ecological variability, season, and management aspects.

Table 13. Prevalence of small hive beetles by study areas and hive types

Variables	**Category**	**Total colony examined**	**Prevalence (%)**	**χ2**	**P-value**
Agro-ecology	Highland	26	7(26.9)	5.0678	0.079
	Midland	24	11(45.8)		
	Lowland	30	17(56.7)		
Hive types	Modern	48	14(29.2)	10.3704	0.001
	Transitional	32	21(65.6)		
Overall prevalence		80	35(43.8)		

Prevalence of bee lice (*Braula coeca*)

The overall honey bee lice (*Braula coeca*) infestation was found to be 33.8% (n=80). The overall prevalence of bee lice observed in the current study was much greater than the previous reports in Wukro wereda by Adeday Gidey *et al*. (2012) with prevalence of 5.5% in adult honey bees. However, the current finding was also much lower than the report by Gemechu Gizachew *et al*. (2013), who found 42% lice prevalence in and around Holeta. On top of that, Donson (1999) reported bee lice with prevalence of 73%, 34% and 50% in the south-west region of England, west region of Wales, and central England, respectively. In a study done in Jordan (Al-Ghzawi *et al*., 2009), Bee lice, *Braula coeca* was detected from 64.3% of inspected apiaries and diagnosed in 45.4% of beehives. In South Africa, bee lice were detected in 92% of apiaries with highly prevalent in all seasons (Strauss, *et al*., 2013). The higher prevalence of bee lice 35.4% was observed in modern hives than in transitional hives 31.3% however, there was no statistically significant difference in prevalence of lice in bees kept in both hive types (P > 0.05) (Table 14). The result was in line with the report of Alemu Tsegaye (2015) who reported that bee lice prevalence was higher in movable frame hives (46.5%) than in intermediate hives (31.9%) and traditional (17%).Contrary to our result Gizachew Gemechu *et al*. (2013) have reported that the highest bee lice prevalence (48.5%) was observed in traditional hives at Holeta and Guesh Godifey (2015) reported 29% bee lice prevalence in traditional beehives. The investigation result also revealed that higher prevalence of bee lice in highland (53.8%) followed by midland 37.5% and lowland agro-ecology 13 with statistically significant difference (P=0.005).

Table 14. Prevalence of bee lice (*Braula coeca*) by study areas and hive types

Variables	Category	Total colony examined	Prevalence (%)	X^2	P-value
Agro-ecology	Highland	26	14(53.8)	10.439	0.005
	Midland	24	9(37.5)		
	Lowland	30	4(13.3)		
Hive types	Modern	48	17(35.4)	0.149	0.699
	Transitional	32	10(31.3)		
Overall prevalence		80	27(33.8)		

Eventhough the honeybee colonies diagnozized for the presence of American Foul brood, Europian Foul brood, Chalk brood disease, Stone brood disease and tracheal mite, however the diseases did not confirmed during the study period.

Conclusion and Recommendations

Beekeeping is important for securing food, poverty reduction, health, environmental protection and plant pollination. However, mainly because of pests and predators, shortage of bee forage, improper application of agrochemicals, high cost of modern hives and accessories, absconding and poor management the study areas in general and rural beekeeping households in particular have not been sufficiently benefited from the beekeeping sub-sector.

The study demonstrated that the occurrence and prevalence of different honeybee pests, predators and disease in all agro ecological zones of the study area. The major honeybee pests and predators detected in the study area were ants, wax moth, honey badger, bee-eater birds, small hive bettles, lizards, spiders, death head hawks moth and wasps. These pests and predators were identified as the major causes of colony absconding, dwindling and honey yield loss. In most cases ants, wax moth,honey badger and bee eater birds were the most destructive pests and predators for the honeybees.

Similarly, laboratory analysis of bee colony samples revealed that Varroa mites, bee lice, Nosema and Amoeba are prevalent in the study area. However, brood colonies are free from European and American foulbrood, Chalk brood, Stone brood and Tracheal mites. The study revealed that bees under the transitional type of hive are found to be more affected by the diseases and pests than modern hives. Regarding agroecologies, highland agroecology was the most affected by *Nosema apis*, while bees in lowland agroecology were found to suffer less from the disease.

Based on the above conclusion the following recommendation forwarded:

- Beekeepers should be made aware of technical knowledge and skills to better manage known honeybee pests and diseases.
- Beekeepers should maintain strong and healthy honeybee colonies via proper seasonal colony management practices enable the natural prevention of honeybee from diseases and pests.
- Good beekeeping practices such as avoiding use contaminated equipment, transfer of infected combs from infected hives were recommended to avoid horizontal and vertical transmission of different honeybee diseases from colony to other near colony.

- Further study on seasonal prevalance and economic importance of honeybee diseases and pests is needed.
- Indigenous knowledge of beekeepers in beekeeping should be scientifically examined

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Ethno-botanical investigation of traditional medicinal plants used to protect honey bee pests and predators in selected districts of East Shewa and West Arsi Zones of Oromia, Ethiopia

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Abstract

Ethiopia has rich flora with different plant species having use in the health care system based on local indigenous knowledge. In this study, plants of traditional medicinal use and indigenous knowledge associated to them in East Shewa and West Arsi were investigated. A total of 146 informants (age≥25) were selected to collect information on medicinal plant use from three sampled districts. Of these, 15 key informants were selected purposely based on recommendation by local elders and authorities. Ethno-botanical data were gathered using semi-structured questionnaires, field observations and group discussions with local traditional medicine practitioners. Data were analyzed using descriptive statistics. Moreover Jaccard similarity index, informant consensus factor, fidelity level, preference ranking and direct matrix ranking were computed. Ethno-medicinal use of 34 plant species distributed in 21 genera and 16 families was documented. Highest number of species (3) was under family Asteraceae and myrtaceae. Habit wise, 50% were Trees followed by shrubs (29.4%),herbs (14.7%) and climbers (5.8%). The most widely used ways of applying plant materials to protect honey bee pests and predators were cutting the leaves and smearing around hive stand and hive entrance (47.6%), smoking(5.7%), cover hives stand with (7.6), put on hives (2.85) and planting around hives(4.76). Ants, lizards, beetles and snakes had the highest ICF value (≥ 90). Therefore, biochemical profiles of plant species used to protect pests and predators with high ICF should be investigated for screening of the active ingredients.

Key words: Ethno-botany, Indigenous knowledge, Informant Consensus Factor, Honey bee

Introduction

Ethno-botany is the study of how people of a particular culture and religion make use of indigenous plants. It accounts for the study of the relationship between people and plants for their use as medicines, food, shelter, clothing, fuel, fodder and other household purposes (Samar *et al.,* 2015). The current account of medicinal plants used in Ethiopia shows that about 887 plant species are reported to be utilized in the traditional medicine (Ermias *et al*., 2013).

Due to ecological shifts and environmental perturbations, plant resources are dwindling at an alarming rate, suggesting the rapid loss of medicinal plants and their associated indigenous knowledge. Ethno-botanical studies are often significant in revealing locally important plant species especially for the discovery of new drugs (Wright, 2005). Despite the agro-ecological and cultural diversity of the country, the documentation of medicinal plants and associated indigenous knowledge appears incomplete (Vechiato, 1993; cited in Mesfin *et al*., 2005). There is not much study in western part of Ethiopia, and particularly no documented study is found from West Arsi and East Shewa zones of Oromia. This suggests that there is still a gap in our knowledge about Ethno-botanical data on medicinal plants from various parts of Ethiopia, although we have rich and diverse ethnolinguistic groups throughout the country (Engdasew*et al.,* 2015). According to Pankhurst (2001), detailed information on the medicinal plant could only be obtained when studies are taking place in the various areas of the country to include places where little or no botanical and Ethno-botanical explorations have been made. Among rural communities of East Shewa and West Arsi zone as would be the case elsewhere, traditional medication is believed to be an important health care system, which mainly involves the use of locally available medicinal plants. However, such knowledge and practices, and plant resources may be threatened due to anthropogenic and other natural factors. Thus, concerted Ethno-botanical research plays a vital role to draw information on plants and related indigenous knowledge for conservation and sustainable utilization. Honey bee, *Apis mellifera L*., is considered as an essential organism to the agricultural sector due to its role in pollination and alleviation of poverty in rural areas. Many pests and predators attack honey bee colonies causing severe damages and economic losses. These pests and predators include Ants, lizards, wax moths, small hive beetles, Snakes and parasitic flies. Using chemical methods to control these pests and Predators causes some negative effects on honey bees and contaminates their products, while using available indigenous knowledge and biological control agents is promising and has no serious hazards (Hossam F and Abou-Shaara, 2019). Decline of honeybee populations is of great concern around the world because of pests, predators and diseases (Panuwan, 2016). Traditional medicinal plants are used to protect Predators, insects and fungi (Jillian, 2015; Cobiac L, et al., 2006; Roy J, 2004). It is well know that in East Shewa and West Arsi, many pests and predators of honey bee colonies cause decreases in honey bee products and productivity. This study is, therefore, designed to collect, identify and document traditional medicinal plants that are used by local people for the protection of honey bees from pests and predators in the study area.

Materials and methods

Survey and Selection of Study Sites

The study was conducted in selected districts of West Arsi and East Shewa zone. Based on beekeeping potential of the area, three districts, Kofale, Wando Genet and Dodola were selected from West Arsi zone. From East shewa zone, Adami TuluJido Kombolcha district was selected. The districts were selected based on the availability of traditional medicine practitioners, traditional medicine use history, and altitudinal variation between districts.

Ethno-botanical Data Collection

Prior to Ethno-botanical data collection, respondents were selected from the selected districts. Totally, 146 respondents (aged ≥25) and 5 key informants (traditional healers) were selected from each district. The key informants were selected by purposive sampling based on the information gathered from the local people while other respondents were randomly selected. Data collection was undertaken using interviews, group discussions and field observations being guided by key informants. Voucher specimens were collected, pressed, and dried for identification. For some species, preliminary identification was done in the field using keys and illustrations. In addition, further identification of all specimens was done by comparison with authentic specimens, illustrations and taxonomic keys from Flora of Ethiopia and Eritrea.

Data collected during the study include the local names of the plants they use to treat pests and predators, pests and predators treated**, p**art(s) of plants used**, m**ethods of gathering, methods of preparation of remedies, **r**oute of administration of remedies**,** application of the remedies**, d**osage and side effects of the treatment**, u**se of the plants other than medicine**, t**ypes of threat and conservation problems and plant habit and habitat.

Data Analysis

The collected data were analyzed by using descriptive statistical, Jaccard's similarity index (JI), Informant consensus factor (ICF), Fidelity level (FL), preference ranking and direct matrix ranking. Percentage and frequency were used to summarize ethno-botanical data.

Jaccard's similarity index (JI)

Jaccard's similarity index was calculated to compare similarity between districts of different altitude with regard to knowledge on medicinal plants. . For this, presence of a given plant species and its utility as medicine or its absence/not considered as medicine are used as data sets.

$$JI = \frac{c}{a + b + c}$$

Where, JI is the Jaccard similarity index, 'c' is the number of species shared by the study sites, 'a' is the number of species in study site A only and, 'b' is the number of species in study site B only. The JI values range between 0 and 1, where a value of 1 indicates complete similarity.

Informant consensus factor (ICF)

Informant consensus factor was calculated for categories of ailments to identify the agreements of the informants on the reported cures using the formula used by (Rodrigo *et al*., 2005). ICF was calculated as follows: number of use citations for each ailment (nur) minus the number of species used (nt) for that ailment, divided by the number of use citations for each ailment minus one.

$$\mathrm{ICF} = \frac{n_{ur} - n_t}{n_{ur} - 1}$$

Fidelity level (FL)

The fidelity level, the percentage of informants claiming the use of a certain plant for the same major purpose, is calculated for the most frequently reported ailments using the following equation (Teklehaymanot,2007).

$$\mathrm{FL}(\%) = \frac{NP}{N} x100$$

Where, Np is the number of informants that claim the use of a plant species to treat a particular pests and predators, and N is the number of informants that use the plants as a medicine to treat any given pests and predators.

Preference ranking

Preference ranking is used to compare the most effective medicinal plants used by the community to treat the particular disease, pests and predators. Preference ranking was conducted following Martin (1995) and Cotton (1996) for six most important medicinal plants. For this, five informants were selected from each district to identify the best preferred medicinal plant species for treatment of the honey bee pests and predators. Each informant was provided with six medicinal plant used being paper tagged. Then they were asked to assign the highest value (6) for the most preferred species against the illness and the lowest value (1) for the least preferred plant and in accordance of their order for the remaining ones.

Direct Matrix Ranking

Direct matrix ranking exercise was done following Martin (1995) and Cotton (1996) to compare multipurpose use of a given species and to relate this to the extent of its utilization versus its dominance. Based on information gathered from informants, multipurpose tree species were selected out of the total medicinal plants and use diversities of these plants were listed for the selected key informants to assign use value to each species. Each key informant was asked to assign use values (5=best, 4=very good, 3=good, 2=less used, 1= least used, and 0=not used). Then, uses values given by each key informant for the selected purposes of each medicinal plant species were summed up and ranked.

Results and Discussions

Socio-demographic of the respondents

Based on the degree of responsibilities to care for honey bee health and assumed accumulation of traditional knowledge of the community, respondents were categorized into four age classes. Age distribution of the informants showed that the majority (61.6%) are between 45 and 60 years of age followed by 31-45 (21.9%), 18-30 (6.2%) and >60 (10.3%). Marriage wise, 95.9% of the respondents were married, whereas 4.2% of them were unmarried. The majority (44.6%) of respondents had no formal education, whereas 32.2, 6.2 and 1.4% of them had elementary school, high school and college level educations, respectively. Majority of the respondents 98.6% were males and only 1.4% were females. From the participants 69.9% were Muslims and 29.4% Christians.

Ethno-medicinal plant species used by people of the study areas

From the study region, a total of 34 kinds of medicinal plants used to combat various honey bee predators and pests were collected. These plants are from 16 families and 21 genera. Out of these plants, 17 species (50%) were utilized to control ants, spiders, snakes, lizards, and beetles, whereas 14 species (41.1%) and 3 species (8.82%) were noted to treat only ants and honey badger, respectively. This implies that local communities in the districts of Kofale, Adami Tulu Jido Kombolcha, Wando, and Dodola use traditional medicine derived from plants. Asteraceae and Myrtaceae were two families with three species each. Other families each have a single or double species (Table 1).

When comparing the number of reported medicinal plant species throughout the investigated districts, Jaccard's Similarity Index (JI) was computed to determine how similar they were. In accordance with the findings, respondents from Kofale (2450 masl) and Dodola (1950 masl) reported the same two species

(Hagenica *abyssinica* and *Syzygiumguineense*) with a JI value of 0.25. The same two species, with a JI value of 0.25, were reported by respondents from Adami Tulu (1558 masl) and Wando (1750 masl). The discrepancy between these two districts may be the result of environmental variations. For instance, the altitudes of Kofale (2450 masl) and Dodola (1950 masl) differ from one another, and the way that information about medicinal plants was shared between residents of the two districts varied. In other words, there may not be much environmental overlap between these two districts, and communication concerning medicinal plants between them has been scarce.

The majority of medicinal plant species (35.2%) were harvested from the wild, followed by home gardens (26.4%), highways (14.7%), agricultural fields (11.7%), and live fences (11.7%). The fact that so many different species of medicinal plants were found growing wild suggests that conserving medicinal plants in the research region should be possible. Various districts of the study area reported using specific plants more frequently than others as medicinal plants to ward against various honey bee pests and predators. *Syzygium guineense*, according to 55% of respondents, is used to defend honeybee colonies against ants, spiders, lizards, and snakes. *Eucalyptus globules*, *Hagenia abyssinica* and *Ruta chalepensis* are employed against ants by 50% of respondents. Similarly, 53% of respondents said *Acacia bussei L* and *Croton macrostachyus* help to keep out lizards, snakes, and ants. On the other side, according to 40% of respondents, *Vernonia amygdalina* is used to prevent ants, while 29% of respondents stated *Ziziphus mucronata* protects honeybee colonies from ants, spiders, and beetles, and 30% of respondents said *Dovyalis abyssinica* avoids honey badgers.

Table 1.Some of the medicinal plants cited most by informants

Botanical Name of Medicinal Plants	**Family name**	**No. of Informants**	**Percentage**	**Districts**
Syzygiumguineense	Myrtaceae	55	55.0	Kofale
Acacia Bussei	Fabaceae	53	53.0	Kofale
*Eucalyptus globulus*Labill	Myrtaceae	50	50.0	kofale
Enseteventricosum(Welw)	Musaceae	45	45.0	kofale
Hageniaabyssinica	Rosaceae	42	42.0	Dodola
Eucalyptus camaldulensis	Myrtaceae	45	40.0	Dodola
Vernoniaamygdalina Dell	Asteraceae	40	40.0	Dodola
Oleaeuropia L.	Oleaceae	37	37.0	Dodola
Cpsicum annuum L.	Solanaceae	34	34.0	Dodola
Vernoniaauriculifera	Asteraceae	31	31.0	Dodola
Ziziphus mucronata	Rhamnaceae	29	29.0	Dodola
Dovyalis abyssinica(A.Rich)Warb	salicaceae	30	30.0	Dodola
Schinus molle L.	Anacardiaceae	50	50	wando
Ruta chalepensis L.	Rutaceae	45	45	Wando
*Aloe macrocarpa*Tod	Aloaceae	35	35.0	A/Tulu
Croton macrostachyus L.	Euphorbiaceae	30	30.0	A/Tulu
Justice schimperiana (Hochst.	Acanthaceae	25	25.0	A/Tulu

Among the 34 therapeutic plants discovered, trees make up the majority, followed by shrubs, herbs, and climbers (Figure 2). This demonstrates that in the study region, trees and shrubs are the most commonly used medicinal plants. This could be as a result of these trees and shrubs being more prevalent in the research areas than herbs and climbers. Alemayehu (2015), who did research on the medicinal plants of the Ada'a District, east Shoa zone, also reported the relatively high number of trees and shrubs used for medicinal purposes.

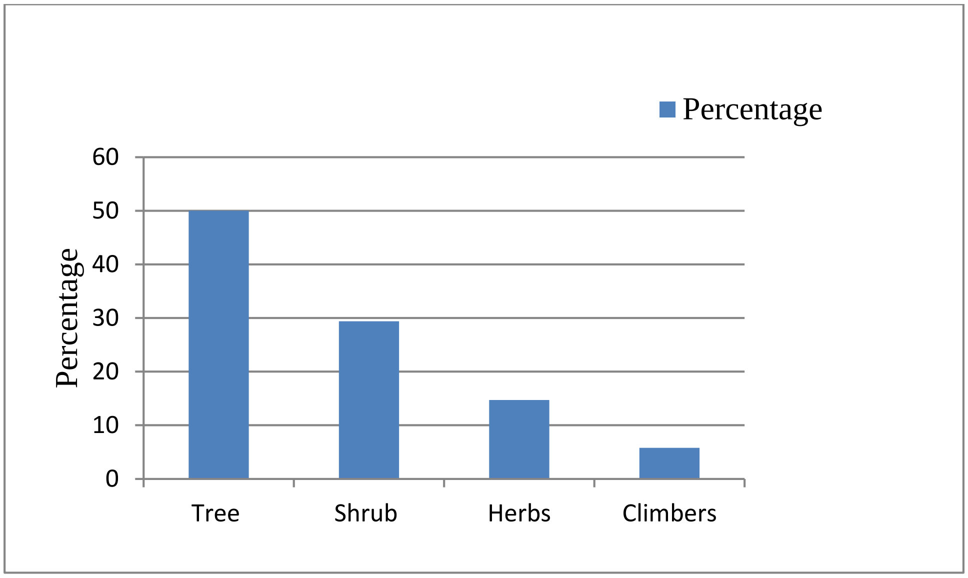

Figure 2: Percentage share of different medicinal plants

Plant Part s Used to Protects Honey bee pests and Predators, Methods of Preparation and Conditions

Despite reports of many plant parts, the leaf, followed by the stem, the whole portion, the seed, and the root, were the most frequently used plant components for remedy concoctions (Table 2). There were additional reports of other plant components, such as berries and bulbs (Table 2). This outcome is in line with some earlier research works carried out across the nation (Mirutse, 1999; Endalew, 2007; Jarrso, 2016, Mekonen 2013; Mulugeta, 2014).

Table 2. Plant parts used for traditional medicine preparations.

Plant parts	Total responses	% of total
Leaf	75	51.3
Stem	25	17.12
Whole parts	20	13.6
Root	11	7.53
Seed	6	4.10
Fruit	5	3.42
Bulb	4	2.73
Total	**146**	**100**

Ways of Applying Plant Remedies

The prepared traditional medicines are applied in a number of methods among which cutting the leaves and smear under hive stand and hive entrance (47.6%), put the leaves in front of hives (19.04%), cover hive stands with (7.60%), smoking (5.7%), Cleaning and smearing by fresh leaves all parts of the hives(6.6%),cover hive stands (7.6), planting around hives as fence (3.80%) and put on the hives

(2.85%) were mentioned. In this study, cutting the leaves and smearing the hive stands and hive entrance accounts for the largest percentage (Table.3).

Table 3. Ways of applying the plant remedies used to protect honey bee pests and predators.

No.	Ways of Applying plant materials	Total Responses	Percentage (%)
1	Cutting the leaves & smear hive stand &hive entrance	50	47.6
2	Put the leaves in front of hives	20	19.04
3	Cutting the stem & put in the hive	2	1.90
4	Planting around hives	5	4.76
5	Put on	3	2.85
6	Smoking	6	5.7
7	Planting around hives as fence	4	3.8
8	Cleaning by fresh leaves smear by fresh leaves parts of	7	6.6
9	Cover hive stands	8	7.6
	Total	**105**	**100**

Informant Consensus Factor (ICF) and Fidelity Level

The pests and predators of the study area have been grouped into different categories based on the site of incidence of the pests and predators, condition of the pests and predators as well as treatment resemblance of the disease by the local people. Analysis of ICF showed that values ranged from 0.85 to 0.98 for the pests and predators categories (Table.4). Of the pests and predators categories, ants, beetles and honey badger had the highest ICF value suggesting the common occurrence of these problems and agreement of the people on their remedy. It has been shown that medicinal plants that are effective in repeling certain pests and predators and well known by the community members have higher ICF values.

Table.4. Informant Consensus Factor (ICF)

Disease categories	Nt	Nur	ICF
Ants	8	98	0.92
Lizards	11	9005	0.89
Beetles	2	20	0.94
Spiders	9	90	0.91
Snakes	10	65	0.85
Honey badger	2	16	0.93

Fidelity level (FL) is an index which shows the specificity of a given plant to effectively treat a particular pests and predators of honey bees. Fidelity level was then calculated for some commonly used medicinal plants to treat pests and predators/ailments. Result showed that *Schinus molle L. Eucalyptus globules*, *Ruta chalepensis Croton macrostachyus* had the highest FL followed by *Acacia bussei*, *Hagenia abyssinica, Olea europea Vernonia amygdalina& Aloe macrocarpa* (Table 5). The medicinal plants that are widely used by the local people to treat one or very few cases have higher FL values than those that are less popular (Tilahun and Mirutse 2007; Mulugeta, 2014). High FL could also be an indication of efficiency of the reported plant to treat specific pests and predators.

Table 5. Fidelity index of some medicinal plants

Botanical Name of Medicinal Plants	Examples of Pests treated	Np	N	FL	FL%
Schinus molle L. Eucalyptus globulus Croton macrostachyus	Ants	46	50	0.92	92
Acacia bussei, *Hagenia abyssinica,olea europea*	Lizards	41	45	0.91	91
Syzigium guineense	Spiders	40	45	0.88	88
Vernonia amygdalina& Aloe macrocarpa	Snakes	35	41	0.62	85
Anoonuu &Hagenia abyssinica	Beetles	22	35	0.81	81
Dovyalis abyssinica	Honey badger	20	30	0.66	66

Where, *Np is the number of informants that claim the use of a plant species to treat a particular pests and predators, and N is the number of informants that use the plants as a medicine to treat any given disease.*

Preference Ranking and Direct Matrix Ranking

When there are different species prescribed for the same health problem, people show preference of one over the other. Preference ranking of six medicinal plants that were reported for protecting ants was conducted after selecting ten key informants. The informants were asked to compare the given medicinal plants based on their efficacy and to give the highest number (6) for the medicinal plant which they think is most effective in protecting ants and the lowest number (1) for the least effective plant in ants. *Ruta chalepensis* scored 46 and ranked first indicating that it is the most effective in treating pests and predators followed by *Eucalyptus globulus* and the least effective was *Syzigium guineense* (Table 6)

Table 6. Preference ranking of medicinal plants used for protecting ants

List of medicinal Plants	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Total	Rank
Ruta chalepensis	4	6	5	2	4	5	4	6	4	6	46	1st
Eucalyptus globulus	6	5	4	5	5	4	4	3	4	4	44	2nd
Croton macrostachyus	5	4	6	3	7	2	6	3	3	1	40	3rd
Hagenia abyssinica	6	2	2	3	5	4	6	3	5	1	37	4th
Eucalyptus camudelencis	3	3	3	8	2	3	1	2	3	3	31	5th
Syzigium guineense	2	2	4	2	1	4	4	2	1	3	25	6th

Key: - R= informant

In the study area, many medicinal plants were found to have different uses other than medicinal purpose. The major uses of plants reported were for firewood, charcoal making, construction, fencing, food, forage, furniture and medicine. The direct matrix ranking result showed that *Acacia bussei* ranked first followed by *Eucalyptus globulus* (Table 7).This result indicates that *Eucalyptus globulus* and *Eucalyp camaldulensis* appear to have more demand than the others as they are used for more diverse purposes. The direct matrix ranking result also shows that the local people harvest the 10 multipurpose plant species mainly for firewood followed by charcoal, fencing, medicinal purpose, furniture, construction, forage and food (Table 7). The ranking was done based on the use criteria rated as 5 = best; 4 = Very good; 3 = good; 2 = less used; 1 = least used and 0 = no value

Table 7. Direct Matrix ranking for the ten Selected Multipurpose Plant Species.

Plant species	Use categories								Total	Rank
	Charcoal	Constriction	Fencing	Fire wood	Food	Forage	Furniture	Medicine		
Acacia bussei	5	4	4	4	2	4	2	4	30	**1st**
Eucalyptus globulus	3	5	4	5	2	3	3	3	28	**2nd**
Eucalypcamaldulensis	5	4	4	3	1	0	5	3	25	**3rd**
syzygiumguineense	3	4	2	2	2	1	5	4	23	**4th**
Croton macrostachyus	4	4	3	3	0	0	3	4	21	**5th**
Vernoniaamygdalina	2	2	2	4	0	4	1	4	19	**6th**
Dovyalisabyssinica	1	0	5	3	1	2	1	3	16	**8th**
Enseteventricosum	2	3	3	4	0	5	2	2	21	**7th**
Schinus molle	0	0	1	0	4	5	0	5	15	**9th**
Oleaeuropea	0	5	2	3	0	5	1	5	21	**7th**

Threats to medicinal plants Rural people need plants for their livelihood in different aspects. In this study several factors both human and natural were found to contribute to the threats that affect survival of medicinal plants species in the study area. From the interview with informants, various factors were recorded as the main threats to medicinal plants in all Districts of study area. Agricultural encroachment, firewood collection, charcoal production, plant use for house and fence construction, overgrazing and urbanization were reported to be factors for the dwindling of natural vegetation in general and medicinal plants in particular. As a result, according to the respondents, the accessibility of medicinal plants has become less when compared to the previous times.

Transfer of knowledge on the use of medicinal plants

Traditional healers also keep their knowledge on medicinal plants for the sake of securing means of income and a cultural belief that telling information may make plants ineffective to cure the pests and predators/ailments. Similar findings were reported elsewhere (Abebe, 2017; Fassil, 2001; Mirutse and Gobena, 2003). However, it was recognized that Ethno-botanical knowledge on uses of some medicinal plants is transmitted orally to one or few family members to use in secrecy. They disclose their knowledge on medicinal plants at old age by the time when they most probably die before teaching the details of medicinal plants or when they are too old to walk to the field to show the plants in their habitats

Conclusions and Recommendations

This study was conducted in Adami Tulu Jido Kombolcha, Dodola, Kofale,Wando and Dugda Districts, with the objective of documenting ethno medicinal plants and indigenous knowledge on their use for medicine. 146 respondents have participated in this study as respondents. Data on medicinal plants use were collected through semi-structured interviews, field observation, and group discussion and guided field walk. Totally 34 medicinal plant species used to protect honey bee pest and predators were documented. The majority of medicinal plant species (35.2%) were harvested from the wild, home gardens (26.4%), highways (14.7%), agricultural fields (11.7%), and live fences (11.7%). *Syzygium* *guineense*, according to 55% of respondents, is used to defend honeybee colonies against ants, spiders, lizards, and snakes. *Eucalyptus globules*, *Hagenia abyssinica* and *Ruta chalepensis* are used against ants by 50% of respondents. Similarly, 53% of respondents said *Acacia bussei L* and *Croton macrostachyus* help to keep out lizards, snakes, and ants.

The prepared traditional medicines are applied in a number of methods, among which cutting the leaves and smearing under hive stand and hive entrance (47.6%) and putting the leaves in front of hives (19.04%) are the major ones. The major threats to medicinal plants and the associated knowledge in the study area are firewood collection, charcoal production, agricultural expansion, uses of plants for construction and using plants for fencing and furniture.

Based on the finding of the study, the following recommendations are forwarded:1) In order to conserve medicinal plants and preserve indigenous knowledge, local people should be aware of cultivating medicinal plants in their home gardens mixing with crops and as live fences, 2) Encourage the local herbal medicine practitioners to enhance the use of traditional medicine through licensing and other incentives, 3) Attention should be given to standardization of measurement and hygiene of the medicines made from plants by training both the healers and other members of the local community, 4) Biochemical profiles of plant species used for diseases categories of high ICF should be investigated for screening of the active ingridients.

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Performance evaluation of herbaceous bee forages for beekeeping development at Adami Tulu Agricultural Research Center and Negele Arsi district

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Abstract

Availability of adequate perennial and annual sources of nectar and pollen is the most limiting factor in the survival, abundance and distribution of honeybees. The study was conducted to evaluate and characterize the best performing bee forages from ten plant species with a view of selecting for honey production for mid and low land agro-ecologies. The planting materials were Nigella sativa, Coriandrum sativum, Dolchus lablab, Brassica carinata, Ocimum sanctum, Lathyrus sativus phaseolus vulgaris, Glycine max and Helianthus annus. The species were evaluated and characterized based on pollen yield, germination rate, number of flower heads per plant, time to set flower, foraging intensity of honeybees and flowering length. Accordingly, Helianthus annus, Brassica carinata, Ocimum sanctum, Coriander sativum and Glycine max were found to be good at Adami tulu and Negele Arsi districts. Mean number of flower heads per 1m^2 for all studied plant species was different. Helianthus annus and Brassica carinata had higher pollen yield at each study site. Honey Bees foraging intensity was higher for Helianthus annus. . From this study, it is concluded that Helianthus annus, Coriander sativum, Brassica carinata, Ocimum sanctum, Nigella sativa, Glycine max, lathyrus satives and Dolchus lablab showed better performance at mid and lowland agro ecology, however, carrying capacity and honey production potential of the selected plants should be investigated in different agro ecology.

Keywords: Bee Forage, Herbs, Flowering Period, Pollen Yield and Foraging Intensity

Introduction

Apiculture is a floral based industry and bees wholly depend on plants for their food requirements. From 250,000 flowering plants in the world, about 40,000 plant species are important for honeybees as a food source and world honey (Crane, 1990). Bee colony performance as well as production of honey, wax and other hive products depends on bee forage plants from which honey bees obtain nectar and pollen as main food source. Among the many bee plants some of plant species supply both nectar and pollen abundantly and others provide nectar or pollen only (Shubharani *et al*., 2004). The diversity of flowering plants and their flowering duration differ from place to place depending on variation in topography, climate and other cultural and farming practices (Alemtsehay, 2011). The extensive knowledge on type of plants, abundance, density and quality of bee flora resources enabled beekeepers to utilize the resources efficiently at the maximum level. This would help to harvest a good yield of honey and other honeybee products in addition to effective pollination for better crop yields.

Thus, the success of beekeeping primarily depends on availability and abundance of potential honeybee flora (Baptist and Punchihewa, 1989). Currently the scarcity of bee flora is a major limiting factor in development of beekeeping in most parts of the country. The central rift valley of Oromia is frequently affected by the recurrent droughts and deforestation and hence honey bees are facing serious food shortage during the dry period leading to colony absconding and loss of honey yield. As a result of the scarcity of bee forage, demand for dearth period bee flora is currently increasing from time to time from different stakeholders. Therefore to respond to the demand, it is necessary to adapt, evaluate and Characterize bee plants those adapt to the short and erratic rainfall conditions of the area, and hence improve the food base of the local honey bee colonies thus contributing significantly to honey production. Herbaceous plants are the major honey source plants in the central lowlands of Ethiopia because majority of the forest land is changed to agricultural land and resettlement. Hence those honey bee forages which have good merit, can be promoted by evaluating their potentiality for honey production in semi-arid rift valley of Oromia where the scarcity of bee forages is the major constraint. Therefore, the aim of this study was to evaluate and characterize performances of selected herbaceous honeybee forages.

Materials and Methods

Description of the study area

The study was conducted at Adami Tulu Agricultural Research Center (ATARC) and Negele Arsi districts under rain fed conditions. Adami Tulu Agricultural Research Center is located 167 km south of Addis Ababa at an altitude of 1650 meter above sea level (m.a.s.l) in mid rift valley. The agro-ecological zone of the area is semi-arid and sub-humid with acacia woodland vegetation type. The mean annual rainfall is 760mm. The mean minimum and maximum temperatures are 12.6 and 27^0c, respectively (ATARC, 1998). On the other hand Negele Arsi district is located between 7.15°-7.75°N and 38.35°-38.95°E. The annual temperature varies from 10-25°C with annual rainfall between 500- 1000 mm. The altitude ranges from 1500-3000 m.a.s.l. (Lowland less than 1600 m with semi-arid climate, midland 1600-2200 m with mild climate and highland greater than 2200 m with cold climate).

Seeds Collection

For this study seeds of *Nigella sativa L, Vigna unguiculata Coriandrum sativum, Dolchus lablab, Brassica carinata, Ocimum sanctum, Lathyrus sativus Phaseolus vulgaris, Glycine max* and *Helianthus annus* were collected .The plant species were selected on the basis of agro-ecology. Apart from agro-ecological adaption of the plants, preliminary field observation and information on parametrs such as similarity of their growth habit (herbaceous) and ease of propagation from seeds were obtained from beekeepers and literature to judge their importance as bee forages. Seeds were collected from each plant species by selecting mature fruits. Immediately after collection, seeds were packed in perforated polyethylene bags and allowed to dry for one day to half weeks at room temperature. The packages were maintained at room temperature until the day of sowing.

Experimental plot preparation

To evaluate the performance of the selected plant materials, seed beds were prepared by digging the ground and smoothing the field. Seeds were then taken out of the packages and planted on prepared seed beds by covering with a thin layer of the same soil of the seed bed. The plot size was 3mx3m arranged in randomized block design with four replications. To keep proper spacing and avoid nutrient competition, spacing used between the plants and rows were 20 and 30 cm, respectively. The necessary agronomic practices (namely, weeding pest control, etc.) were carried out. No fertilizer was applied to keep its natural growing state. The planting was done under rain fed conditions.

Then, data on days to germinate and average flower opening time was recorded. At 50% flowering, the number of flower heads was counted for each species by taking 1m^2 plot area as well as foraging intensity of honeybees on flowers was counted starting from 6: 00 a.m. to 6: 00 p.m. for ten minutes at every 2 hour interval. Also pollen yield of each species was determined by collecting 50 matured flower heads having similar age and was kept for certain days to dry. For removal from the flower, pollen was shaken on a paper tray and weighed using a sensitive weighing balance. Moreover times from blooming to shedding and shedding were also recorded.

Statistical Analysis

The collected data were statistically analyzed by GenStat and descriptive statistics using Statistical Package for the Social Sciences (SPSS) version 20.

Results and Discussions

In this study plant species, *Brassica carinata* , *Coriandrum sativum*, *Vigna unguiculata*, *Dolchus lablab*, *Glycine max*, *Helianthus annus*, *Lathyrus sativus*, *Nigella sativa* L and *Ocimum sanctum* were planted in two districts (Adami Tulu and Nagele Arsi) and mean values for the investigated traits were indicated in Tables 1 and 2.

Germination date

Values for germination dates (GD) of the plant species are shown in Figure 2 and 3. There were different germination dates among different experimental sites between the same plant species. *Brassica carinata*, *Dolchus lablab*, *Helianthus annus*, *Lathyrus sativus* and *Ocimum sanctum* had the shortest germination date at Adami Tulu Research station.

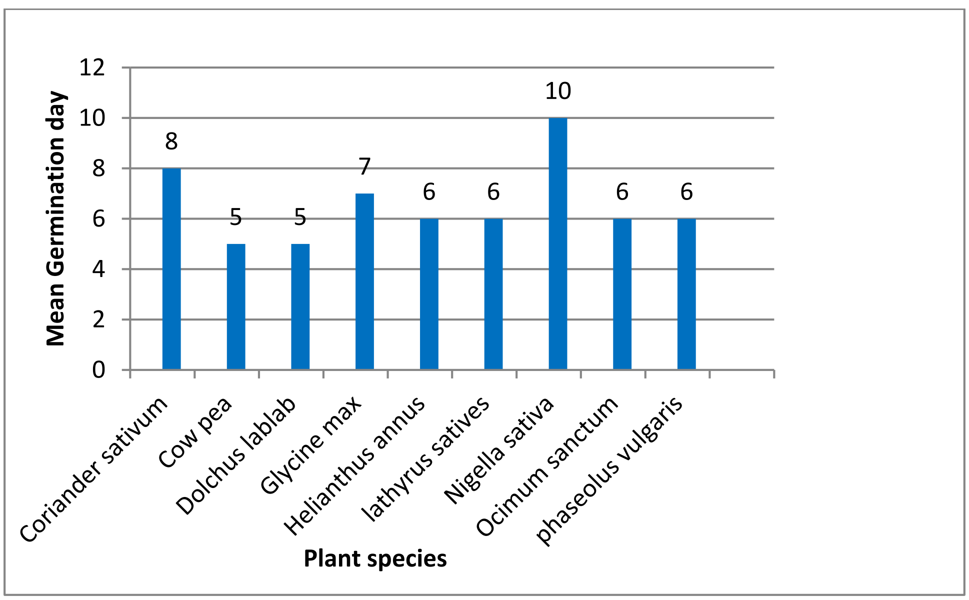
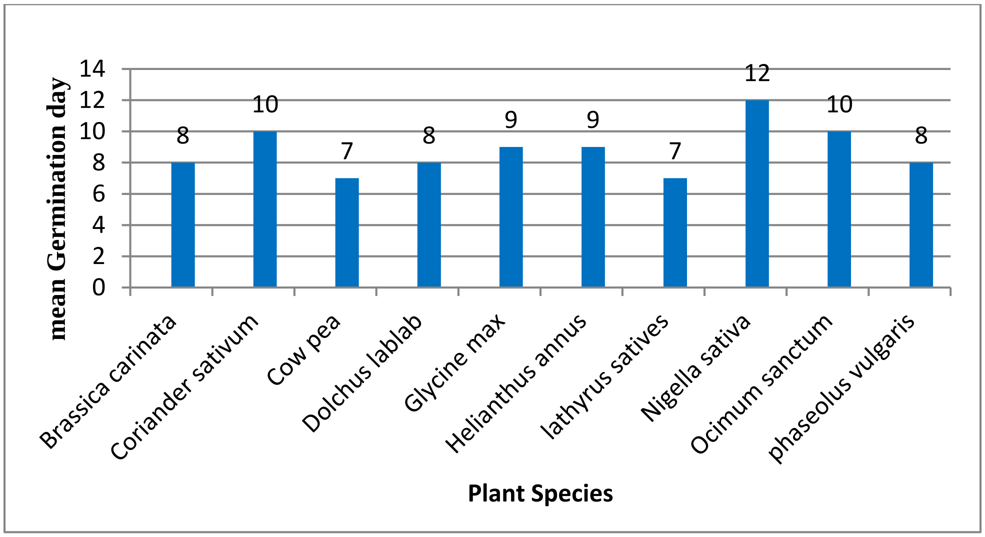


Figure 1. Mean germination days for bee forages at AdamiTulu Agricultural Research Center

Most of the plants had a long germination date at Negele Arsi (Ashoka) as compared to Adami Tulu which might be related to low temperature in the later site (Figure 2). The longer duration of germination is due to the variation of the temperature, soil condition and germination behavior of the plants (Girma C, 2014). This is in agreement with (Rizzardi, 2009) who reported that in the life cycles of plants, germination and emergence of plants are the two most important factors that determine the efficient use of the nutrients and water resources available to plants. *Helianthus annus*, *Lathyrus sativus*, *Brassica carinata*, *Ocium sanctum* and *Dolchus lablab* started Seed germination 5-6 days at Adami Tulu. In contrast, *Brassica carinata*, *Helianthus annus*, *Negella sativa* began to germinate after 8, 7 and 12 days, respectively at Negele Arsi (Figure 2.)



.Figure 2. Mean germination days of bee forages at Negelle Arsi

Time required to set to flower

Mean time taken to set flowers was 45 days for *Lathyrus sativus. T*his is the shortest as compared to that of the other plants. Flower shedding occurred after 18 and 25 days at Adami Tulu and Ashoka, respectively. Time taken to set flower was 78 days and flower shedding occurred after 28 days at Adami Tulu, Ninety days flower shedding occurred for *Hellianthus annus* after 35 days at Ashoka (Table 1 and 2). Mean time taken to set flower for *Brassica carinata* was 46 and 68 days at Adami Tulu and Ashoka sites, respectively. These shows that mean times taken to set flower at each agro ecology were statistically different ($P<0.05$). The mean time required to set flowers was different among plant species because the plants are of different species.

Number of flower heads per 1m^2 and their flowering length

The number of flower heads per 1m^2 for *Ocimum sanctum* and *Dolchus lablab* were 9721.4 and 8118.2, respectively at dami Tulu (Table 1). The number of flower heads produced for *H. 0annus* per 1m^2 at Adami Tulu was 4294.6 compared to that of the *H. annus which* produced at Asoka site produced the mean numbers of flower heads per 1m^2 were similar for *H.annus* at each study site. Mean number of flower heads per m^2 for *Brassica carinata* , *Coriandrum sativum* and *Nigella Sativa were* 7762.2, 5244.2 and 1237.4, respectively at Ashoka site (Table 1and 2). More branching produces more flower heads per plant. John *et, al.* (2014) showed that the more vegetative growth the plant attained, the more flowers and seeds it develops, and also plants that grow longer vegetative before flowering are typically bigger and able to support more reproductive growth. Mean time taken from the start of blooming to shedding was similar for *Brassica carinata* , *Coriandrum sativum Dolchus lablab, Glycine max, Nigella sativa and Ocimum sanctaum* with the range of 30 to 47 days at Adami Tulu (Table 1). Lower blooming period were observed for *Lathyrus sativus* and *Phaseolus vulgaris* with values of 18 days for each of them (Table 1). *Brassica carinata* , *Coriandrum sativum, Dolchus lablab Glycine max, Nigella sativa, Helianthus annus and Ocimum sanctaum* took long days from flower opening until shedding at Ashoka site(Table 2). These occur due to different factors such as growing temperature; photoperiod (Evans 1957). Moreover availability of moisture in the soil also increases the duration of flowering. Bee forage plants which take long time from blooming to shedding are very important for honey production where as those that have short flower shedding time may be only used for honey bee colony buildup.


1. Ocimum sanctum *2.Helianthus annus*



3. Nigella sativa *4. Coriandrum sativum* *5.Brassica carinata*

Figure 3. *Performance of planted plants*

Pollen Yield

Pollen yields, expressed as yields from 50 flower heads in grams, were higher for *Helianthus annus* and *Brassica carinata* compared to other plant species at Adami Tulu and Ashoka site (Table 1and 2). Pollen feeding is essential for the survival of honey bee colonies. To collect nectar and pollen honeybees, have to learn and remember not only the color and shape of flowers that contain nectar and pollen, but also how to get to them (Menzel et al., 1996; Wehner, 2003; Collett *et al.,* 2003).

Table 1. Mean time taken to set flower (MTSF), total number of flower head per 1m^2 (TNFP$^)$, pollen yields (PY) and time taken from blooming to shedding (TBSH) of the plant species at ATARC

Plant species	**PY+ SE**	**MTSF+ SE**	**FIB+ SE**	**TNFPM^2+ SE**	**TBS+ SE**
Brassica carinata	0.76±0.6a	46±5	32.4±2.46	7762.2±2219.3	47±0.0
Coriandrum sativum	0.25±0.6c	53 ± 0.0	8±1.26	5244.2±807.58	31±0.0
Vigna unguiculata	0.01±0.0f	62 ± 0.0	0.8± 0.2	8118.2±131.74	36±0.0
Dolchus lablab	0.012±0.0b	56± 3.92	3±0.316	6235.4±1324.82	26±0.0
Glycine max	0.21±0.0g	67± 0.0	8±1.22	5024.6±200.68	35±0.0
Helianthus annus	0.85±0.2e	78±0.0	36±4.84	4294.6±371.59	28±0.0
Lathyrus sativus	0.02±0.0h	45± 0.0	20.4±1.47	2083.2± 72.310	18±0.0
Nigella sativa L	0.11±0.0ab	66 ± 0.0	7.6±0.98	1227.4±202.379	30±0.0
Ocimum sanctum	0.4±0.0d	51±0.0	26.2±3.23	9721.4±1411.82	42±0.0
Phaseolus vulgaris	0.023± 0abc	46±0.0	0.6±0.4	3064.2± 80.952	18±0.0

Table 2. Mean time taken to set flower (MTSF), total number of flower head per 1 m^2 (TNFP^2), pollen yield (PY) and time taken from blooming to shedding (TBS) of the plants at Ashoka site

Plant species	**PY+ SE**	**MTSF+ SE**	**FIB+ SE**	**TNFPM^2 + SE**	**TBS+ SE**
Brassica napus	0.66±0.0	68±5	32.4±2.46	8762.2±1219.3	67±0.0
Coriandrum sativum	0.26±0.0	65 ± 0.0	8±1.26	5290.2±799.58	45±0.0
Cow pea	0.01±0.0	69 ± 0.0	0.8± 0.2	7721.4±301.82	45±0.0
Dolchus lablab	0.012±0.0	59± 3.92	3±0.316	5235.4±124.82	33±0.0
Glycine max	0.21±0.0	74± 0.0	8±1.22	4024.6±80.68	38±0.0
Helianthus annus	0.83±0.0	90±0.0	36±4.84	4289 ±271.59	35±0.0
Lathyrus sativus	0.02±0.0	56± 0.0	20.4±1.47	3083.2± 720.310	25±0.0
Nigella sativa L	0.11±0.0	68 ± 0.0	7.6±0.98	1237.4±52.379	41±0.0
Ocimum sanctum	0.1±0.0	63±0.0	26.2±3.23	9566.2±231.74	62±0.0
Phaseolus vulgaris	0.023± 0.0	57±0.0	0.6±0.4	3078.2± 31.952	21±0.0

Foraging intensity of Bees

The number of bee visits within ten minutes per 1m^2 at each study site was different for each species. *Helianthus annus* was highly visited by bees followed by *Ocimum sanctum*, *Coriandrum sativum*, *Brassica carinata*, *Nigella sativa* and *Lathyrus sativus* at Adami Tulu and Ashoka sites. According to observations recorded on the foraging rates of the pollinators, they were few in the early morning and late in the evening. The foraging time of honey bees to different plant species varied, with peak foraging time ranging from morning 8AM to 4PM and 4AM to 4PM at Adami Tulu and Ashoka sites, respectively.(Figure5 and 6). *Helianthus annus* and *Brassica carinata* were visited by bees starting from early morning at both the study sites. These plants also produced a high amount of pollen. As a result of their pollen yields, they were visited starting from early morning up to late afternoon because honey bees usually collect pollen in the morning and nectar in the afternoon. Early in the morning, the concentration of nectar is low due to higher humidity to attract the bees. Bees synchronize their behavior with daily floral rhythms foraging only when nectar and pollen are at their highest levels. The variation of number of bee count is associated with different factors such as attractiveness of the flower, number of flower heads per plants, nectar and pollen yields and weather condition. This is also in agreement with Crane (1990). The intensity of bee visit is a measure of the potentiality of plants for nectar and pollen production.

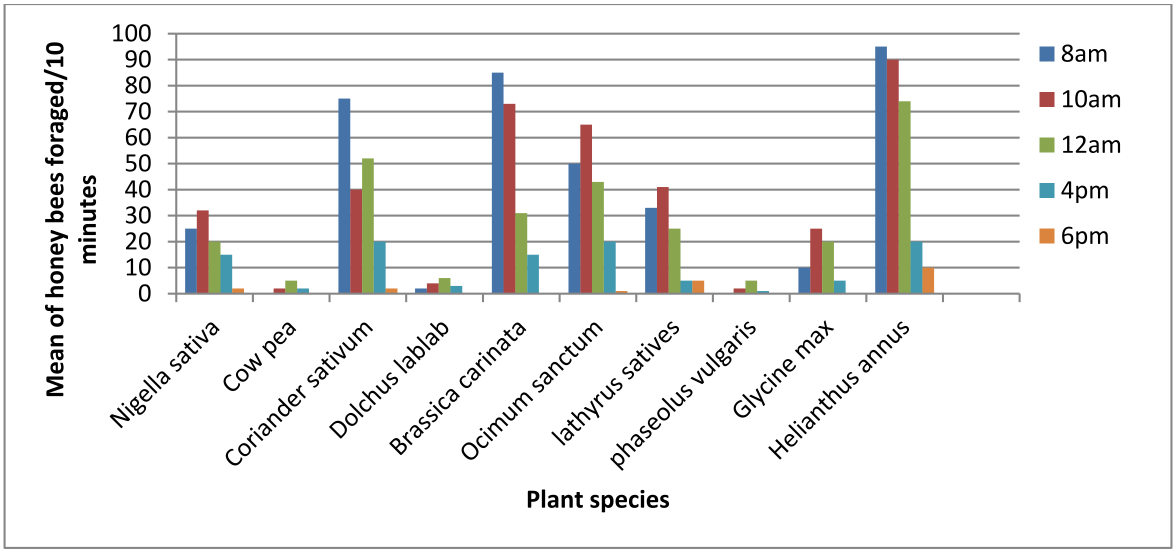


Figure 5. Foraging time of honeybees at different time of day on different species of plants at ATARC

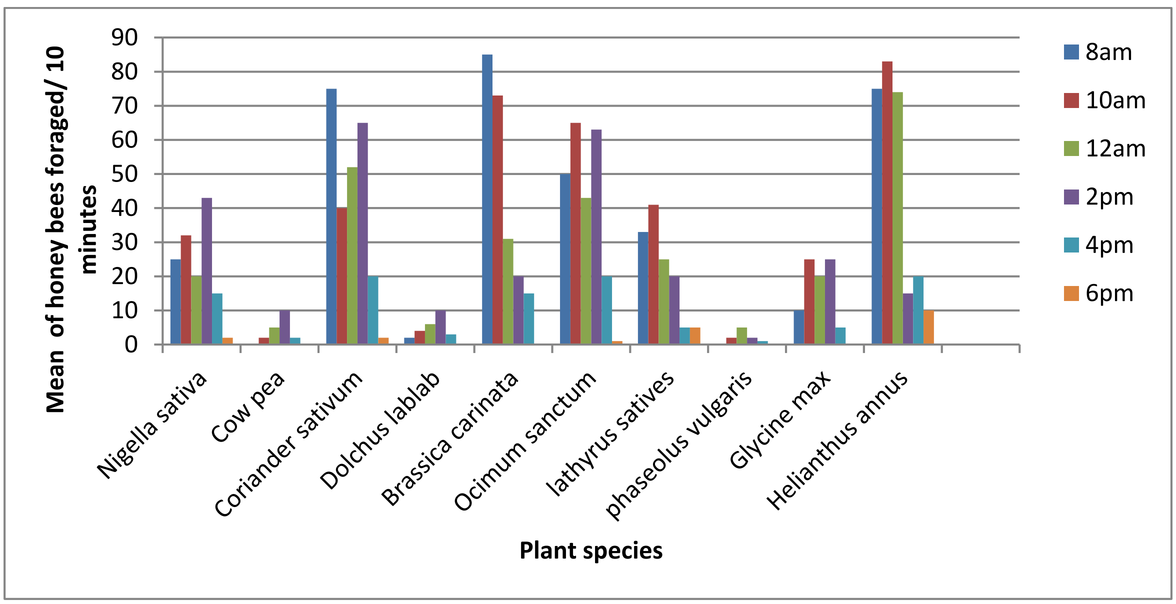


Figure 6. *Foraging time of honeybees at different time of day on different species of plants at Negele Arsi*

Conclusion and Recommendation

The study revealed that *Helianthus annus*, *Coriander sativum*, *Brassica carinata*, *Ocimum sanctum*, *Nigella sativa*, *Glycine max*, *lathyrus satives* and *Dolchus lablab* performed well at Adami Tulu and Ashoka sites. *Helianthus annus*, *Brassica carinata* and *Ocimum sanctum* were highly visited by bees at both the study sites. The time spent by bees for foraging on the flowers depends on the amount of nectar and pollen present in the flower. The peak foraging time is associated with nectar and pollen potentiality and floral preference of honeybees. Finally *Helianthus annus*, *Brassica carinata*, *Ocimum sanctum*, *Coriander sativum* and *Nigella sativa* performed very well both at Adami Tulu and Nagelle Arsi Districts whereas *laythrus satives* and *Dolchus lablab* performed well at Ashoka site because it needs long rainy season for seed seting. From this it is concluded that *Helianthus annus*, *Coriander sativum*, *Brassica carinata*, *Ocimum sanctum*, *Nigella sativa*, *Glycine max*, *lathyrus satives* and *Dolchus lablab* showed better performance at mid and lowland agro ecologies, however, further evaluation of the plants particularly, carrying capacity of the colony and honey production potential, should be tested under different agro- ecologies of the country

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Evaluating the efficiency of different stingless bee honey harvesting methods

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Abstract

Several stingless honey harvesting techniques have been developed and are used by stingless beekeepers around the world to extract stingless bees' honey. However, each technique has its own advantages and disadvantages. This study was conducted to evaluate different stingless bee honey harvesting methods in terms of their efficiency and the level of suffering they inflict on stingless bee colonies. During honey flow seasons, honey was harvested using cutting and squeezing, perforating and flipping, pipette filler suction pump, and disposable syringe from stingless bee colonies kept in pot hives. The volume and weight of honey extracted/pot, the time elapsed to extract honey from each pot and the honey extraction efficiencies of each technique were compared. In addition, observations were made during each honey extraction time to notice the effect of honey harvesting techniques on stingless bee colonies' well-being and honey quality. The highest weight (8.56 g) and volume (6.38 ml) of honey/pot were obtained with the cutting and squeezing technique, which were statistically ($p < 0.001$) higher than the pipette filler suction pump and disposable syringe methods, but not statistically ($p > 0.05$) different from the flipping method (8.44 g and 6.03 ml). Similar results were also obtained when comparing the honey extraction efficiencies of the techniques. The pipette filler suction pump method (21.70 ± 2.02 s), and cutting and squeezing methods (19.56 ± 2.55 s) took significantly longer time, while the disposable syringe method (12.26 ± 0.26 s) took the shortest time. Unlike cutting and squeezing, the pipette filler suction pump, disposable syringe, and perforating and flipping methods allowed honey extraction with minimal injury and disturbance to the bees when visually observed. These results suggest that the flipping of perforated honey pots method likely has more place for Ethiopian stingless beekeepers because of its high efficiency, less time consuming, inflicts less damage to the bees, its independence from other additional equipment and other sources of energy, and the possibility of increased yield through induced stress suffered on the colony to stimulate it to collect more honey.

Keywords: Stingless bee, squeezing, flipping, disposable syringe, pipette filler

Introduction

Stingless bees (*Apidae*, *Meliponini*) are one of the most common groups of bees in tropical and subtropical areas, which are highly social bees that live in colonies of thousands of individuals (Heard and Dollin, 2000). A total of 20 species are known to live in Africa (Eardley, 2004). Out of these, only five species have so far been reported in Ethiopia (Pauly and Hora, 2013), of which *Meliponula beccarii* (*M. baccarii*) is the most common species.

Meliponula beccarii is an inimitable species that lives in perennial colonies by constructing its nest, harboring underground nest where the bees reproduce each other, and store honey and pollen materials. These bees are very docile in their behavior, and their non-stinging nature helps for easy management and adaptation around home gardens, both for the production of sustainable quality honey and pollination services. Additionally, the honey of these bees has been highly regarded by local people for many years, playing an important role as traditional medicine for different ailments in Ethiopian local communities.

Because of this, stingless bees' honey has been known as a product with high market demand, fetching higher prices than the honey produced by the genus *Apis* and locally marketed in different regions of Ethiopia. As a result, stingless bees are of great potential for small-scale farmers as a source of income generation. Despite these positive attributes, stingless beekeeping (meliponiculture) has not been practiced. Rather, the traditional method of stingless bees' honey hunting is a common practice in Ethiopia.

Recently, Bayeta and Hora, (2021), reported for the first time the possibility of domesticating stingless bees using pot hives for the sustainable honey production and conservation of *M. baccarii* in Ethiopia. Moreover, this work provides valuable baseline data on how to transfer wild colonies into pot hives and manage the bees. Furthermore, characterizing stingless bee nest biology and ecology (Hora et al., 2021), quantifying antioxidant contents (Gemeda et al., 2021) and physico-chemical properties of *M. baccarii* honey (Alemayehu et al., 2021) have been done. Consequently, this works has raised a surge of interest in keeping native stingless bees and it is also believed that the business of keeping stingless bees will be expanded . Despite the success in domestication and the ability to manage stingless honeybee colonies on a sustainable basis, almost nothing has been done on the stingless bees' honey harvesting techniques to improve the traditional way of honey collection, which has been practiced since ancient times.

On the contrary, there are various stingless bees' honey harvesting techniques that affect the volume of the harvest as well as the wellbeing of the bees. Honey harvesting methods influence the quantity and quality of honey produced as well as the level of colony losses (Jaffé et al., 2015). As the colonies of *M. baccarii* are housed deep underground, the nests are not easily accessible for honey hunters, which finally ends up in whole nest destruction during the course of traditional honey harvesting (Halcroft et al., 2013a; Vit et al., 2018). As a result, colonies suffer greatly from the harvesting method. Moreover, in this traditional stingless bees' honey harvesting method, honey and pollen pots are removed together, and honey is squeezed out. Since honey pots are mixed with pollen pots and some soil particles, the honey harvested is generally poor quality (Villa-Boas, 2012). There are also other alternative harvesting techniques such as cutting and squeezing, perforating and flipping, disposable syringe, and mechanical and electrical suction pumps with varying efficiency (agility of collection), asepsis (cleaning), and negative effects on the colony's worker population and even on the colony itself. However, evidence has shown that improving harvesting methods could increase the efficiency on some stingless bee species by up to 20% (Yusoff, 2015). Thus, improving methods of honey harvesting for stingless bees is suggested as an area that would benefit from further research (Cortopassi-Laurinoa et al., 2006; Halcroft et al., 2013b; Koffler et al., 2015). Despite the easy management and adaptation of stingless bees around home gardens, the high honey market price and the role of stingless honey in traditional medicine, and the possibility of increasing collection efficiency, insignificant effort has so far been done to evaluate stingless bee honey harvesting techniques. Therefore, the aim of this work was to evaluate different stingless bee honey harvesting methods in terms of efficiency and the level of grief they are inflicting on stingless bee colonies.

Materials and methods

Experimental colonies and colony management

The study was conducted at Holeta Bee Research Center apiary sites (Holeta and Gedo). The previously established stingless bee colonies in pot hives following Bayeta and Hora (2021) method at both sites were used for the evaluation. Stingless bee colonies for the study were kept under shade to protect them from rainfall and sunshine. Regular colony follow-up was carried out and sugar syrup in a 1:1 ratio was fed to the colonies during dearth periods (during rainy and dry seasons when pollen and nectar were not available for bees).

Evaluating honey harvesting techniques

During honey flow seasons, honey was harvested using four different harvesting techniques: cutting and squeezing, perforating and flipping, pipette filler suction pump and disposable syringe, from stingless bee colonies kept in pot hives at the meliponaries of Holeta and Gedo. In this experiment, all harvesting techniques were evaluated based on honey pots rather than the whole hive content. Because removing the whole storage pots can lead to colony absconding or dwindling. Moreover, getting colonies with uniform honey storing potential is difficult.

Cutting and squeezing method: In this method, the pot hive was carefully opened with the help of a chisel without damaging the nest chambers containing the brood nest in the middle, and honey and pollen pots surrounding the brood nest (Figure 1). Ten pots of approximately equal size were selected. The pots were cut using a sharp knife and removed from the nest. The cut and removed pots were squeezed using the hand to separate honey from propolis in a plastic container. When the honey draining from the honey pots was over, the time elapsed to drain the honey was recorded and the collected honey was measured by a graduated falcon tube and digital scale with precision of 0.01 g. The portion of honey pots after draining of the honey was weighed, to determine the amount of honey left in pots, the pots was washed with water and kept until dry, then weighed using a digital scale.

Figure 1. Careful opening of pot hive from the top and locating of storage pots.

Perforating and flipping method: The pot hive was taken up from the earth (underground) and its external body was carefully cleaned to remove soil particles. The lid of the hive was opened with the help of a chisel, as in the cutting and squeezing method. The hive was tilted/flipped approximately 45^{o} and ten honey pots of approximately equal size were punched with a pointed knife or wooden skewer so that the contents could be drained into a plastic jar. When the honey draining from the honey pots was over, the time elapsed to drain the honey was recorded, and the volume and weight of honey collected were measured. Then, the pots were cut and removed to estimate the efficiency of the technique. This was done by weighing the drained pots, then washing and drying them, and measuring the weight of dried pots

Pipette filler suction pump: The suction pump was an RPI rechargeable battery operated pipette filler fitted with a 5 mm plastic tube connected to a container with 100 mL to create vacuum space (Figure 2). The procedures to make ready honey pots for harvest were done in the same way as for the perforating and flipping method. The honey was sucked by pressing the sucking button of the pipette filter and the content was directly moved into the honey container. The time elapsed to suck honey from each pot was recorded and the volume and weight of the collected honey were measured. Like for the perforating and flipping technique, the pots were cut and removed, weighed, washed, dried and weighed again to estimate the efficiency of the technique.

Disposable syringe: All the steps were carried out in the same way as in the pipette filler suction pump method. To calculate the efficiency of the techniques, first the initial weight of the pot was determined by adding the weight of honey extracted and the weight of the pots after honey was removed, then total weight of honey in the pots was calculated by subtracting the weight of washed and dried propolis/cerumen from the weight of the pot before extraction. Finally, the harvesting efficiency (%) of each method was calculated as:

(WHE/TWH)*100,

Where, WHE = weight of honey extracted (g), THW = Total weight of honey in the pot (g).

Furthermore, subjective observations were made to determine whether the techniques had caused any grief to the workers or the colonies as a whole.

Figure 2. Stingless bee honey harvesting techniques: Cutting and squeezing method (a), perforating and flipping method (b), pipette filler suction pump method (c), and disposable syringe method (d).

Data analysis

Data on the volume of honey extracted/pot (ml), weight of honey extracted/pot (g), time elapsed/pot (s), total weight of honey in each pot and extraction efficiency of each technique were entered into Microsoft Excel. Then the volume and weight of honey extracted/pot, time elapsed to extract honey from each pot, the honey extracting efficiency of each technique were compared using one-way analysis of variance (ANOVA) procedure of IMB SPSS statistics version 20. For mean separation, Tukey's Honest Significant Difference test at 95% confidence interval (= 0.05) level of significance was used.

Results

Evaluation of extracting efficiency

To compare the extraction efficiency of four different harvesting techniques (cutting and squeezing, perforating and flipping, pipette filler suction pump and disposable syringe) the weight of honey in a pot, the weight of honey extracted using these techniques and the weight of honey left in a pot were analyzed. The average weight of honey in a pot, the weight of honey extracted and the weight of honey left in a pot are shown in Figures 3 and 4. We found significant differences (p <0.001) among the techniques in the weight and volume of honey extracted. The highest weight (8.56 g) and volume (6.38 ml) of honey (Figure 3 and 5a) were obtained with the cutting and squeezing, which were statistically (p <0.001) higher than the pipette filler suction pump and disposable syringe methods, but not statistically (p >0.05) different from the perforating and flipping method (8.44 g and 6.03 ml). Similar results were also obtained when comparing the honey extracting efficiencies of the techniques (p <0.001). The result indicated that the cutting and squeezing method was significantly more efficient (91.12%) than the pipette filler suction pump and disposable syringe methods. However, it was not statistically higher than the perforating and flipping method which was 89.71% efficient.

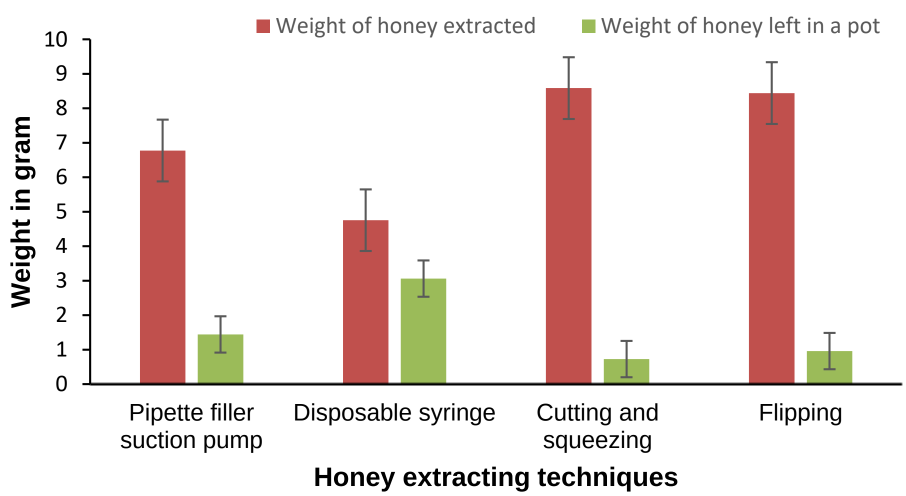

Figure 3. Weight of honey harvested per pot and left in a pot after extraction using four different techniques based on honey pots. Data is shown as a mean $\pm SE$. Vertical bars indicate 0.95 confidence intervals.

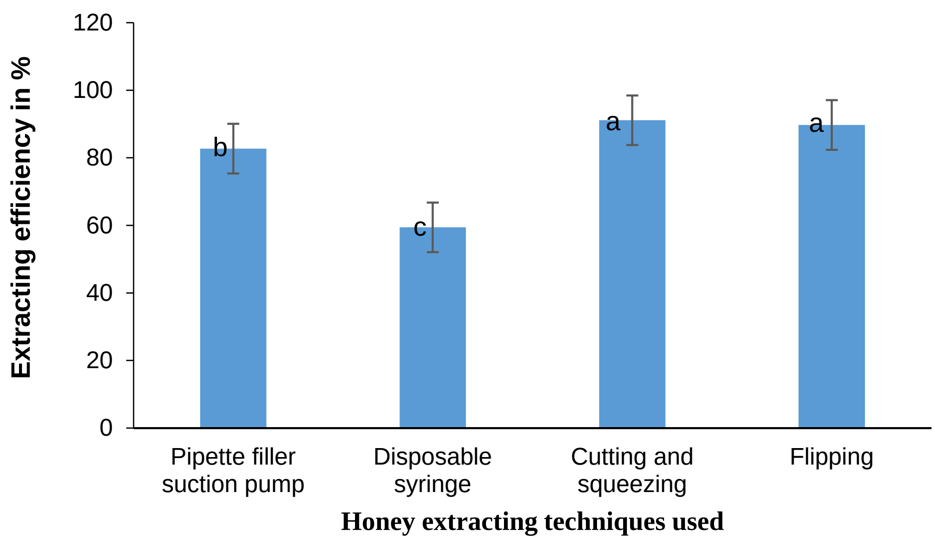

Figure 4. Honey extraction efficiency using different techniques. Data is shown as a mean $\pm SE$. *Vertical bars indicate 0.95 confidence intervals. Different letters denote a significant statistical difference at p < 0.001.*

The effect of honey harvesting techniques on the time required to extract honey from a honey pot

The average time required to extract honey from a honey pot regardless of the technique used was 15.74 $\pm$ 0.74 s with a range of 2.93 – 42.50 s (n = 133) (Figure 5b). The average time required to extract honey from a single honey pot varied significantly (p 0.001). Significantly longer time was recorded for pipette filler suction pump, and cutting and squeezing methods, while the shortest time was for the disposable syringe method (12.26 $\pm 0.26\,s$). However, there was no significant time difference between the cutting and squeezing, and perorating and flipping methods.

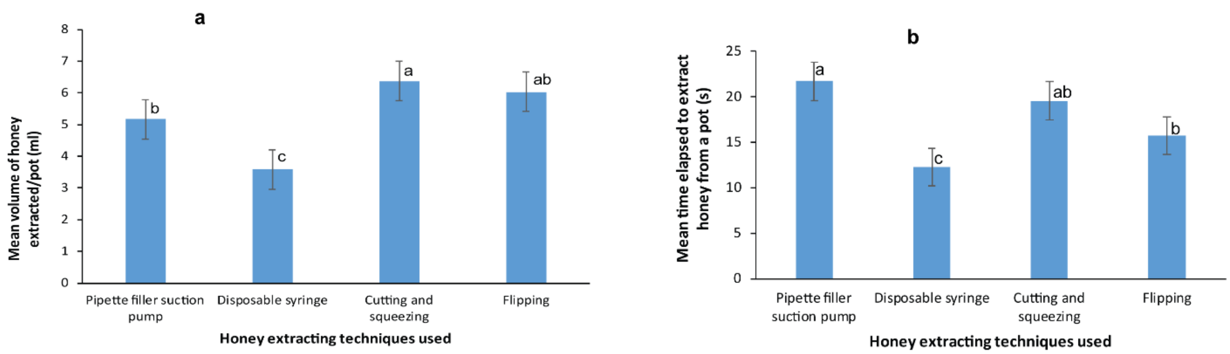

Figure 5. Evaluation of four different honey extracting techniques: *mean volume of honey extracted/pot for each technique (a), mean time elapsed to extract honey from a honey pot (b). Data is shown as a mean* $\pm SE$. *Vertical bars indicate 0.95 confidence intervals. Different letters denote a significant statistical difference at p < 0.001.*

Effects of the harvesting techniques on worker bees and honey quality

To notice the effect of honey harvesting techniques on stingless bee colonies and honey quality, observations were made during each honey extraction time. Stingless bee colonies subjected to the cutting and squeezing method, were suffered from the harvesting method during cutting, removing and squeezing of honey pots. As a result, a considerable number of worker bees were killed during these steps. However, the pipette filler suction pump, disposable syringe, and perforating and flipping methods allowed honey extraction with minimal injury and disturbance to the bees when visually observed. Moreover, in this traditional stingless bees' honey harvesting method (cutting and squeezing) honey pots, some pollen pots and worker bees were all removed together and squeezed, which was less hygienic (Figure 2a).

Discussion

Despite the fact that stingless beekeeping has the potential as a means for sustainable development, the activity is largely traditional and informal, technical knowledge is scarce and there are no standard management practices as in apiculture (Koffler et al., 2015; Leão et al., 2016). However, significant efforts have been made to develop sophisticated and standardized management and honey harvesting techniques. In this regard, several stingless honey harvesting techniques have been developed and are used by stingless beekeepers around the world to extract stingless bees' honey. Given the diversity of stingless bees and socio-environmental contexts in which stingless beekeeping is manifested in the world, the application of a single and standardized harvesting technique, as with apiculture, tends to be unsuccessful (Villas-Bôas, 2012).

Each technique has its own advantages and disadvantages (Jaffé et al., 2015; Villas-Bôas, 2012). A tangy taste due to the pollen mixing with the honey during extraction; the cutting and removing of pots resulting in colony disturbance and damage; extraction efficiency; and the time-consuming and difficult nature of harvesting methods are among the disadvantages of the techniques (Dulce Jovillano-Mostoles, 2019). In this result, this is reflected by the significantly varying honey weight and volume using different harvesting techniques. Honey harvesting using cutting and squeezing, and perforating and flipping methods relatively extracted higher amounts and was found more efficient compared to pipette filler suction pump and disposable syringe methods, which is consistent with the previously reported extraction efficiency (Dulce Jovillano-Mostoles, 2019). The higher amount of honey produced by cutting and squeezing may be attributed to the complete removal of the resources (Delgado et al., 2020). However, this traditional method of harvesting honey involves the destruction of honey and pollen pots, and surrounding cerumen (beeswax mixed with resin) (Gostinski et al., 2017; Villas-Bôas, 2012). The destructive method with complete removal of honey and pollen pots frequently results in the dearth of the colony (Delgado et al., 2020). This method of extraction requires the bees to rebuild the pots after harvesting, which may take a lot of effort. Moreover, honey from stingless bees harvested by cutting and squeezing the honey pots with bare hands is often contaminated with pollen and bodies of killed worker bees, which may result in less hygienic and lower quality honey (Dulce Jovillano-Mostoles, 2019; Villas-Bôas, 2012) The method not only kills the colony's working force but also makes the harvested colonies more attractive to phorid flies. This is because the technique involves the rupture and exposure of honey and pollen during the cutting of the pots and the harvested colony is left with exposed pollen, which may attract pests, particularly phorid flies (Jaffé et al., 2015). Thus, although the cutting and squeezing method allows high efficiency, the possibility of resulting in a depleted colony, a killing of work force and less hygienic honey shades a negative light on sustainable honey production. Finally, such conditions give less room to recommend the method for stingless beekeepers.

Now a day, stingless beekeepers try to harvest honey with minimal damage to the nest and pots during the harvest. These methods help the bee produce more honey in a sustainable way. To this end, employing a less destructive, simple and easy perforating and flipping method compared the traditional cutting and squeezing technique (Villas-Bôas, 2012) was resulted in a significantly higher amount (weight and volume) and efficiency of the extracted honey. This easy and non-destructive honey harvest minimizes excess pollen pot rupture, reduces contamination and the possibility of fermentation (Halcroft et al., 2013a). Moreover, it may reduce one of the main factors that results in low production in destructive harvesting method (Delgado et al., 2020). Because stingless bee colonies recently subjected to non-destructive honey extraction has been found to have more honey pots and a greater amount of honey three months following the first harvest (Gostinski et al., 2017). Accordingly, post-harvest stress suffered colonies of *M. fasciculata* were stimulated to collect more honey in the initial months following the extraction. Similar behaviour has also been observed in *M. eburnean*, in which honey gathering increased after the first batch of honey had been removed (Delgado et al., 2020). This could create a chance of multiple honey harvests for *M. baccarii* colonies rather than the traditional yearly basis. However, further study will be required for *M. baccarii* colonies. Furthermore, the method has the advantage of combining independence from using other additional equipment and other sources of energy but with possibility of honey oxygenation, as in the case of the squeezing method. This, together with its non-destructive nature, high efficiency and possibility of increased yield through induced stress on the colony, suggests that using the perforating and flipping technique to extract honey from domesticated *M. baccarii* colonies is more viable for stingless beekeepers.

In general, the time of hive operation during honey extraction is an important factor because of its cost implications if there are many colonies. Honey extraction with a pipette filler suction pump, as well as cutting and squeezing methods, took considerably longer time compared with a disposable syringe. The longest time to extract honey using the pipette filler suction pump may associate with low suction flow in the hose due to less vacuum generating capacity of the pump in the collection container (Villas-Bôas, 2012). In the squeezing and flipping methods, sampling was done based on 10 pots, but in reality, time of extraction does not depend on pot numbers. Once the pots are subjected to squeezing and flipping, draining takes the same amount of time regardless of the number of pots. As a result, squeezing and flipping are considered the quickest methods. Hence, this finding reals that flipping methods enable stingless beekeepers to harvest honey from domesticated *M. baccarii* colonies in a shorter time frame, inflicting relatively less suffering on bees.

Conclusion

This study takes the first steps in evaluating the efficiencies of different stingless bee harvesting techniques in terms of harvest time and the level of suffering inflicted on bees. Our results suggest that the flipping of perforated honey pots method likely has more place for Ethiopian stingless beekeepers for its high efficiency, less time consuming, inflicts less damage to the bees, and the possibility of increased yield through induced stress. Based on this finding, the method of flipping perforated honey pots is recommended as efficient honey extracting technique for domesticated *M. baccarii* colonies with minimal worker bee suffering. However, further research will be needed for *M. baccarii* colonies to determine whether honey gathering increased after the first batch of honey was removed, indicating the possibility of multiple honey harvesting from a stingless bee colony.

Conflicts of Interest

The authors of this paper do hereby disclose that there are no conflicts of interest whatsoever in relation to this article.

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Assessment of diastase levels in different monofloral and multifloral honey from Oromia, Ethiopia

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ABSTRACT

Diastase is an enzyme that is found naturally in honey and reduces over time, particularly when exposed to heat. It can be used to indicate the oldness and exposure of honey to heat. Though the enzyme activity in honey is relatively low, it plays a very vital role in honey quality. Thus, the aim of this study was to assess the diastase levels of the main mono-floral and multi-floral honey in potential areas of Oromia region. In this study diastase activity was investigated in different floral honey from different study areas. Free acidity was determined based on titration and while botanical origin was identified by microscopic method. All parameters determined in this study were based on international honey commission 2009 methods. Diastase activity was determined using Schade methods and it ranged from 4.61±1.50 DN to 12.75±4.78 DN. When compared to other monofloral and multifloral honey collected from the study area, the diastase activity of Erica arborea honey from Wenchi revealed significantly different ($P<0.05$). The results of the pH and free acidity ranged from 3.22±0.13 to 4.17±0.97and 22.43±6.37 to 35.10±10.51 $mgkg^{-1}$ respectively, which indicated the analysis test demonstrated that the honey samples used in this investigation were fresh. Certain monofloral and multifloral honey have diastase levels that are below those required by international standards. The low level of diastase may be due to the intrinsic properties of honey. Diastase activity is an inherent property of honey and a good indicator of the quality of honey. Honey bees geographical race, location and harvesting time affected diastase activities. However, it was not possible to identify the real factor influencing diastase activities in this study which needs further study starting from the harvesting time to the reach of the honey to consumer.

Keywords: Botanical Origin, Monoflora, Multiflora, *Apis melifera*, Schade Method, Starch

INTRODUCTION

Enzymes are the most important honey components. They are responsible for the conversion of nectar and honeydew to honey and serve as a sensitive indicator of the honey quality. In some countries, the specification of enzymes is a binding legal indicator (Oddo et al., 1999). Enzymes play an important role in honey and contribute to its functional properties. Honey holds small amounts of enzymes of which diastase and invertase are the most important because they are carefully chosen for the validation of honey quality. However, the predominant ones are diastase (amylase) (Vorlova & Pridal, 2002). The higher the content of this enzyme, the higher the quality of honey Diastase activity and HMF content are well used as criteria to assess the quality of the honey (Thrasyvoulou, 1986).

Diastase is found in nectar and is also added by honeybees during the collection and ripening of nectar. Like HMF, the diastase activity of honey can be used as an indicator of aging, overheating, and the degree of preservation /storage (Kędzierska-Matysek et al., 2016). The activity of diastase in honey is affected by storage and is sensitive to temperature increase and can thus be used as an indicator of storage time/freshness. Various studies indicated that honey shows significant variation in Diastase content based on composition, pH value, and floral source (White, 1992; Babacan and Rand, 2007). Free acidity and pH are also important parameters for the evaluation of honey quality, and indicators of freshness and adulteration of the honey (Silva et al., 2016).

Ethiopia produces both mono-floral and multi-floral honey (Adgaba, 2007), with Oromia accounting for the majority of these forms of honey (Gemechis Legesse, 2013). Several studies have been done so far on the physical and chemical characterization of the honey varieties produced in various regions of the nation (Adgaba, 1996; Belay et al., 2013; Gebremedhin et al, 2013; Legesse, 2014).

However, a few studies that examined the enzyme content of various honey varieties gathered from various regions of the nation and simply looked at the diastase level revealed that the average diastase level of honey samples from the entire nation was lower than the EU and global standard. Ethiopia now sells honey to European Union (EU) nations, Middle Eastern nations, and African nations (Legesse, 2014). According to feedback from its overseas buyers, which honey processing and exporting enterprises have been complaining about, the enzyme content, especially the diastase level, of various honey kinds shipped from Ethiopia is below the generally recognized average set for international trade (personal communications). However, based on composition, pH level, and floral resource (White, 1992; Babacan and Rand, 2007). As of yet, all evidence available from Ethiopia indicates that the amount of enzymes is minimal (Adgaba, 1996). Some monofloral honey naturally has a lower diastase value due to its botanical origin and is accepted on the world market (Bogdanov et al. 1999). However, due to a low quantity of enzymes, the honey from forest areas was the target of numerous complaints in the European market. Investigating the diastase level of honey from various plant sources as well as other crucial elements like pH and acidity level is therefore vital to determine quality. Thus, the aim of this study was to assess the diastase levels of the main monofloral and multifloral honey in potential areas of Oromia region.

MATERIALS and METHODS

Honey sample collection sites

Honey samples were collected from major honey-producing zones of Oromia to represent the major mono-floral and multi-floral honey types of the region. Accordingly, Gera and Goma districts of Jimma, Bacho, Didu, Mettu and Yayo districts of Ilu Ababora, Ejere, Ada Berga and Tokke-kuttaye districts of West Shoa and Ammaya, Waliso and Wenchi district of Southwest Shoa zones were selected.

Honey sample collection and Straining

A total of sixty-nine crude honey samples each weighing 1 kg were collected from beekeepers and brought to Holeta bee research center, bee product quality analysis laboratory. The collected honeys were categorized as multifrola and unifloral honey based on pollen analysis results. The purchased crude honey samples were strained to separate the pure honey from the wax using a drainage system using moderate warming.

Botanical sources of each honey type

The Louveaux et al. (1978) recommended methods for honey pollen analysis were used to examine the honey's pollen spectrum. This was accomplished by dissolving ten grams of honey in twenty milliliters of warm, distilled water. The sediment was then concentrated by repeated centrifugation at 3800 rpm for ten minutes, and the supernatant was decanted. After adding 20 ml of distilled water to completely dissolve the remaining sugar crystals for 5 minutes, the supernatant was completely removed. A sterile micro spatula was used to evenly distribute the sediment on a microscope slide, and the sample was then allowed to air dry for some time. After adding one drop of glycerin jelly and covered with the coverslip, the pollen grains were then identified under magnification power of 40X. Pollen atlas was used as reference to identify plant origin (Adgaba, 2002). Based on the total number of various types of pollen grains counted in each sample, the percentage of pollen types in each sample of honey was computed. The honey is classified as monofloral honey when the percentage of a pollen grain is greater than 45%, while named multifloral honey when there is no dominating pollen or less than 45% (Louveaux et al., 1978). The pollen count was performed using a computer-linked light microscope high power 400 x.

Determination of diastase activity

Diastase activity was evaluated spectrophotometrically (UV-visible Spectrometer) using the Schade method at Holeta bee research center bee product quality Laboratory. As the diastase number, the diastase activity is computed (DN). DN expresses diastase activity units (Gothe unit). A 50 ml beaker weighed out with 10 g of honey, 5 mL of acetate buffer, and 15 mL of water was used to measure the diastase activity. After the material had dissolved, three milliliters of NaCl were added, and then distilled water was used to dilute the solution to 50 ml. Iodine solution was also used to standardize a starch solution. 40 ^{o}C was used to heat both solutions. The stopwatch was started after adding five milliliters of starch solution to ten milliliters of honey solution. Five mill liters (5 mL) of the iodine solution received an aliquot every five minutes. A calibration curve was formed once the absorbance was recorded. According to the IHC 2009 method, the number 300 was expressed as a DN, or diastase number, by dividing it by the amount of time required to reach the absorbance value of 0.235 (IHC, 2009). The diastase activity was calculated as diastase number (DN) as follows:

$$\mathbf{DN} = \frac{60\ \text{minute} * 0.10 * 1.0}{tx * 0.01 * 2.0} = \frac{300}{t\,x}$$

Where, t_x is the reaction time in minutes obtained as absorbance values of the test sample solutions plotting against the corresponding reaction times in minutes after subtracting the absorbance of the blank value control.

Determination of pH and free acidity

The pH and free acidity of honey samples were analyzed according to the International Honey Commission (2009) by pH meter (Mettler Toledo, China).To determine pH and free acidity, from each honey sample, ten grams of honey was dissolved in 75 ml of distilled water in a 250 ml beaker and stirred using a magnetic stirrer. The electrode of the pH meter was immersed in the solution and the pH of the honey was recorded. For measurement of free acidity, the solution was further titrated with 0.1 M NaOH solution to pH 8.30. FA is expressed as milli equivalents per kilogram of honey and is equal to ml of 0.1M NaOH x 10.

$$\mathbf{FA = 10\ V_{NaOH}\ consumed,}$$

Where: V = the volume of 0.1N NaOH in 10 g of honey used

Data management and analysis

The results of each experiment were expressed as a mean standard deviation, and each laboratory analysis was carried out in triplicate (n = 3). The one-way ANOVA and Tukey tests were used to analyze the data and separate mean differences. The SPSS Software version 20 was used to perform all statistical analyses, and a significance level of 5% (P 0.05) was adopted.

Results and Discussion

Botanical sources of honey samples

The honey samples dominantly originated from nine different nectar source plant species (*Guizotia scabra, Eucalyptus globulus, Eucalyptus camaldulensis, Sheffleria abyssinica, Vernonia spp, Erica arborea, Coffea arabica, Lathyrus sativus* and *Terminalia schimperiana* (Table 1). These plant species are also known as major and minor honeybee flora. The relative pollen frequency of honey samples indicated that *Schefflera abyssinica* was a predominant source of honey samples collected from the Bacho and Didu, Metu, and Yayo districts of I/A/Bora and Gera and Gomma districts of Jimma Zones. *Schefflera abyssinica* is an abundant bee forage plant in moist highlands of southwestern and southeastern parts of Ethiopia and it provides monofloral honey in these areas (Bareke and Addi, 2019). It was also reported that *Schefflera abyssinica* is a major monofloral honey source in the Gera area (Degaga 2017). This supports the current study results. *Guizotia* species was predominant in honey samples collected from Ada Barga, Toke Kuttaye districts of the West and Waliso and Amaya districts of Southwest Shoa Zones, while, *Eucalyptus globules* were predominant in honey samples collected from Woliso of Southwest and Ejere, Adaa berga and Chalia of West Shoa Zones. Admasu et al., (2018) reported that most of the Ethiopian highlands are covered with golden-yellow flowers of *Guizotia spp.* with many different colors.

On the other hand, the majority of honey samples from the Gera districts of Jimma Zone were produced dominatly from *Coffea arabica* which was identified as monofloral honey (Table 1). *Coffea arabica* is a common cash crop in Western Oromia and contributed much to the production of monofloral honey. Admasu et al. (2014) stated that *Coffea arabica* flowers provide abundant pollen and nectar in January for honeybees. In addition, *Erica arborea* was a predominant honey plant in the honey sample collected from the Wenchi district of the Southwest Shoa Zone which is supported by Admasu and Tura (2019) studies that *Erica arborea* honey is commonly found in Wenchi district in West Shoa

Table 1: Botanical origin of bee honey samples

Site	**Predominant (>45%),**	**Secondary (16-45%)**	**Important minor (3-15%**	**Minor pollen source (< 3%)**
Ada'a Barga	*Eucalyptus globulus, Guizotia scabra*	*Guizotia spp*	*Acacia spp, Ocimum spp,* and *Plantago lanceolate*	*Brassica carinata, Vicia faba,* and *Trifolium spp*
Ejere	*Eucalyptus globulus* and *Lathyrus sativus*	*Guizotia spp*	*Corandrum sativum* and *Vernonia spp*	*Rumex spp, Brassica carinata, Hyposthus trifolium, plantago lanceolta,Trifolium*

				spp, and *Echinops macrochatusi*
Chalia	*Vernonia spp* and *Eucalyptus globulus*	-	*Guizotia spp, Brassica carinata, Hyposthus trifolium* and *plantago lanceolta*	-
Tokke kuttaye	*Guizotia spp*	*Plantago lanceolta*	*Eucalyptus globulus, Brassica carinata,* and *Pisum sativum*	*Trifolium spp,* and *Hyposthus*
Ammayya	*Guizotia scabra*	-	*Eucalyptus Clamaldulensis, Brassica carinata* and *plantago lanceolta*	-
Waliso	*Eucalyptus Camaldulensis* and *Guizotia spp*	-	*Hyposthus trifolium,* and *Trifolium spp*	
Wenchi	*Erica arborea* and *Eucalyptus globulus*	-	*Bidens spp* and *Rumex spp*	*Guizotia spp, Brassica carinata,* and *Corandrum sativum*
Bacho	*Schefflera abyssinica*	*Coffea arabica*	*Eucalyptus globulus*	*Rumex spp, Pisum sativum, Echinops macrochatus,* and *Croton macrostachys*
Didu	*Schefflera abyssinica*	-	*Eucalyptus globulus,* and *Croton macrostachys*	*Rumex spp,* and *Croton macrostachys*
Gera	*Schefflera abyssinica* and *Coffea Arabica*	-	-	-
Gomma	*Schefflera abyssinica* and *Terminalia schimperiana*	*Eucalyptus globulus*	*Coffea Arabica*	*Guizotia spp.*
Yayo	*Schefflera abyssinica*	-	*Vernonia spp* and *Croton macrostachys*	-
Mettu	*Schefflera abyssinica*	*Eucalyptus globulus, Vernonia spp,* and *Croton macrostachys*	-	-

P=predominant pollen source (>45%), S= Secondary pollen Source (16-45%), I= Important minor pollen Source (3-15%), M= Minor pollen source (< 3%)

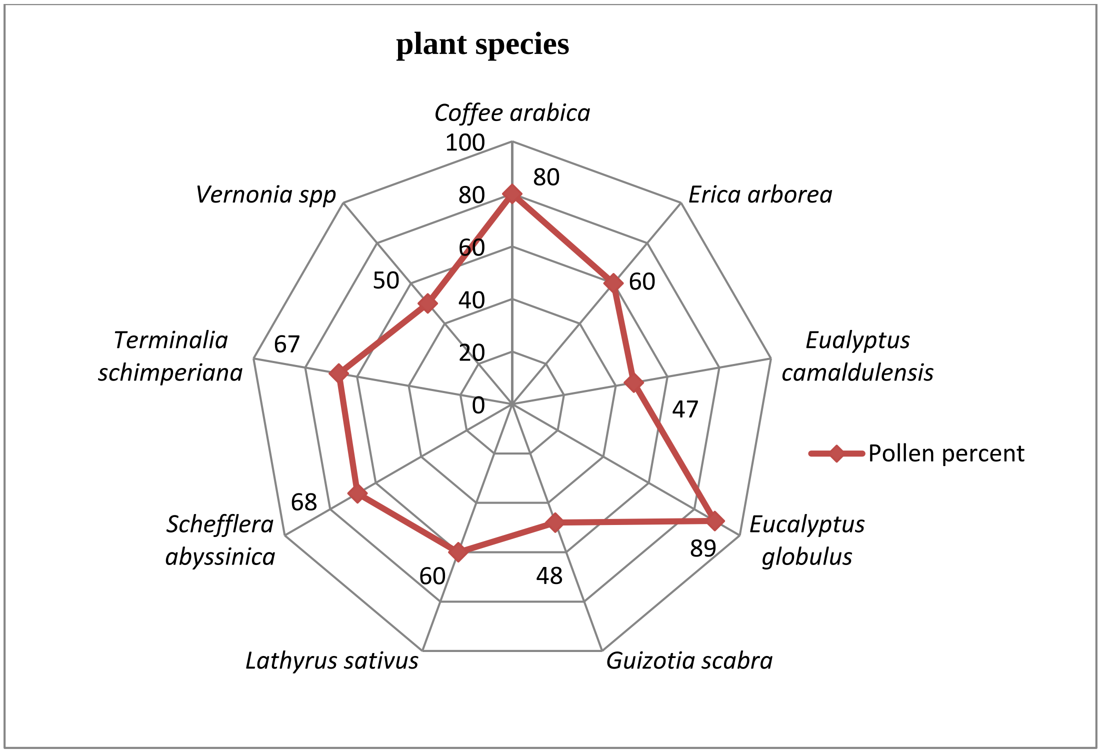

Figure 1: *Spider distribution of predominant pollen sources in honey samples from the study area*

Diastase activity

Diastase activity ranged from 4.61±1.50 (*Lathyrus sativus*) to 12.75±4.78 DN in *Erica arborea* honey. The diastase activity of *Erica arborea* honey showed significant differences ($p < 0.05$) from that of other honey collected from study areas. Abera et al. (2017) reported that the diastase activity of *Eucalyptus globulus* honey was 5.86 ± 0.890 Schade Unit which is lower than the present study finding. This showed that honey from the same botanical origin can probably differ in diastase activity, due to the influence of the environment, in which the honey plant species grow and the occurrence of different geographical races of bees, which are mostly governed by biotic and abiotic factors (Adgaba et al., 2017). Similar values for diastase were also reported in Argentina honey which averaged 19.7 Schade units (Cantarelli et al., 2008). The diastase activity of multifloral honey for the current study is 6.84±1.75 which is comparable with the finding of (Al-Farsi et al., 2018) who found the diastase activity ranging from 0.78 to 5.55 for multiflora samples in Muscat of Oman. According to Oddo et al. (1999), the level of diastase activity in honey varies depending on its sugar content, its floral and geographic origins, the length of time it was collected, the age of the bees, and the bee colony. In addition, the variation in reported diastase values could be due to different factors, such as processing, storage conditions, bee species, and harvesting time (Belay et al., 2017; Al-Farsi et al., 2018; Seraglio et al., 2018).

Table 2: Mean ± SD for DA, FA, and pH values of monofloral and multifloral honey (n = 69)

Honey type	DA (DN)	FA (meq/kg)	pH values
Coffee arabica	9.67±1.20[c]	35.10±10.51[a]	3.66±0.40[a]
Erica arborea	12.75±4.78[a]	26.18±1.73[c]	4.01±0.21[a]
Eualyptus camaldulensis	4.99±0.85[h]	28.18±7.00[c]	4.08±0.16[a]
Eucalyptus globulus	10.34 ±2.60[b]	30.10±4.82[c]	4.17±0.97[a]
Guizotia scabra	6.03±2.81[f]	23.83±7.14[c]	3.99±0.56[a]
Lathyrus sativus	4.61±1.50[g]	25.00±9.81[c]	4.05±0.22[a]
Multiflora honey	6.84±1.75[e]	34.80±9.33[b]	3.88±0.59[a]
Schefflera abyssinica	7.43±1.34[d]	22.43±6.37[d]	3.99±0.29[a]
Terminalia schimperiana	8.01±1.06[c]	33.00±0.51[c]	3.22±0.13[b]
Vernonia spp	6.56±0.79[e]	23.94±4.29[c]	3.95±0.517[a]

a, b, c, d, e, f, g, h= Means with different superscripts within the columns are statistically different at P<0.05.
DA= diastase activity, FA = Free acidity, SD =Standard deviation and DN =Diastase number

The diastase activity is a very interesting enzyme to know the freshness of honey (Oddo et al., 1990). The Location also affects the Diastase value. Waykar et al. (2022) found that the enzyme values in honey varied from location to location.

Coffee Arabica, Eucalyptus globulus, Terminalia schimperiana, and *Erica arborea* honey were found to be the honey within requirements based on different regulations (Bogdanov et al., 1999). Based on international legislation (Codex Alimentarius Commission, 2001), the permitted minimum diastase activity limit for honey is ≥8 DN of diastase per gram of honey.

The other honey samples showed lower diastase levels than the acceptable range. As a result, the obtained values can be regarded as characteristics of the examined honey and not dependent on external factors. The lower diastase level of some of the honey samples in the current study may be related to the internal characteristics of the honey and their botanical origin. Low levels of diastase activity are not indicative of adulteration or a lack of quality, according to Cortopassi-Laurino & Gelli (1991), who also supported the findings of the current study. Instead, they are specific characteristics of certain types of honey. The current study supports Wang and Li's (2011) finding that fresh honey from different plant origins that was not heated had lower diastase activity. According to White (1994) and Bogdanov et al (1999), honey is also used as a quality parameter even though some have a lower level of enzymes intrinsically. Diastase, a heat-sensitive enzyme found in honey, is a parameter that shows the freshness of honey and inappropriate heat treatment and storage conditions (Anklam, 1998; Marquele-Oliveira et al., 2017)

Free acidity and pH values

Free acid and pH values for monofloral and multiflora honey are presented in Table 2. The free acidity ranged from 22.43±6.37 (*Schefflera abyssinica*) to 35.10±10.51 meq/kg (*Coffee arabica* honey) taken from Gera Districts (Table 2). On the other hand, the free acidity of *Schefflera abyssinica* honey was significantly different from all honey types (p<0.05) which is the lowest free acidity recorded for this study. According to Belay et al. (2017), *Schefflera abyssinica* honey had a free acidity of 23.90 ±1.85 meq/kg, which is comparable to the current study. Even though enzymes are present in very small amounts, they have a significant effect on the quality of honey. This is because the enzymes would significantly affect the protein content, free amino acid profile, and acidity of honey samples. A variation in free acidity among the monofloral honey types might be because of the differences in honey harvesting conditions and seasons. All of the investigated samples (monofloral and multifloral) met the demands imposed by the regulations, which require in general not more than 50 meq/kg/1 (Codex, 2001). This indicates the absence of unwanted fermentations and honey sample freshness.

The pH values for the current study ranged from 4.17±0.97 (*Eucalyptus globulus*) to 3.22±0.13 (*Terminalia schimperiana*) honey. The pH of *Terminalia schimperiana* honey was significantly different from that of other honey types at ($p < 0.05$). According to previous reports, the pH of honey is between 3.2 and 5.5 (Bogdanov et al., 2004; Karabagias et al., 2014). The variation in the pH might be due to the effect of extraction, storage conditions, and floral types.

The pH of the honey was reported by other scholars and is in line with the current investigation. Accordingly, Belay *et al* (2017c) and Adgaba *et al*. (2020) reported the pH value of 3.61–3.77 and 3.5–3.7 respectively for Ethiopian monofloral honey. In addition, Saxena et al (2010) stated the pH of monofloral honey ranged from 3.7–4.4. The low level of diastase found in the current results may be attributable to an inherent characteristic of the honey samples, as both the pH and free acidity of honey indicates the freshness of the honey sample examined in this study. Besides affecting the pH value, the activity of enzymes might change the flavor and aroma of honey after fermentation (Chua et al., 2014)

Conclusion and Recommendation

The highest diastase level among the investigated honey samples is found in *Erica arborea* honey from the Wenchi district in the South West Shoa zone, while the lowest diastase level is found in Lathyrus sativus honey. Some of the monofloral and multifloral honey did not meet the international standard for diastase level, except for *Erica arborea, Coffee arabica, Eucalyptus globulus,* and *Terminalia schimperiana* honey. It may be concluded that variations in the diastase level may be caused by various uncontrollable intrinsic and external factors as well as by the botanical origin of honey. Although the honey sample examined in this study was fresh and unheated, the results of the pH and free test also demonstrated the honey sample's freshness. However, some monofloral and multifloral honey have lower diastase levels than are normally required. The lower levels of diastase could be inherent characteristics of monofloral and multiflora honey. It should be recommended to conduct further research on the diastase activity of various monofloral honey gathered from various agroecology around the country.

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Assessment of Stingless Bee (*Apidae Meliponini)* Production Systems and Indigenous Knowledge in West Arsi and Bale Zones of Southeastern Oromia, Ethiopia

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Abstract

The study was aimed to assess the stingless bee production systems, distribution and indigenous knowledge in the Bale and West Arsi Zones of South Eastern Oromia. Three districts were identified from the two zones. From each district, three Rural Kebeles were selected and a total of 65 stingless bee honey hunters were interviewed and field observations were also made. According to the study results, the honey hunters were categorized as Meliponula species (underground nesting stingless bees) and Trigona species (tree trunk cavity nesting stingless bee). Different methods were used to find the ground-nesting stingless bees and these include: by chance when walking along forest or grazing land or to home steady, direct observations of nest entrance or devoted searching for the presence of holes on the ground, use of honey smell to locate the nest site on ground and use of their enemies like wasps as indicators. The average amount of honey harvested per nest was 2.88±0.23 Liters and it was differing from place to place depending on the availability of flowering plants, age of nests and season of the year. The study also indicated that harvesting honey in the area was a totally a destructive method. Regarding the frequency of stingless bee honey harvesting, 49.2% of sample respondents stated that they harvest honey twice per year. During the study period the price of stingless bee honey ranged from 250 to 1000 Birr per liter with mean price of 507.89 Birr per litter at local market. Furthermore, honey was used for home consumption, treatment of different kinds of diseases and for income generation. About 87.7% of the respondents stated that they search stingless bees early in the morning and afternoon when sun gets on set. The study indicated that Honey badger, Aardvark, Sugar ant, Ant, Termites and Wasps were the major stingless bee enemies in the study areas. From the results it was noted that there is deep indigenous knowledge on wild stingless bee honey collection practices. In addition, the study area has a unique and diverse fauna and floras among which dominant flowering plants that make conducive environment for stingless bees exist. Hence, adopting stingless bee colony domestication technology is critical for increasing stingless bee production and productivity and conservation of the species resources.

Key words: *Stingless bee, Ground-nesting, Indigenous knowledge, honey hunters, production system*

Introduction

Stingless bees which belong to the family Apidae and sub family Meliponinae are a group of eusocial small to average sized honey producing bees with vestigial stings. Stingless bees are widely distributed in the tropical and subtropical regions of the world (Michener, 2007). Stingless bees comprise about 56 genera, more than 600 species and are known to occur in various ecological areas of the world (Eardley, 2005, Cortopassi Laurino *et al*, 2006,). Thus, only few eusocial bees occur permanently in tropical dry forests, among them some are highly adapted species (Michael *et.al*, 2019). Stingless bees are mainly associated with tropical dry and humid forests in low and warm areas, although some species can be found in cloud forests and pine-oak forests in the highlands (Ayala, 1999).These bees are responsive all year round and do not sting instead, they defend by biting if they are disturbed. Stingless bees usually nest in hollow tree trunks, tree branches, underground cavities, or rock crevices, but they have also been found in wall cavities, old rubbish bins, and storage drums (Kumar *et al.*, 2012). Stingless bees are true generalists, collecting nectar and pollen from a vast array of plants (Heithaus, 1979; Biesmeijer *et al*., 2005).

Ethiopia has only six species of stingless bees known to occur (Pauly and Zewdu, 2013). These species are commonly found in low to medium altitudes in Ethiopia. Stingless bees nest in different habitat types, preferentially in cultivated lands, adjoining forest areas, protected forests, grass lands, and woody shrubs along field edges in central and western Oromia (Zewdu *et al*., 2022). Some of these stingless bees are known for their honey, which are found from wild nests of stingless bee colonies. Honey hunting from stingless bee colonies in Ethiopian farming communities has a longstanding practice. In honey hunting, local hunters search for natural nests of stingless bees which is a common practice all over the country in general and in Oromia in particular. Bale and West Arsi Zones of Oromia, honey hunting has been a long tradition of collecting honey from wild nests of stingless bees. In addition to the long lasting tradition of stingless bees honey collection, the Bale zone is known for its abundant forest cover including the Harenna forest which is the second largest forest in Ethiopia with a coverage of 3500 to7000 km^2 (Bussmann, Rainer. 1997). The existence of this forest coverage in the area may create the suitable conditions for stingless bee honey production.

Stingless bees play an important role in the ecology, economy, and culture of local communities in the area. They act as the main pollinators for many wild and cultivated crops (Alemayehu and Zewdu 2021). Stingless bees' honey has been used as a source of income generation and is highly attached to the culture of the local community. Honey from the stingless bees is used as traditional medicine for treating different kinds of ailments in different parts of Ethiopia (Birhanu and Anualem, 2013). Like in other parts of Ethiopia, stingless bee honey has played a role in traditional medicine in local communities of Bale and West Arsi Zones.

Despite the ecological, economic and medicinal roles of stingless bees, information on their distribution and indigenous knowledge of hunting them has not been documented yet in the rural areas of Bale and Arsi zones. Hence, documenting the distribution and traditional production systems of stingless bees in the area is highly important for the development of appropriate stingless bee management practices and conservation. Therefore, this study aimed at identifying their distribution, production practices, and indigenous knowledge with regard to the bees in Bale and West Arsi Zones of South Eastern Ethiopia.

Materials and Methods

Description of the study area

The study was conducted in Dellomenna and Harenna Bulluk Districts of Bale Zone, and Nensabo District of West Arsi Zone which were located in the South eastern part of Ethiopia (Figure 1). The study areas cover the agro ecologies from lowland to highland. There are two rainy seasons in the area. The first and the main rainy season, extends from August to December with an annual rainfall of 270 to 560 mm, and the second, the short rainy season, goes from April to July with annual rainfall of 250 to 560 mm. The dry season covers the months from December through March (SARC, 2001). Honeybee floral diversity is found from lowland to highland. That creates conducive environment for providing year-round forages to stingless bees in the area.

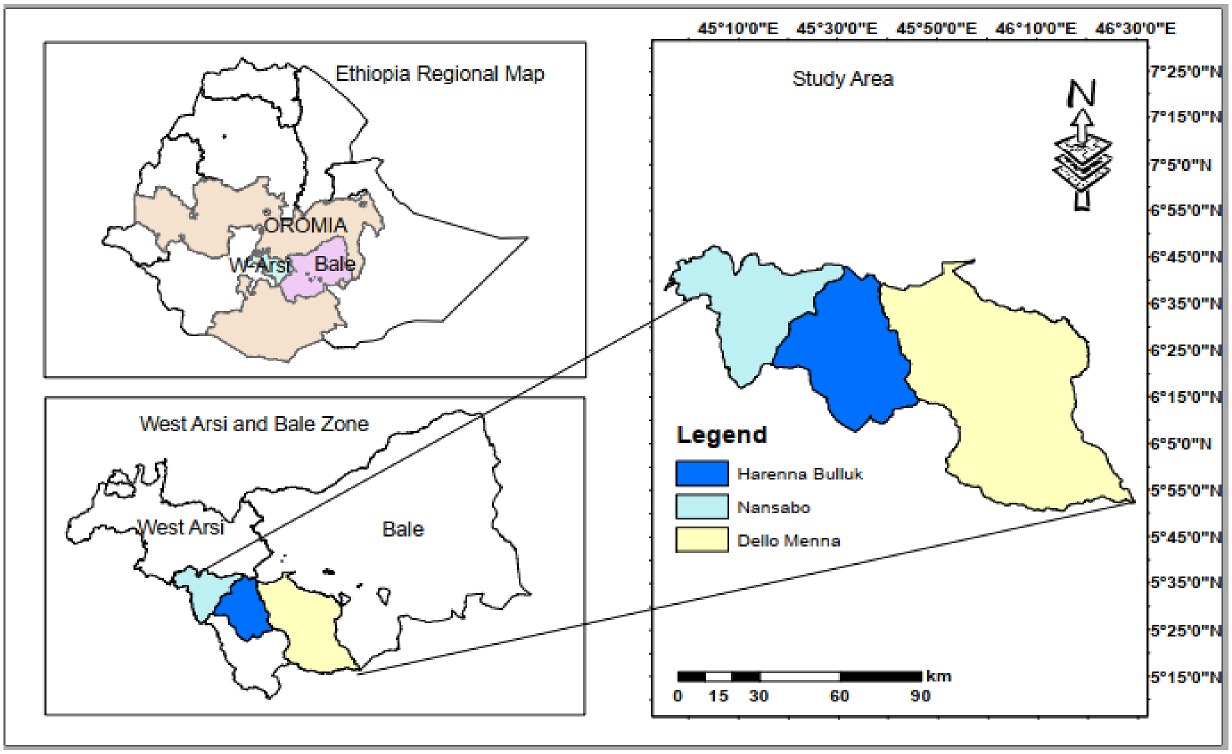

Figure 1. Map of the study area

Sampling design and methods

A cross-sectional study was employed to document the production system and indigenous knowledge regarding stingless bees and their honey production in Bale and West Arsi zones. The study was conducted for two years (2021-2022) in Dellomenna, Harenna, and Nensabo Districts. From each district, three Peasant Associations (PAs) were selected based on prior information obtained from experts, development agents, and elders in the districts about the potential areas for stingless bee honey production. A total of 65 male households participated in the study. Informal discussions with elders and local administrators in each Peasant Association (PAs) were held to identify those individuals who are knowledgeable about stingless bee hunting practices. Thereafter, the purpose of the study was clarified, and agreed to undertake an interview with honey hunters individually.

Method of Data Collections

A semi-structured questionnaire was developed, pre-tested with a few farmers, and reframed in such a way that it could be used to collect reliable data. The aspects covered during the interview focused on how to search or locate the nest when to search the nest, the number of nests harvested per year, the amount of honey harvested per nest, honey production/collecting practices, the honey flow season, major challenges of stingless bees and their distribution, and marketing of honey. In addition, the secondary data were collected from the Zonal and District Office of Agriculture. The focused group discussions (FGD) were also held with experts, community groups (elders), development agents, and key informants. During

Focused group discussion, representation of gender was considered and the checklist on which the participants are guided was prepared and the responses were taken as true information upon consensus.

For each collected data, ranking index (RI) was calculated by using the formula by (Musa *et al.*, 2014) as follows:

$$\textbf{Rank index} = \sum W/A*N$$

Where: W: Weight given to each factor by the respondent
A: the highest weight in the research
N: Total number of respondents

Data Management and Statistical Analysis

All collected data were entered into MS-Excel spread sheets. The ranking index, descriptive statistics (Means, standard errors, and percentage) logistic regression, and one way ANOVA were computed using the procedure of Statistical Package for Social Sciences (SPSS) version 23.

Results and Discussions

Socio-economic characteristics of the Respondents

From the result of characterization of the traditional knowledge of stingless bee honey hunting in Bale and the West Arsi Zone of the Oromia Region, the respondents stated that stingless bees honey hunting was undertaken by male-headed households. This arises from the traditional believe that stingless bee honey hunting practice is a men's activity and thus women are not allowed to collect honey from stingless bees in the study areas. This is due to a cultural taboo that restricts women from collecting honey from stingless bees. Similarly, (Amenaya Assefa, Kidane, *et al*., 2018) reported that nest hunting, and hanging wooden logs hives on large trees for the honeybees (*Apis mellifera)* are generally considered the work of men.

The age of the respondents ranged from 22 to 96 years old, with a mean age of 43.91 years. This result showed that stingless bee honey collection can be performed by all age groups reasonably without any difficulties but more actively performed by the younger age groups. The results also indicated that there were significant differences ($P<0.05$) in stingless bee honey collection experience among districts, with the total mean of 20.22 years' experience with a range of 1 to 81 years. Furthermore, the correlation between the age of stingless bee honey hunters and their experience indicated a strong positive and highly significant relationship ($r = 0.74$, $N = 65$, $P = 0.00$), showing that the engagement in stingless bee honey collection starts from an early age. Similarly, Tesfaye *et al.* (2017) reported that beekeepers in Bale started beekeeping at their early age.

Indigenous knowledge on stingless bee honey hunting

The stingless bees and their products represent one of the main natural resources known to the communities and it is one of the most appreciated natural products in areas of Bale and West Arsi zones of Oromia. The current study showed that there is a deep and diverse indigenous knowledge of the local community on stingless bee nest location, honey collection, and the use of its products. This practice has long been an integral part of the community's culture and their way of life in the study area. However, the method of harvesting stingless bee honey is completely destructive. This is agreeing with Eardley (2004) who stated that meliponic ulture is relatively uncommon in Africa and harvesting of meliponine honey is mostly destructive.

The majority of respondents (47.7%) stated that the knowledge of stingless bee honey collection and use of their products is obtained orally from their parents and elders, about 32.3% only from their parents and 20% only from elders. It is in this way that knowledge of stingless bee hunting is passed from generation to generation and honey from ground-nesting stingless bees has been collected by their ancestors for many years in the study area.

In the area, stingless bees were traditionally classified as *daamu* (underground nesting stingless bees*)*, which are known as *Meliponula* ***beccarii,*** and *bookee* (tree trunk cavity nesting stingless bees*)*, which are known as *Trigona spp.* In addition, the Majority of the respondents (83.1%) stated that the underground nesting types are the most common types of stingless bees and only 16.9% of the respondents stated both the underground nesting and tree trunk cavity nesting stingless bees are widespread in the area. Likewise, from the species recorded in Ethiopia so far, only *Meliponula beccarii* is known to build its nests underground (Pauly and Zewdu, 2013; Zewdu *et al*., 2021).

As reported by the respondents, the age of stingless bee nests was determined by the number of guard bees on the nest entrances and the amount of honey produced. For instance, if four bees sit on the nest entrance, the nest is four years old. On the other hand, the amount of honey obtained from a nest is proportional to its age. However, the number of guard bees at the entrance varies due to several factors, including time of day, weather conditions, presence of pests and predators, and colony strength (Griuter *et al*., 2011, Alemayehu and Zewudu, 2021). Therefore, the traditional method of estimating the age of a given nest based on the number of guard bees around the nest entrance and honey yield doesn't produce real information about the age of the nest.

Stingless bee honey harvesting practices

It is known that stingless bee honey is a non-timber forest product that is highly appreciated by local communities in the area. The respondents mentioned different kinds of materials such *as spade, knife*, *axe*, '*sefed'* or collection plate, and small plastic or glass bottles as the materials used for harvesting stingless bee honey. After preparing all these materials, the process of collecting honey is carried out starting by placing an indicator, such as a stick or symbol at the entrance of the nest and cleaning the ground surface up to 30 to 50 cm around the nest entrance. Next, they excavate around the nest until they reach the bottom of the nest, which has a depth of 30 to 60 cm. The entire nest was then removed and placed on a *sefed* or other materials such as broad leaves or large tree barks. Finally, they cut the brood nest to separate it from the honey and pollen pots. The honey is then strained. The majority of the respondents (82.3%) stated that they crush and squeeze the honey pots and then use clean clothes to retain any impurities and allow liquid honey to pass through the cloth, Only a few respondents (17.7%) made a hole in each individual honey pots so that the pure honey runs directly into the honey container they prepared.

The use of stingless bee honey in the communities

The sampled respondents throughout the study areas stated similar use of stingless bee honey. 78.55% of the respondents said that the community use stingless bee honey for several purposes such as for home consumption, treatment of different diseases, and sale in the study areas. From the total of 65 sampled respondents, about 18.5% mentioned that honey is used for the treatment of different kinds of diseases like asthma, coughing, tuberculosis, diabetes, cancer, malaria, and tonsillitis. Few respondents (3.1%) stated that they use honey only for home consumption because of its medicinal and nutritional value. This might be due to use in indigenous knowledge or tradition of the communities to use stingless honey for medicinal values which passed from generation to generation or from parents and elders in society. Stingless honey has also similar use in other countries. For instance, stingless bee honey is believed to have a medicinal value and has a higher market demand in India, where it is 20 times more expensive than *Apis* honey (Kumar *et al*., 2012).

Methods and time of searching stingless bee nests

According to the responses of the sample respondents, there were several methods of searching for the nests of the ground-nesting stingless bees in the study districts. The first method is seeing nest holes on the ground by chance when they walk along forest or grazing land or their home range. The second method is looking for the presence of stingless bees carefully and searching ground nests or holes on the ground where they observe any bee activity in the areas. They also keep silent, sit down on the ground, and look for forager stingless bees returning to or leaving their nest. The third method is the use of honey smell to locate the nest sites on the ground during the active season. The fourth method is the use of stingless bee enemies like wasps as indicators to locate stingless bee nests on the ground. If the bees are attacked by enemies like ants, they said the bees make a buzzing sound and then they will be able to carefully find for the nest entrance. These practices are consistent with the findings of Kidane *et al.*(2018) who described the practices of Sheka communities of Southwestern Ethiopia.

Regarding the time of searching stingless bee nests, about 87.7% of the respondents said searching is performed in the morning and late in the morning (8:00 to 11:00 AM), late in the afternoon (4:00 to 5:30 PM) while only 10.8% of the respondents said they search only in the morning (8:00 to 11:00 AM) and, 1.5% said only in the afternoon (4:00 to 5:30 PM). The respondents stated that the stingless bees prefer cool weather condition for foraging. After searching or sudden identification of a stingless bee nest, the owners place their own unique mark around the nest to keep the nest in great secret and avoid other people spotting it and then they leave it untouched until honey collection time. They use different symbols like stones, sticks, animal dung, and any other material to mark the place, and allow returning during the harvesting season.

Stingless bee honey harvesting frequency and price

Almost half of the respondents (49.2%) said they harvest honey twice per year, which extends from December to February and June to August (two seasons). Others (38.5%) said they harvest only once per year. Moreover, a few respondents (12.3%) stated that they harvest honey throughout the year depending on the availability of forage resources in the area.

The price of stingless bee honey ranged from 250 to 1000 Birr per litter with a mean price of 507.89 Birr per litter at the local market during the study period. This was up to three times greater than the price of *Apis* honey, which ranges from 200 to 300 ETB per kilogram. This indicates that stingless bee honey is, traditionally, highly demanded by the local community as a cure for different kinds of diseases in the area.

Stingless bee distribution and production practices

According to focus group discussion (FGD) with apiculture experts in Bale and West Arsi Zone, stingless bees are found in twelve districts out of 18 and five districts out of 11 in the Bale and West Arsi Zones, respectively. It is stated that about 35.4% of stingless bees are distributed only along forest areas, 7.7% in farmland, 1.5% only in caves, 26.2% in forest areas and farmland, and 29.2% in forest areas, farmland, and around home steady. According to Kajobe (2008), *Meliponula beccar*ii is distributed in protected areas, non-protected areas, and open farmlands in Uganda.

About 98.5% of the respondents stated that ground nesting stingless bees are commonly distributed in their locality while only 1.5% mentioned that tree trunk nesting bees are also found in the area. The ground-nesting stingless bees are known for their high honey yield as stated by sample respondents. This is in agreement with the report by Kidane *et al*., (2018), in which it is indicated that the ground nesting stingless bees are more abundant, and honey hunters frequently collect the honey in the Sheka zone of South Western Ethiopia. Similarly, grounds nesting stingless bees are found in many localities in Gojjam and Tigray region (Pauly and Zewdu, 2013). The greater abundance of ground-nesting stingless bees in the survey area could be related to the altitude. In most of the studies conducted in medium altitude areas (eg. Fichtl and Admasu Adi, 1994) the ground-nesting stingless bees are more frequent. The tree trunk nesting stingless bees are rare in such areas as the bees prefer lower altitudes. Furthermore, during a focus group discussion with elders, experts, and local administrates, they stated that ground-nesting stingless bees were previously found to be abundant, but nowadays decreasing in population and production due to deforestation, overgrazing, expansion of agricultural land, and the use of different agrochemicals.

Harvesting of honey from stingless bees

Honey is a non-timber forest product that is highly appreciated by the local communities in the study area. While the respondents are familiar with some types of products (honey, pollen, and cerumen) from stingless bees, they commonly collected the honey. According to the respondents the amount of honey harvested per nest per year was higher (2.97 ±0.51 liters) at Harenna Bulluk followed by at Nensebo (2.96±0.28 liters) and the lowest (2.42±48 liters) was from Dellomenna (Table 1). As the respondents mentioned, the amount of honey yield per nest differ from place to place based on the availability of bee forage, age of the nest, colony strength, and season of the year. The honey yield of the ground-nesting bee colonies can differ based on nest age. A nest older than one year can produce up to 5 litters of honey (Kidane *et al*, 2018).

Table 4. Annual honey yields in the study districts (in litters per nest)

Districts	Total sample size (N=65)		
	Minimum	**Maximum**	**Mean ± SE**
Dello Menna	1	6	2.63±0.47
Harenna Bulluk	0.5	9	2.97±0.51
Nensebo	1	6	2.96±0.28
Overall mean	**0.83**	**7**	**2.88±0.23**

Challenges and threats in stingless bee honey hunting

The main challenges and difficulties of hunting stingless bee honey as reported by respondents are stingless bee enemies, lack of extension services, lack of government attention, poor knowledge of sharing culture in the community, lack of awareness by farmers about meliponiculture and lack of improved technologies. A similar finding was reported by (Amssalu and Teshome, 2021) from western Oromia. The major enemies of the stingless bees threatening the stingless bee honey production as stated by 24.8, 16.9, 14.46, 13.8, 12.3, 9.3, 8.2% of the respondents are honey badgers (*Mellivora capensis*), Aardvark (*Orycteropus afer*), sugar ant, ant (*Dorylus fulvus*), wasp (*Vespula germanica*), termites and snakes, respectively. The major enemies were ranked by respondents according to their relative importance as enemies of stingless bees in the study areas (Table 2). Different scholars (Awraris *et al*, 2012; Amenaya *et al.*, 2018) similarly reported that grounds nesting stingless bee colonies were affected by honey badgers, ants, moles, wasps, termites, foxes, and snakes.

Table 2: Rank index for major pests and predators of stingless bee colonies confirmed by respondents

Stingless bees enemies	1	2	3	4	5	6	7	**Rank index**	Rank
Honey badger	32	36	24	18	32	16	32	0.418	1st
Aardvark	16	22	24	0	22	44	22	0.330	2nd
Sugar ant	19	26	22	26	19	16	18	0.321	3rd
Ant	36	18	0	18	14	18	9	0.248	4th
Wasps	24	16	16	0	32	0	16	0.229	5th
Termites	12	0	12	24	0	12	12	0.158	6th
Snakes	0	22	11	0	22	0	11	0.145	7th

Index = *sum of (7*ranked 1st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5 th+2* ranked 6th+1* ranked 7th) for individual pests and predators divided by the sum of (7*ranked 1 st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2*ranked 6th+1* ranked 7 th) for overall pests and predators.*

Stingless bees are also threatened by a number of environmental and anthropogenic factors under natural conditions. In this study, as it can be seen in Table 3, deforestation took the lion's share (46.7%), followed by application of agro-chemicals (27.2%) and over grazing of range lands (25.8%). Reports by some authors (Coll *et al.*, 2010 and Eardley *et al.*, 2009) have shown that stingless bees are threatened by a number of anthropogenic factors, the most common threats being frequent fires, honey hunting, logging for timber and livestock overgrazing.

Table3. Rank index for major factors threatening stingless bee colony declared by respondents

Threatening factors	1	2	3	4	Rank index	Rank
Deforestation	32	8	24	64	0.492	1st
Application of agro-chemical	40	0	20	38	0.377	2nd
Overgrazing rangeland	36	18	27	9	0.346	3rd
Unlimited stingless bee hunting	16	32	8	32	0.338	4th

***Index** = sum of (4*ranked 1st+ 3* ranked 2nd+3* ranked 3rd+4* ranked 4th+3* ranked 4 th+2* ranked 3th+1* ranked 4th) for individual and threatening factors divided by the sum of (4*ranked 1st t+ 3* ranked 2nd+3* ranked 3rd+2* ranked 2th+3* ranked 3th+2*ranked 3th+1* ranked 4 th) for overall threatening factors*

Opportunities for meliponiculture in the study area

According to respondents and field observation, the study areas have unique and diverse fauna and flora, which makes a conducive environment for stingless bees. In this regard, the major opportunities for keeping stingless bees in the study districts include the high medicinal value of stingless bee honey, the high demand and high price of the honey, the non-stinging nature of the bees, availability of bee forages, drought-resistant behaviors of stingless bees and indigenous knowledge of farmers on locating stingless bee nests Index ranking of such factors is shown in Table 4. The finding of this study is therefore supported by another study (Amssalu and Teshome, 2021). Meliponini species are dry spell resistant than honeybees. Because mated queens are unable to fly, colonies would have to leave their queen behind when abandoning the nest (Michael *et al*, 2016). As result, resource-induced absconding is rare in the Meliponini, and they need to adopt alternative strategies such as high demand of honey, and drought resistant behaviors, to sustain their permanent colonies during the extended dearth period they experience in tropical dry forests (Michael *et al*, 2016).

Table 4. Rank index for major opportunities of keeping stingless bee colonies in the areas

Opportunities	1	2	3	4	5	6	Rank index	Rank
High medicinal value of SH honey	30	15	30	60	0	90	0.577	1st
High demand and prices of honey	40	60	20	0	20	20	0.410	2nd
Non-aggressive and no side effect	16	32	0	16	0	48	0.287	3rd
Availability of bee forage	48	12	24	12	12	0	0.277	4th
Drought resistant behaviors	27	0	9	18	27	18	0.254	5th
Indigenous knowledge of farmers	22	11	22	0	0	33	0.226	6th

Index = *sum of (6*ranked 1st+ 5* ranked 2nd+4* ranked 3rd+3* ranked 4th+2* ranked 3th+2* ranked 5th+1* ranked 6th) for individual opportunities divided by the sum of (6*ranked 1 st+ 5* ranked 2nd+4* ranked 3rd+3* ranked 4th+2* ranked 5th+1*ranked 6th) for overall opportunities*

Conclusion and Recommendations

The present study showed that hunting honey of stingless bees and use of products of stingless bees are very traditional, the method of honey harvesting is totally destructive and the yield is very low. Thus adopting stingless bees domestication technology is critical for increasing stingless bee production and productivity. From the survey result, stingless bee colonies were categorized as *Meliponula beccarii* (*daamu),* ground-nesting stingless bees, and a *Trigona* spp (*bookee), above ground-nesting stingless bees*. In the area, the underground nesting stingless bees are the most dominant ones accounting for 83.1%

while *Trigona* spp accounts for 16.9%. As the major anthropogenic factors threatening the stingless bee colonies are found to be deforestation, application of agro-chemicals and overgrazing of range lands, the government and non-government organizations should raise awareness on the conservation of forests, proper grazing management and use of agrochemicals and also on sustainable use of stingless bee resources.

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Indigenous practice on behavioral and productive characteristics of local honeybee (*Apis mellifera*) sub species at Southern Oromia region, Ethiopia

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Abstract

This study was designed with the objectives of identifying and documenting indigenous practices on behavioral and productive characteristics of different local honeybees. Structured questionnaire for interview, Focus Group Discussion (FGD) and key informant interview were employed to generate data. The majority of beekeepers (44.62%) were between 32 and 40 years of age, while 26.15% were between 16 and 24 years. with a range of 16 to 65 years. Beekeepers predominantly used their indigenous knowledge for honey production. The majority of beekeepers keep traditional bee hives by hanging on trees nearby their homestead. According to the indigenous knowledge of the beekeepers, locally available honey bee colonies were classified into two groups based on their physical characteristics like color, body size and honey production potential. Accordingly local honeybee colonies are categorized as black and red based on their body color. Black colored honeybee ecotype has been preferred by beekeepers for their better productivity, adaptability, lesser absconding and migration tendency. Generally it is advisable to test behavior and productivity of local honeybees from each agro-ecology under controlled experiments. Practical beekeeping training should be given on biology and behavior of honeybee to beekeepers in order to capitalize on their indigenous knowledge for improving honey production and to conduct breeding of honey bee colonies with desired merits.

Key words: *Indigenous knowledge, behavior, local honeybee, ecotype*

Introduction

Indigenous knowledge is the unique knowledge confined to a particular culture or society. It is the understandings, skills and philosophies developed by societies with long histories of interaction with their natural surroundings.

Ethiopia has a longer tradition of beekeeping than any country in the world even though the sector is still undeveloped subsector of agriculture (Melaku et al., 2008). The contrasting geomorphic landscapes of Ethiopia create favorable environment for the existence of a large and unique biodiversity (Kerealem E., 2005). Owing to the varied ecological and climatic conditions, the country is home to some of the most diverse flora in Africa. Ethiopia is the fifth major country in tropical Africa in terms of the diversity of flora; This diversity makes it highly suitable for sustaining a large number of bee colonies (Nuru A., 2007).

Beekeeping is an important agricultural activity in Ethiopia. It is especially suitable in the semi-arid areas where other modes of agriculture cannot be sustained effectively. Five honeybee races are recorded in Ethiopia and, it is reported that *Apis mellifera scutellata* can be found in the southern part of the country (Amssalu B. *et al*., 2004). Traditional beekeeping is a part of cultural heritage and has been practiced by rural people. This technique of beekeeping has been passing from generation to generation since time immemorial. The beekeeping is predominantly based upon the indigenous knowledge and use of locally available materials. Different studies showed that inappropriate skill of bee management practices, colony absconding, poor design of modern beehives, low honey yields and bee pests are the major problems that impede the full use of apiculture resources.

The indigenous knowledge and practices of beekeeping could be significant basis for development of modern beekeeping in the rural communities (Rameshwor P., 2018). Farmers in Ethiopia have developed their own selection criteria from their long years of beekeeping experience. Selection of honeybee colonies adapted to local conditions with its appropriate management practices is an important step for the success of the beekeeping sector and hence harvesting of desired products from honeybees. Generally it is expected that there is ample indigenous knowledge on beekeeping among our communities. It is very imperative to identify and document the available indigenous knowledge and practices on beekeeping for further improvement and development of the beekeeping sector. Therefore, the objectives of the study was to assess and document the local knowledge and experience of beekeepers, and to characterize the behavioral and productive performance of the local ecotypes of honey bees for honey production in Borana zone.

Materials and Methods

Description of the study area

The study was conducted in Taltale, Yabello and Arero districts of Borana zone, Oromia regional state. (Figure 1). The Borana Zone is located at about 565 km from Addis Ababa, in the southern Ethiopia. The zone lies between 4˚3΄N to 5˚N and 37˚4΄ E to 38˚2΄ E with a total area of approximately 95,000 km². The elevation of the area ranges from 1000 to 1600 m a.s.l. (Coppock, 1994). Borana zone has a bimodal rainfall pattern with the main rainy season (Ganna) between March and May, the peak being in April (Coppock, 1994). The short rainy season (Hagayya) extends from September to November with a peak in October. There is a shift in time and in duration and hence in total amount of rainfall. The mean annual rainfall of the zone ranges from 352 mm in the southern part to 605 mm in the northern part of the zone. The mean annual rainfall is 587.2 mm. The mean monthly minimum and maximum temperatures of Yabello are 15.6 and 18.8^{0}C, respectively, with a mean annual temperature of 18.3^{0}C.

Sampling and data collection method

Three representative districts were purposively selected based on their potential for beekeeping, agro ecological representativeness and accessibility to transport facility. From each district, three potential peasant associations (PAs) were selected. From each PAs 22 beekeepers were randomly selected and interviewed using structured and semi-structured questionnaires. Beekeepers were given the opportunity to list ecotypes of honeybee races, colony selection criteria for honey production and characterize the behavior of each ecotype of the honeybee race.

In addition, focus group discussion (FGD) and key informant interview were used to collect information. In each target study area (Kebeles), participants for FGD and key informant interviews were represented by both gender with different ages. The checklist by which the participants are guided was prepared and the values were taken as true information up on consensus. The discussion focused on the importance of honeybee, types of local honey bee ecotypes, selection criteria and their management practices.

Method of data analysis

Simple descriptive statistics (mean, standard deviation, and percent values) were used to summarize the nature of respondents, their experience on beekeeping and their management practices.

Result and Discussion

Socio-economic characteristics of the sampled households

From the total respondents about 96.9% were male, while the remaining 3.1% were female (Table1). A few number of female household were engaged in beekeeping activities due to low level of awareness in encouraging women in the community Beekeeping is taken as only the men's occupation. This is in agreement with previous reports by Workneh Abebe (2011). Regarding the age of the respondents, majority of beekeepers (44.62%) were between 32 and 40 years of age, while 26.15% were between 16 and 24 years, the range being between 16 and 65 years. This indicates beekeeping is practiced by active age groups. Educational background of the bee keepers indicated that the majority (60%) were illiterate. The remaining 27.7, 9.2, 1.5 and 1.5% were with primary school, secondary school, informal education and 12 plus Levels of education, respectively.

With regard to the marital status, majority of them (83.1%) were married, while the remaining 15.4% and 1.5% were unmarried and divorced, respectively. With regard to religion, majority of the respondents were *Wakefata* (67.7%) and the remaining were *Muslim* (20%) and *Protestant* (12.3%).

Table 1. Personal characteristics of respondents

Social characteristics	Frequency	Percent
Gender		
Male	63	96.9
Female	2	3.1
Age		
16-24	17	26.15
24-32	29	44.62
32-40	4	6.15
40-48	7	10.77
48-56	5	7.69
Above 56	3	4.62
Education level		
Illiterate	39	60
Primary school	18	27.7
Secondary school	6	9.2
Informal education	1	1.5
12 plus	1	1.5
Marital status		
Married	54	83.1
Unmarried	10	15.4
Divorced	1	1.5
Religion		
Wakefata	44	67.7
Muslim	13	20
Protestant	8	12.3

Beekeepers experience and sources of colonies

Beekeeping is one of the major agricultural activities used for income generation, poverty reduction and biodiversity conservation. In the study area, beekeeping has been practiced as part-time activity. There were no any respondent who depend solely on beekeeping business. Beekeepers use their indigenous knowledge for honey production using traditional log and basket hives. Majority of the beekeepers (63.1%) get beekeeping experiences from their neighbors while others (32.3%) get from parents. The remaining 4.6% stated that they get the beekeeping experiences both from parents and neighbors. Beekeeping experience of of the respondents ranges from 6 to 10 years (Table 2). This indicates that indigenous beekeeping practice has been passing from generation to generation.

With regard to sources of honeybee colonies to start beekeeping, catching natural swarm was reported to be the only sources of honeybee colonies in the area. According to Mohammed Tilahun *et al*, (2016), other sources of honeybee colonies in addition to catching swarm include purchasing and inheritance. The overall average number of colonies owned by beekeepers is 8 with a minimum of 1 and a maximum of 32 colonies per beekeeper. The majority of beekeepers use traditional bee hives for their colonies by hanging the hives on trees near their homestead (Fig 1).

Table 2. Beekeeping activities and the bee keepers indigenous knowledge in the areas

Knowledge attribute	Frequency	Percent
Years of experience		
Less than 5	21	32.3
6-10	19	29.2
11-20	018	27.7
More than 20	7	10.8
Sources of experience 0000		
Parent	21	32.3
Neighbor	41	63.1
Parent and neighbor	3	4.6
Nature of beekeeping		
Part time	65	100
Full time	0	0
Harvest frequency per year		
Once	3	4.6
Twice	37	56.9
Three times	25	38.5
Indicators for harvesting time		
Accumulation of bees around hive entrance	6	9.23
Less/no traffic and accumulation of bees, end of flowers	21	32.3
Ends of flowering	31	47.69
Ends of flowering and opening the hive	7	10.76

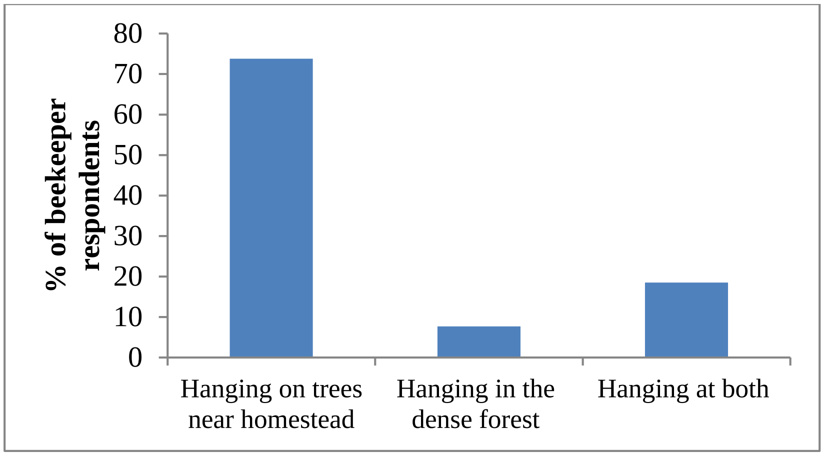

Fig 1. Placement of honeybee colonies

Beekeepers' Knowledge on local honeybee races and selection

In the surveyed area, beekeepers recognized honeybee castes in the colony by their size, stinging and numbers in the colony. But the majority of them at all districts did not know clearly the role of each caste in the colony. Though the majority of beekeepers were not aware of common honeybee races, they are able to categorize them based on different parameters (Table 3).

Table 3. Beekeepers knowledge on biology of bees

Variable	**Arero**		**Yabello**		**Taltalle**	
	Freq	Percent	Freq	Percent	Freq	Percent
Cast identification						
• Can identify	23	71.87	30	68.18	45	83.33
• Can't identify	9	28.13	14	32.82	9	16.67
Total	**32**	**100**	**44**	**100**	**54**	**100**
Role of each caste						
• Know role	11	34.38	5	11.36	15	27.77
• Don't know role	21	65.62	39	88.64	36	72.23
Species of Honeybees						
• Can differentiate	31	96.87	40	90.9	45	83.33
• Can't differentiate	1	3.13	4	9.1	9	16.67
Classification based on some criteria						
• Yes	31	96.87	42	95.45	53	98.15
• No idea	1	3.13	2	4.55	1	1.85

Subspecies of common honeybees

Beekeepers have their own methods of categorizing their honeybee colonies, mostly based on color of the bees. Based on the indigenous knowledge of the beekeepers locally available, honey bee colonies were classified into two groups considering physical characteristics such as color, body size, honey yield history, aggressiveness and direction of comb building. Accordingly body color, body size and colony population were the first three parameters used for classification of local honeybees (Table 4). Likewise beekeepers in the Tigray and Amhara regions carried out honeybee selection based on body color, colony population and production potential (Mohammed Tilahun *et al*., 2016 and Tasema Ayele and Zeleke Makuria, 2017) respectively.

Beekeepers recognized that honeybee colonies in the study area construct their combs in two directions, locally named as *Qurxaa* and *Dheerrina* irrespective of the types of .honeybees *Dherina* is the one where combs are built straight along the length of the traditional hive and *Qurxaa* is perpendicular to the length of the hive. In traditional hives, beekeepers prefer honeybee colonies that construct their comb in perpendicular pattern to the length side '*qurxaa*', as this facilitates easy harvesting and better honey yield. In Tigray, three comb construction directions were recognized by beekeepers (Mohammed Tilahun *et al.*, 2016) namely the *Salah*, where combs are built along the length of traditional hive, the *Difoe,* where combs are built in perpendicular to the traditional hive length and the *Goni/Seyaf*, where combs are built neither parallel nor perpendicular to the length of traditional hive, but slanting along the length by some angle to the width. In this regard, beekeepers can guide bees to build combs following their preference by providing strips of wax in the internal part of the traditional hive to make honey harvest from it easy and to maximize honey yield (Workneh, 2011).

Table 4. Beekeepers' colony selection criteria and categories

Selection criteria	**Categories**	**Score**	**Rank**
Body size	Small & big	23	2nd
Body color	Red & black	54	1st
Colony population	Strong & weak	22	3rd
Aggressiveness	Aggressive & less aggressive	15	5th
Honey yield history	Productive & less productive	16	4th
Direction of the comb building	Round *(qurxa)* and straight *(dherina)*	11	6th

Beekeepers' knowledge on behavioral and productive characteristics of honey bees

The study showed that beekeepers were very much experienced in characterizing their local honey bee colonies. Accordingly majority of respondents described black colored bees as being more productive and aggressive, having lesser swarming tendency and exhibiting higher rate of absconding than red colored bees (Table 5). Majority (60%) of the beekeepers in the study areas prefer black colored local bees for beekeeping, because they believe that black honeybee colonies are more productive and adaptable, survive long dry periods and build up early. Thirty five percent of the respondents provided the red bee colonies as their first choice whereas the remaining 5% of the beekeepers did not respond for both. The results did not agree with the findings of Tasema Ayele and Zeleke Makuria (2017), in which the brown red colored honeybee ecotypes has been selected as best for honey production. These authors also found that black local honeybees are preferred for their high drought tolerance or feed shortage tolerance which is in agreement with the current finding. Though beekeepers show selection for their colonies they do not manage their colonies differently according to their type or category.

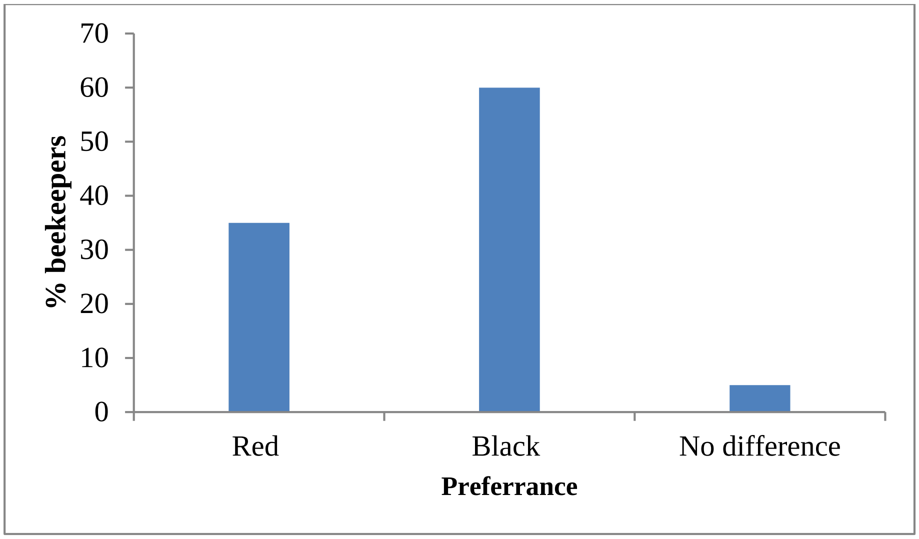

Fig 2. Beekeepers' honeybee colonies preference

Table 5. Respondents' view on distinctive behaviors of the two types of bee colonies

Red colored honeybee ecotypes	Black colored honeybee ecotypes
✓ Less productive	✓ More productive
✓ Docile compared to black	✓ Aggressive
✓ Lower swarming tendency	✓ Higher Swarming tendency
✓ Higher absconding and migration tendency	✓ Lesser absconding and migration
✓ Adaptable	✓ Higher adaptability
✓ Physically smaller than black	✓ Bigger than red

Conclusion and Recommendation

In the study areas, beekeepers have developed experience and indigenous knowledge on behavioral and productive characteristics of their honey bee colonies. Accordingly they are able to categories their honey bee colonies mostly based on appearance of their color and categorized them as black and red.

Despite their aggressiveness and higher swarming tendency, black colored ecotypes of honeybees were highly preferred by beekeepers for their higher productivity and low absconding and migration behavior. Practical beekeeping training should be given on biology and behavior of honeybees to beekeepers in order to capitalize on their indigenous knowledge for improving honey production and to start breeding of honey bee colonies with desired merits.

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Stingless bee production systems in Borana Zone, Southern Oromia

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Abstract

Survey was conducted in selected districts of Borana zone to identify stingless bees' production system, distribution and constraints for stingless bee honey production. About 73 indigenous stingless bee honey hunters were purposively selected and interviewed using structured questionnaire. The results reveal that all sampled respondents (100%) were male with an average age of 39 years old. They had been collecting stingless bees honey for the average of 22.75 years, but the number of years varied from 2 to 60 years. The average age at which the honey collectors started to collect honey was 13 years, ranging from 8 to 22 years. Regarding the nests of stingless bees mainly found in forest (bushes) and farm land which are far away from residential areas. Respondents identified type of stingless bees by looking body size, colour, and type of nest. Accordingly the majority of respondents were distinguished stingless bees that nested below and above ground. Based on color undergrounding nesting stingless bees were classified as red and black. Red stingless bees are productive with an average 2.24 liter honey per colony per harvest. The average honey yield obtained from natural nest varied based colony strength, age of nest and types of stingless bees.The study revealed that the study area is very potential for the existence of different species of stingless bees and their resources, for the production of significant volume of stingless bee honey. However, in the study areas, local people do not keep stingless honey-bees either traditionally or improved keeping practice, but they hunt for honey. Therefore, avoiding discarding of stingless bee colonies and their nest after honey harvest, use of appropriate technology while creating awareness of pastoralists, is important to improve stingless bee honey production system in the area.

Keywords: *stingless bees, ground nesting, tree trunk nesting, honey hunting*

Introduction

Stingless bees are a large group of bees in the family Apidae, belonging to the tribe Meliponini and they are social insects without functional stings (Eardley, 2004). Like any other living organism, stingless bees live and interact with the natural environment. They are found in tropical and subtropical regions of the world and they are believed to be native to Africa (Kwapong *et al*., 2010). In Africa, about six genera and 19 species of stingless bees are known to exist (Eardley, 2004). According to Pauly and Hora (2013), six species of stingless bees has been reported in Ethiopia. Stingless bees are active all year round and do not sting instead biting if their nest are disturbed. They usually nest in hollow tree trunks, tree branches, underground cavities, or rock crevices, but they have also been reported from wall cavities, old rubbish bins, water meters, and storage drums (Kumar *et al*., 2012). Stingless bees are true generalists, collecting nectar and pollen from a vast array of plants (Heithaus, 1979; Biesmeijer *et al*., 2005).

The six species of stingless bees are common in Ethiopia's low to medium highlands. They prefer to nest in a variety of nesting habitats including cultivated lands adjacent to forest areas, protected forest, grasslands, and woody shrubs along field edges (Zewdu *et al*., 2022). Some of these bees are well-known for their honey, which is produced by hunting honey from wild stingless bee colonies. Honey hunting from stingless bee colonies is a long-standing tradition in Ethiopian farming and pastoralist communities. Traditional honey hunting involves local hunters searching for natural stingless bee nests, which is a common practice throughout the country and in Oromia in particular.Stingless bee honey hunters of Borana pastoral communities have a long tradition of collecting honey from wild colonies (Personal communication).

Stingless bees play an important role in the ecology, economy and culture. They act as the main pollinators for many wild and cultivated tropical and subtropical plants (Slaa et al., 2006). Their products, such as honey, pollen and cerumen have been used as a source of income generations. In Ethiopia, people have used stingless bees' honey for many centuries. The uses of stingless bees' honey are attached to the culture of local community in different regions of Ethiopia.

Stingless bee honey, like in other parts of Ethiopia, has long been used in traditional medicine in the Borana pastoral communities. Despite the ecological, economic, cultural, and traditional medicinal roles of stingless bees and their products in the pastoral communities of Borana, little is known about the production systems, indigenous knowledge and constraints of stingless bee honey collecting. As a result, documenting the traditional production systems, indigenous knowledge and constraints of stingless bees' honey hunting in the area is important for the development of proper stingless bee management and conservation practices. Hence, the aim of this study was to identify the production practices, indigenous knowledge and constraints of stingless bee honey production in the pastoral communities of Borana Zone of South Oromia.

Materials and Methods

Description of the study area

The study was carried out in Borana Zone, Southern Oromia (Figure 1). Borana Zone is located about 565 km from Addis Ababa. The zone lies between 4˚3΄N to 5˚N and 37˚4΄ E to 38˚2΄ E with a total area of approximately 95,000 km². The elevation of the area ranges from 1000 to 1600 m a.s.l. (Coppock, 1994).

Borana zone has a bimodal rainfall pattern with the main rainy season (Ganna) between March and May with the peak in April (Coppock, 1994). The short rainy season (Hagayya) extends from September to November with peak in October. There is a shift in time and reduction in duration and subsequently in total amount of rainfall. The mean annual rainfall of the zone ranges from 352 mm at southern part 605 mm to the northern part of the zone. The mean annual rainfall is 587.2 mm. The mean annual temperature varies from 15-24˚C and shows little variation across the seasons (Coppock, 1994). The mean monthly minimum and maximum temperatures of Yabello were 15.6 and 18.8^{0}C, respectively, with a mean annual temperature of 18.3 0C.

Borana range land comprise of three main soils,53% red sandy loam soil, 30% black clay and volcanic light colored silty clay and 17% silty (Coppock, 1994).Plant communities on flat and hilly plains of central Borana Plateau consist of diverse mixture of woody and herbaceous vegetation (Coppock, 1994). According to Solomon Tefera et al., (2006) cited in (Addisu, 2009), four plant community types were described namely: evergreen and semi evergreen bush land and thickets, rangeland dominated by Acacia and Commiphora, rangeland dominated by shrubby Acacia, Commiphora and allied genera, and dwarf shrub grassland to shrub grassland.

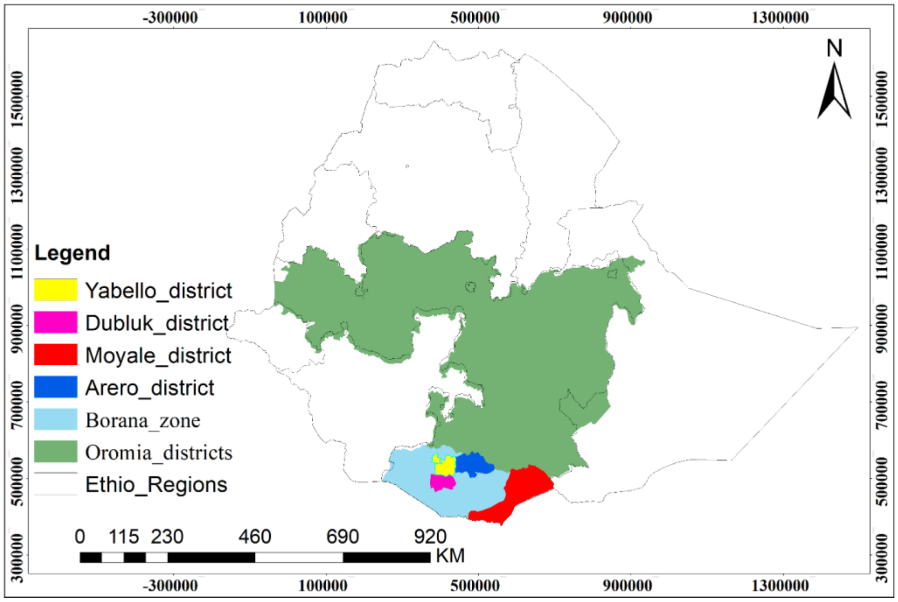

Fig. 1. Study site

Sampling techniques and sample size

For this study, four districts (Elwoye, Yabello, Moyale and Dubluk) were selected purposively based on secondary information obtained from district experts, development agents and elderly community members on the potential of the areas for stingless bee honey production. Then two peasant associations (PAs) per each district were randomly selected. Structured questionnaire was developed and used for the interview. A sample of seventy three (73) households was selected. These were from Elwoye 12(16%), Yabello 11 (15%), Moyale 28, (38%) and Dubluk 22 30%). Only households with prior experience in stingless bee honey hunting from wild stingless bee colonies were included in the sampling frame.

Data collection methods

Data was collected between May to July of 2022. The information was collected through interviews and direct observation, particularly emphasizing on the production systems, distribution, indigenous knowledge and constraints of stingless bee honey production.

Statistical analysis

The collected data were coded and tabulated for analysis. Descriptive analysis (i.e., mean, percentage, ranking and standard deviation) using SPSS version 20.0 was used to analyze the collected data.

Result and Discussions

Demographic Characteristics of the Respondents

The result in Table 1 revealed that all the respondents were male. There are no cultural taboos for female not to collect stingless bees' honey, but because of difficulties of collecting honey from wild nest as the result females do not participate in stingless bees' honey collection. Thus they need assistance from male when they found stingless bees' nest. Regarding educational level, respondents were categorized into five groups, where the majority of the respondents were illiterate (69.9%), followed by respondents in grades

5-8 (16.8%). The respondents were also categorized under basic education, grade 1-4, and grade 9-12 with percentages of 1.4%, 11% and 1.4%, respectively. In terms of marital status, majority of the respondents (89%) were married, while the remaining (11%) were single.

Table 1. Demographic characteristics of stingless bee honey hunting households

Categorical variables	**Frequency**	**Percent**
Sex		
• Male	73	100
• Female	0	0
Educational level		
• Illiterate	51	69.9
• Basic	1	1.4
• Grade 1-4	8	11.0
• Grade 5-8	12	16.4
• Grade 9-12	1	1.4
Marital status		
• Single	8	11
• Married	65	89
Total	73	100

The continuous variable of demographic characteristics of stingless bee honey hunters in the study area is depicted in Table 2. The sample respondents' mean age was 39.71 ± 15.42 years old. In addition, the mean family size of the respondents was 6.84 ± 3.87 (Table 2).

Table 2. Continuous variable of demographic characteristics of stingless bee honey hunters in the districts

Continuous variables	Minimum	Maximum	Mean	Std
Age of respondents	17	76	39.71	15.42
Family size	0	18	6.84	3.87

Stingless bee nesting habitat and substrate

The result of this study showed that nests of stingless bees were mainly found in bushes, bushes and farmland, which are far away from residential areas (Figure 2). Although stingless bees are considered generalists when it comes to nesting site selection, they still show some flexibility in their nesting habitats and nesting substrates (Njoya et al. 2019). Amssalu Arega and Tashoma Gudata (2021) also described natural vegetation as the preferred nesting habitat for the existence of more stingless bees than any other habitat.

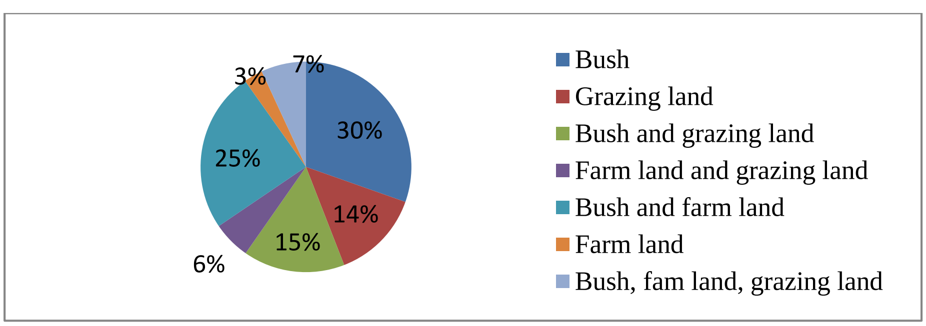

Figure 2. Types of stingless bee nesting habitats in the study area

Stingless bees' honey hunting practices and indigenous knowledge

The study revealed that the respondents had been involved in stingless bee honey hunting activities between 2 and 60 years of experience. They had collected stingless bee honey for an average of 22.75 years (Table 3). The average age at which the honey hunters started to collect honey was 13 years old, ranging from 8 to 22 years (Table 3) which is in the range of the previous finding (Amenay et al., 2021), who reported a mean age of 29 years old with a range of 10 to 62 years.

Table 3. Age of the respondents and years of experience with stingless bee honey hunting

Variables (year)	N	Min	Max	Mean	Std
Experience of hunting stingless bee's honey	73	2	60	22.75	12.95
Length of duration in exposure to stingless honeybee products	73	2	60	22.34	13.01
Usual age of involvement in honey harvest	73	8	22	13.01	3.156

Table 4. Respondents' indigenous knowledge of stingless bee honey hunting

Variables	Categories	Frequency	Percent
Types of stingless bees based on nesting habit	Below ground nesting	10	13.7
	Above ground nesting	0	0
	Both types	63	86.3
Abundance	Above ground nesting	28	38.4
	Below ground nesting	45	61.6
Types of stingless bee based on their body color	Red	1	1.4
	Black	0	0
	Red and black	72	98.6
Productive type	Red	67	91.8
	Black	6	8.2
Preference for honey harvest	Above ground nesting	7	10.8
	below ground nesting	58	89.2
Honey difference	Yes	66	95.7
	No	3	4.3
Determinants for honey difference among stingless bee type	Volume of honey	27	39.7
	Volume and color	19	27.9
	Volume and medicinal value	11	16.2
	Volume, medical value, price	7	10.3
	Volume, color, medicinal value	4	5.9

Stingless bee types were identified with the help of local stingless bee honey hunters. They distinguish stingless bee types based on body size, color, and type of nesting substrates. Accordingly, the majority of respondents distinguished stingless bees depending on where the bees used to nest, i.e., underground nesting type and tree trunk cavity nesting type. The stingless bees nesting underground and in tree trunk cavity are referred to as 'Daamuu' and 'Xunale' respectively, in the local language, Afan Oromo. According to the respondents, the above ground nesting stingless bees known to build their nests mainly in tree trunk cavities than any other cavities (Figure 3), which is different from previous report (Kumar et al., 2012) that stated the above ground nesting stingless bees construct their nest in wall cavities, old rubbish bins, water meters, and storage drums in addition to tree trunks. Trees, such as 'Hamesa' *(Commiphora africana)*, 'Hallo' (*Acacia bussei)*, 'Dakara' *(Boswellia neglecta)*, 'Agarsu' (*Commiphora erythraea*), 'Sapahnsa' (*Acacia mellifera*) and 'Dhadacha' (*Acacia tortilis*) were the major tree species used for the bees to build nests in. This finding is in agreement with the previous finding that tree trunks are the major natural nest sites for most above ground nesting stingless bees (Kajobe, 2007).

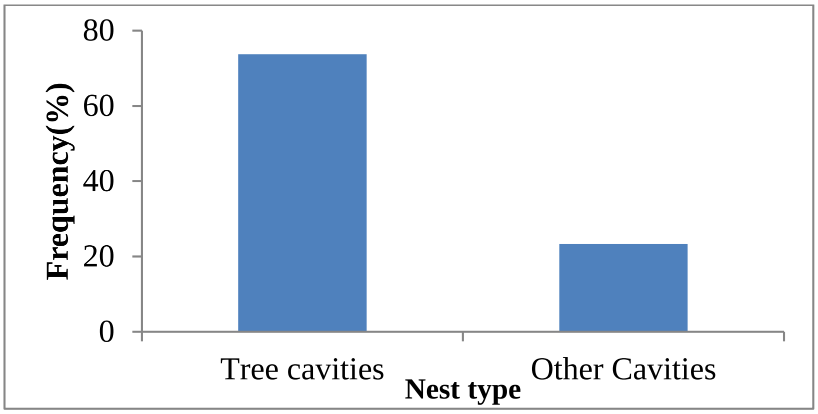

Figure 3. Nest types of above ground nesting stingless bees.

The respondents classified underground nesting types of stingless bees as red and black based on their color. They build their nest in the soil, where the red type prefers red soil, 'woyama', whereas the black type builds its nest in black soil, 'koticha'. The respondents said the red type is the more abundant and productive type of stingless bee in the study area. However, the current finding contradicts previous findings that the occurrence of ground nesting stingless bee nests in soils with different physical properties suggests that soil color has no effect on nesting cavity selection, as black type stingless bees from central and western Oromia were found to nest in both red and loamy soil (Hora *et al*., 2021). Furthermore, other subterranean stingless bee species, such as *Geotrigona subterranean* and *Geotrigona nombuca* nesting cavities are not affected by soil colour (Barbosa et al., 2013). Therefore, though the ground nesting type of stingless bee in the Borana has different colours, the colour is not due to soil colour rather may be because of varietal variation (Pauly and Hora, 2013).

In the study area, majority 89.2% of respondents prefer to collect honey from below ground nesting stingless bees, while the reaming from above ground nesting bees. They replied that honey harvested from different types of stingless bees differs by volume, color and medicinal values (Table 4).

Honey harvest and method for locating natural nesting sites

Table 5 presents the occasion and method for locating natural nesting sites for stingless bees. The stingless bee hunters recognize the natural nesting site for underground nesting bees by looking for stingless bees and then conducting a dedicated search for nests when bees enter and leave the nest. They also used stingless bees enemies like honey badger (*Mellivora capensis*), in locating stingless bee nest's location. As shown in the figure 4 below, stingless bee hunters search for bees during in the late morning and late afternoon because they believe that stingless bee foragers forage more actively in these times in mass, which makes it easier for them to get their natural nests. Only some hunters search during morning time. In general, the majority of the hunters look for stingless bee during flowering season, which lasts from March to May (Figure 5).

Table 5. Occasion and method for locating natural nesting sites

Identification methods of nest site	Freq	Percent
Looking for stingless bees and then dedicated search for nest when bees entering and leaving the nest	5	6.8
Use stingless bee enemies as an indicator to locate their nest	3	4.1
Looking for stingless bees and then dedicated search for nest when bees entering and leaving the nest, use bees enemies	65	89.04

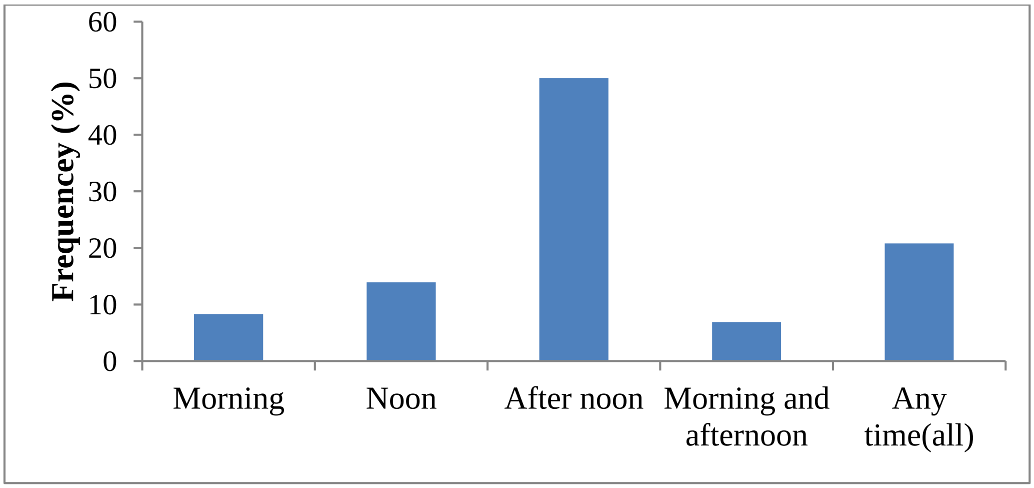

Figure 4. Time of the day for searching for stingless bee colony

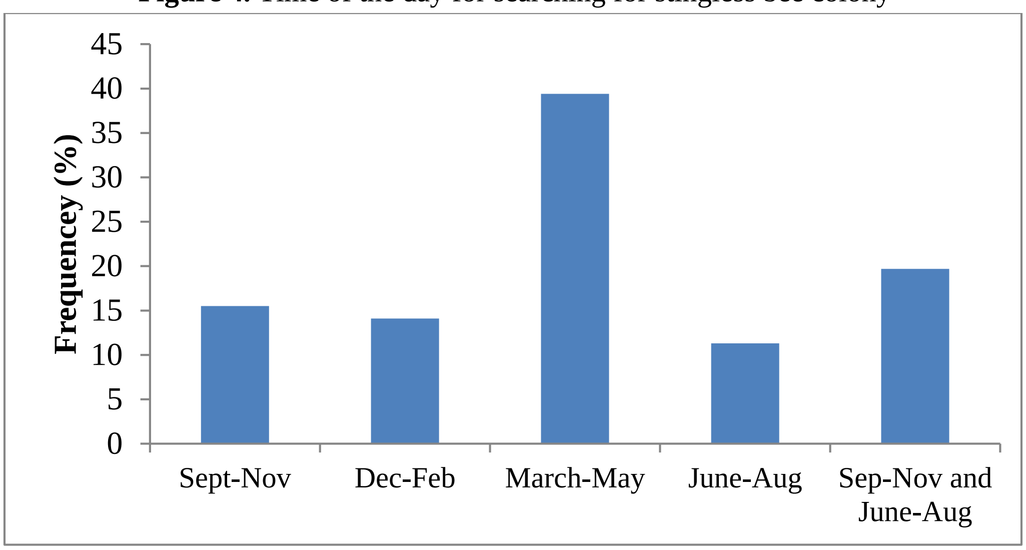

Figure 5. Season for searching stingless bee colonies

The majority of respondents said that they get bees on the occasion of livestock keeping (74%), followed by livestock and farm practice (20.5%), and the rest (5.5%) during other activities (Table 6). Contrary to the current results, Amssalu Arega and Tashoma Gudata (2021) found that the majority of respondents had been found stingless bee nests during farm practice. This could be livestock keeping is the primary activity in the pastoral areas as opposed to crop farming in the west. Once they obtained the nest, majority (48.6%) of them placed a symbol around the nest and left untouched until honey harvest time. Others used their own mark to locate its nest, such as large known trees, tree marking and so on.

Table 6. Occasion of nest site location finding

Occasion of nest finding	**Freq**	**Percent**
Keeping livestock	54	74
Farm practice	15	20.5
Fuel wood collection and other activities	4	5.5

In the study areas, local people do not keep stingless honey bee, but they hunt for honey. Similar result has been reported in Tanzania, where people in the Udzungwa area do not usually keep stingless honey bees but they hunt for honey, which is used for purposes of worship and medicine (Njau et al, 2010). During honey harvest, they harvest only once from a colony, as the nest is totally destroyed at the time of harvest. Respondents stated that they harvest honey from wild stingless bee’s nests using various local materials such as a spade, machete, axe and or knife, as well as collecting materials like plastic or glass, as suggested in southwestern Ethiopia (Amenay et al., 2021).

The overall average number of wild stingless bee nests harvested per respondent per year was 2.4 colonies, with minimum and maximum of 1 and 10 colonies, respectively (Table 7). According to the present study, the maximum numbers of colonies were recorded in the Moyale and Dubluk Districts. The abundance of stingless bees’ nests is usually related the availability of appropriate nesting site Sakagami and Starr as cited by Siqueira *et al*., 2012.The average amount of honey harvested for underground nest stingless bees’ type per nest seems similar for all districts, with an overall average of 2.24 liters per colony (Table 7). According to respondents, the productivity of stingless bees depends mainly on the colony strength and types of stingless bees (23.29%), the age of the nest (19.18%), the age and types of stingless bees (12.33 %), colony strength and the age of the nest (9.6%) Table 8.

Table 7. Average number of stingless bee colonies hunted and the honey yield per nest.

District	No of colonies used per HH per year			Honey yield per colony (Liter)		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**
Yabello	1.91	1	5	2.77	0.5	5
Moyale	2.5	1	10	2.95	2	5
Dubluki	3.32	1	10	2.23	1	4
Elwoye	1.83	1	4	1.75	0.5	3
Oveall mean	**2.39**	1	7.25	**2.4**	1	4.25

Table 8. Factors affecting for honey yield per colony

Factors	Freq	Percent
Colony strength and types of stingless bees	17	23.29
Age of the nest	14	19.18
Age and types of stingless bees	9	12.33
Colony strength	8	10.96
Colony strength and age of the nest	7	9.59
Age of nest and age of queen	7	9.59
Types of bees	6	8.22
Colony strength, type of bees, age of the queen, size of nest, forage availability and season of harvest	5	6.85

The respondents replied that they obtained their knowledge of stingless bees and the uses of their products from their parents (34.2%), relatives (23.3%) and relatives and neighbors (17.8%), while the rest got it from elders (16.4%) Figure 6. This shows that the practice of honey hunting and the indigenous use of honey have been passed down from generation to generation, which is in line with Amenay *et al*., (2021).

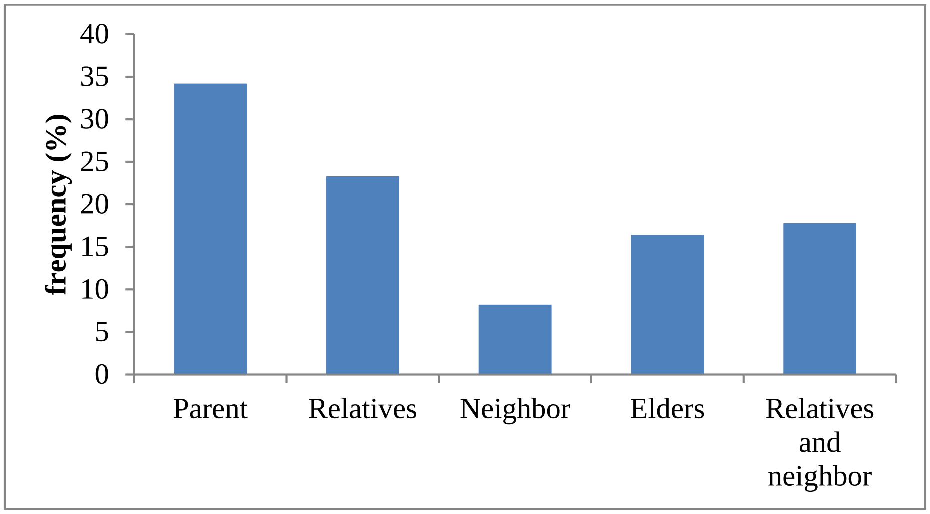

Figure 6. *Respondents' source of indigenous knowledge on stingless bees*

Uses and marketing of stingless bee honey

Out of the total interviewed respondents, 35.6% use stingless honey for home consumption and treatment of ailments; 34.2% use it for home consumption; and 19.2% use it for home consumption, treatment of diseases and sale. While the rest, 9.5% and 1.5%, use it for home consumption and sell and for the sale and treatment of diseases, respectively (Figure 7). People have traditionally used stingless bee honey to treat, 'Birte', cough and wound healing. According to the survey results, consumers (100%) were the only actors involved in stingless bee honey marketing and sold at a good price.

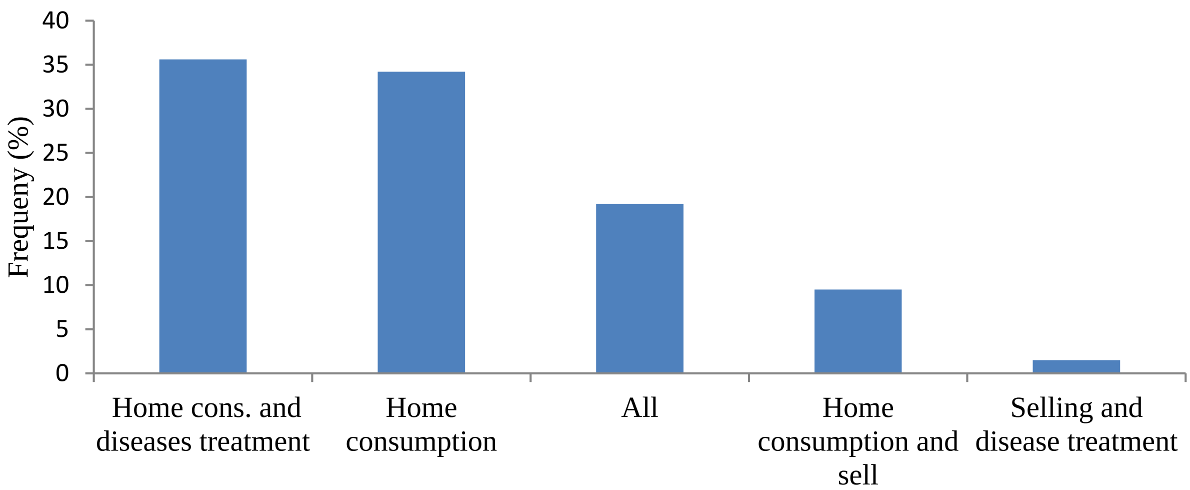

Figure 7. *Uses of stingless bees' honey*

Constraints and opportunities

In the study area, though honey collectors had some knowledge gained from their grandparents or parents, no evidence was found of the tradition of stingless beekeeping that had been practiced in the past or in the present. According to most respondents, the major challenges for stingless bee honey domestication were, in order of importance, lack of awareness about their domestication (39.7%), difficulty in finding a nest site (27.4%), lack of technologies (16.4%), low attention and effort by the government (12.3%) and low productivity (4.2%) (Table 9). In addition, honey badger is also a stingless bee enemy found in the study areas.

Table 9. Major constraints of stingless bee honey production

No	Factors	% respondents	Rank
1	Lack of awareness by pastoralists	39.7	1st
2	Difficult to find nest	27.4	2nd
3	Lack of appropriate technology	16.4	3rd
4	Low attention and effort of government	12.3	4th
5	Low productivity	4.2	5th

Respondents were identified potential opportunities for the domestication of stingless bees. As the respondents replied medical value (31.1%), high demand and price value of stingless bee honey (21.6), non-stinging (14.9%) and drought resistance (14.9%) were the major opportunities for the domestication of stingless bees (Table 10). Meliponines are relatively harmless because of their vestigial sting, and this helps for easy management compared to honeybees (Crane, 1992). Furthermore, there was evidence that the majority of respondents were showed high degree of interest to practice stingless bee domestication in the future. For better utilization and conservation of stingless bees, they suggested that every hunter should minimize the loss of these bees by showing great care for colonies during harvesting of honey.

Table 10. Major opportunities of stingless bee honey production

No	Factors	% respondents	Rank
1	Medical value	31.1	1st
2	High demand and price value	21.6	2nd
3	Non aggressive no side effect	14.9	3rd
4	Drought resistance	14.9	3th

Conclusion and Recommendation

The study revealed that the area has great potential for the existence of different species of stingless bees and their resources, which could lead to the production of significant volumes of stingless bee honey. During honey harvesting, care is given only for the quantity of honey, not for the colony and its nest. Moreover, the practices of wild honey hunting and nest destruction seem to threaten stingless bees in the area. Therefore, to avoid discarding stingless bee colonies and their nests after honey harvest, the introduction of Meliponiculture) using appropriate technologies while creating awareness among pastoralists is important to rescue and conserve stingless bees, thereby making the stingless bee honey production system in the area more economical and enhancing its products for National and International needs. Besides, further research is required in stingless bee species identification and potential threats for stingless bees as well as their domestication needs to be encouraged in the area.

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Effect of Storage time on honey quality in selected districts of west Hararghe zone, Oromia regional state, Ethiopia

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Abstract

This study was conducted to identify the effect of storage time on honey quality in West Hararghe zone. A structured questionnaire interview and checklist were the main tools used to gather primary data from the households. The household data were collected from 96 beekeepers in three districts of west hararghe zone. For the physicochemical analysis, honey was harvested from frame beehive of a single farmer and stored in a glass jar for 3, 6 and 9 months and analyzed for moisture, pH, free acidity, glucose, fructose , sucrose and HMF. Majority of the beekeepers owned traditional beehives. The average beekeeping experience of the sample beekeepers was 10.25 years with the range varying from 1 to 40 years of continued engagement in beekeeping. From the total number of respondents 79.2 were store honey for different purposes up to one year. Beekeepers store their honey in different sizes and types of materials available in the area with the most common being Plastic bucket (46.4%), followed by Plastic bucket and "qil" (29.8%), Plastic bucket,"qil" and "Tanika" (11.9 %) and "Tanika" and plastic bucket (6 %). From the respondents 97.9% were smoke the hive during honey harvesting and 53.1 said it has no effect on honey quality. Beekeepers identified Olea spp, Vernonia spp, cow dung, Juniperus procera and Carissa spinarum as major smoking material in the study area. The results of the physicochemical analysis indicated all the samples are within the acceptable range of world and Ethiopian honey quality standards with moisture (20.6%), free acidity (34.4meq/kg), pH (4.6), glucose (30.8%), fructose (33.4%), sucrose (3%) and HMF (8.5 mg/kg). From this study it was revealed that the quality of honey produced in the area was good during the nine month storage time.

Introduction

Honey, a natural product gathered by honey bees from either nectar or honeydew. Nectar is the most common source of honey worldwide, while honeydew is only common in European countries such as Greece and Austria (ref). Honey is a sweet, viscous food substance made by bees and some related insects. The variety of honey produced by honey bees (the genus *Apis*) is the best-known, due to its worldwide commercial production and human consumption. Honey is collected from wild bee colonies, or from hives of domesticated bees, a practice known as beekeeping or apiculture.

Because honey contains a variety of different carbohydrates, amino and organic acids, minerals, aromatic compounds, colors, waxes, and pollen grains, it has a complicated chemical composition (Sanz ML, et al., 2004). A physicochemical examination of honey's individual components is typically used to evaluate it. For the honey industry, these components which also contribute to the medicinal quality of honey include storage quality, granulation, and texture. Flavor and nutritional quality of the honey are also very important. The International Honey Commission (IHC) has therefore proposed certain constituents as quality criteria for honey. These constituents include PH, moisture content, electrical conductivity, reducing sugars, sucrose content, minerals, free acidity and hydroxymethylfurfural (Bogdanov SC, *et al.*, 1999).

PH is an important parameter during the extraction and storage of honey. It influences honey texture, stability and shelf life (Terrab A, *et al*, 2002). In general, a low pH of honey inhibits the growth and proliferation of microorganisms. According to Codex Alimentarius (2001) standards, the normal range of pH in honey is 3.4 to 6.1. If the pH is increases or decreases above or below the normal range, then several undesirable changes occurs in honey and therefore the honey is not suitable for human consumption.

In honey, several harmful and toxic compounds such as HMF may be present and they possibly showed their effect when consumed by humans. Several studies have been shown that the compound has adverse effects of causing mutation, toxic genetically and carcinogenic to mice (Teixido E, *et al*, 2006) and it also has adverse effects on blood cells. It was also reported that, it induced tumors and colon cancer also (Lakka NS and Gosewami NA, 2011). Due to potential toxic effects, HMF is essential for assessing the conformity of honey (Lakka NS and Gosewami NA, 2011). Honey quality is significantly influenced by storage time and heating. The increasing concentration of the HMF compound on the limit refers to the increasing age of the honey (Tosi EA, *et al*, 2004). HMF content in honey is an important parameter for determining the quality of honey, its age, antioxidant activity as well as its nutritional value (Nozal MJ, *et al*, 2001).

The West Hararghe zone, the study area, has a significant potential for the production of honey. Because of the various kinds of bee forage that are present in the region, bees produce honey with a variety of ingredients, flavors, and colors. When honey was tested in a lab after being stored for a specific amount of time, its physiochemical qualities vary. The farmer keeps honey in storage for several months in order to anticipate the moment when it will become more expensive and to utilize as medication when necessary. Therefore, the length of storage has an effect of its own. As a result, the goals of this study were the goals of this study were to understand how long honey is stored for and how that affects its physiochemical characteristics in the study area, and to evaluate how long honey is stored for in the study area using different storage methods and materials.

Materials and methods

Description of study area and data collection

The study was conducted in three districts (Gemachis, Oda Bultum and Daro Labu) of West Hararghe Zone of Oromia National Regional State, Eastern Ethiopia. The districts were selected based on agro ecology to represent the high land mid land and lowland. Gemachis district has mean annual temperature of 17 degree Celsius. The area has also 1280 mm annual rainfall. Oda Bultum district is characterized by 28 and 13 degree Celsius maximum and minimum daily temperature and 1053 mm mean annual rainfall. Daro Lebu district has 21.5 mean daily temperature and 1120 mm mean annual rain rainfall (Wasihun Gizaw, 2021 unpublished**)**.

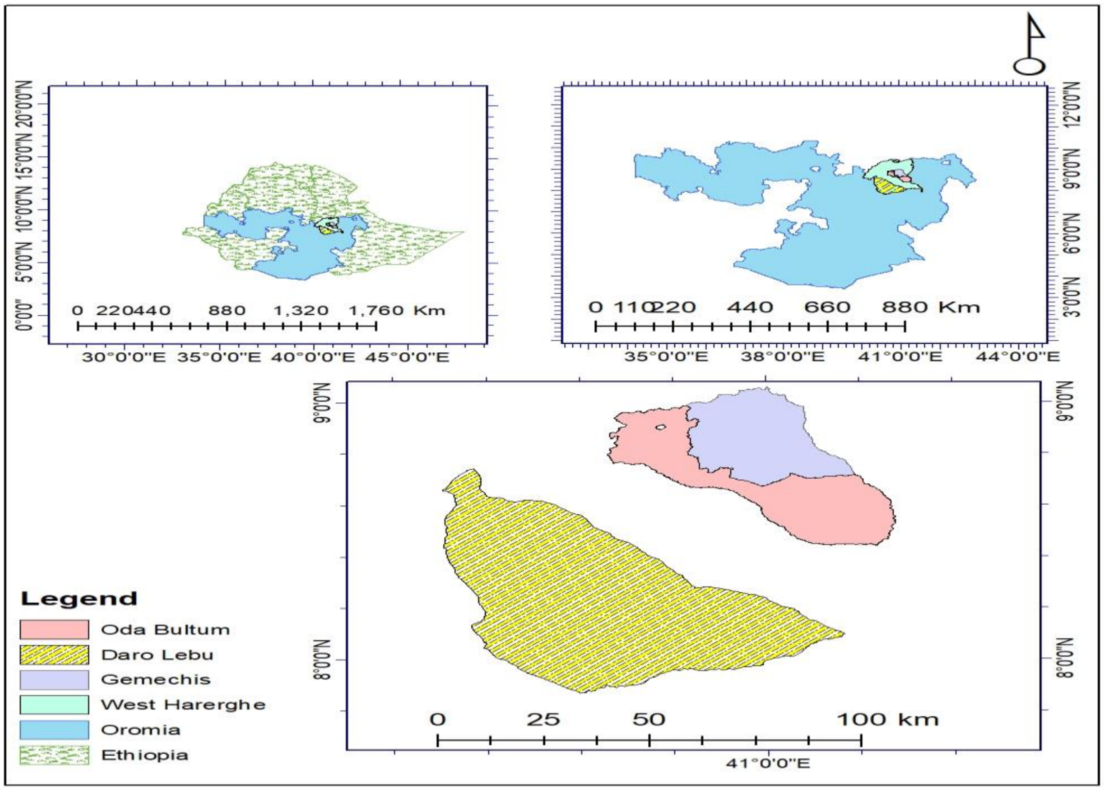

Figure 1 map of the study area and sampling district

Phase I: Survey Parts

Primary and secondary data was collected by using questionnaire and checklist. And again data like market price of honey, honey harvesting Method, honey handling, Storage material that farmer's use, for how many month or year that farmers are store honey and constrainer of storage were collected. Additional data was collected through focused group discussion from experts, community groups, development agents and farmers. All necessary information like socio-economic description of the household, honey production potential, honey flow season was also collected. 32 farmers per district were interviewed totally 96 from the zone.

Phase II: Laboratory Parts

Honey samples were collected from three agro ecologies (high land, mid land and Low land) of West Hararghe zone for laboratory analysis. All the samples was collected freshly in sterile containers of glass jars (labeled with numbers, place and date of collection) and was stored under room temperature (25-29°C) in the laboratory until analysis. Then, 126 samples were prepared and analyzed by three month interval from harvesting time, for physicochemical in order to know whether the component that exist in the honey like, Ash content, PH, HMF, glucose content, fructose content, sucrose content, moisture content, acidity and so on are increase or decrease by using International Honey commission (IHC, 2009) procedure.

Laboratory procedure

Determination of pH and of free acidity by titration to pH

The samples were ensured for representativeness. 10 g sample was dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker. Stirred with the magnetic stirrer, the pH electrodes were immersed in the solution and the pH were recorded. Titrated with 0.1M NaOH to pH 8.30 (a steady reading obtained within 120 sec of starting the titration; in other words, the titration was completed within 2 minutes).

Determination of hydroxymethylfurfural after White

5g of honey accurately weighed approximately into a 50 ml beaker. The sample was dissolved in approximately 25 ml of water and transferred quantitatively into a 50 ml volumetric flask. 0.5 ml of Carrez solution I added and mixed. 0.5 ml of Carrez solution II added, mixed and made up to the mark with water (a drop of ethanol may be added to suppress foam). Filtered through paper; the first 10 ml of the filtrate rejected. Pipette 5.0 ml in each of two 2 test tubes (18 x 150 mm). 5.0 ml of water added to one of the test tubes and mix well (the sample solution). 5.0 ml of sodium bisulphite solution 0.2% added to the second test tube and mix well (the reference solution).

Honey Storage

Honey is stored in a cool place for longer shelf life. It is stored at very low (-20°C) and close to ambient (20°C) temperatures resulted in fine crystals and coarse grains, respectively. While storing in the mild temperature range (4–10°C) resulted in mixed size crystals. Therefore, the honey can be processed into a number of value added products ranging from intermediate moisture to dried products, to overcome the problems of storing liquid honey and of crystallization during its storage. Honey in the dried form could overcome these problems, and therefore, has good commercial potential in the bakery and confectionary industry. Usage of honey powder in dry mixes for cakes and bread found to improve the sales appeal, as well as flavor, color, aroma, texture, and the keeping quality of the product.

Effect of Storage

During storage honey becomes crystallized sooner or later. Crystallized honeys are not popular with consumers and can only be marketed when it is liquefied. In order to change to a liquid, heat is needed. Gentle heating (32–40 °C) is mostly used to liquefy crystallized honey and to destroy yeast. Besides long period of storage high temperatures can adversely affect the chemical composition of honeys (Castro-Vazquez *et al.*, 2008).

Physiochemical properties of honey

Sugar Content

Honey is supersaturating sugar solution, where carbohydrate is the main constituent's accounts for about 95% dry. The most important physiochemical and nutritional properties of honey, such as sweetness, viscosity, granulation specific rotation and energy value depends on sugars composition (Cavia *et al.*, 2002).

Hydroxy-methylfurfural

HMF is a furanic compound produced by sugar degradation, from dehydration of hexoses in acidic medium and to a lesser extent as an intermediate in the Millard reactions. HMF is the quality index which is a good model to estimate shelf life of honey (Biagio *et al*., 2009).Several factors such as temperature, time of heating during processing, storage conditions, aging of honey products and sources of floral were found to influence the HMF content in honey (Mohamed *et al.*, 2013).Hydro material increases during honey processing by heat treatment and also by adulteration with a commercial sugars and thought storage (Belay *et al*., 2013).

Free acidity

Honey could have be difference in its free acidity. This difference in free acidity among different honeys can be attributed to floral origin or to variation in the harvest season. The limit for honey acidity according to EU honey standard (2002).

Moisture Content

After the carbohydrates, water is the second most important component of honey. Moisture content ranges between 15 and 23% and it substantially affects some physical properties of honey (crystallization, viscosity, specific weight).Moisture contents of honey from different locations and hive types are depends on the environmental conditions such as temperature, relative humidity of the area and the manipulation of honey during harvesting period by beekeepers and seasonal variation (Acquarone *et al*., 2007; Argentine Chemical Society, 2008).

Data Analysis

In order to analysis the above data, SPSS. 20 versions Software and excel spreadsheet were used.

Results and Discussions

Household Socio-Economic Characteristics

From the total of 96 sample households interviewed, about 80.2% and 18.8% were male and female headed household, respectively (Table 1). The mean age of the respondents was 41.76±12.117 years and ranged from 20 to 70 years (Table 2). The result showed that beekeeping performed by different age groups and in most cases people at younger and older ages are more engaged in beekeeping. The average family size 5.33 persons, with minimum and maximum family size of 1 and 12 persons, respectively (Table 2). The average beekeeping experience of the sample beekeepers was 10.25 years with the range varying from 1 to 40 years of continued engagement in beekeeping. The average landholding during the study year was 0.82703 hectares with minimum and maximum holding of 0.125 and 3.000 hectares per household, respectively. From the total beekeepers, 96.9 % were married while 1% and 1% were single and divorced, respectively (Table 3). Based on the study result, beekeeping activities could be performed by every social class of the community regardless of their marital status. Among the respondents 31.3%, 27.1% and 34.4 % were illiterate, read and write and other, respectively. The survey result showed that all the beekeepers in the area are using all types of beekeeping practices. Majority of the beekeepers practice traditional beekeeping having maximum number of 30 colonies.

Table 1 sex of the household

Sex of the HH	Frequency	Percent
Male	77	80.2
Female	18	18.8
Total	96	100.0

Table 2 Family size, experience, age, and landholding of household heads (N = 96)

Socio-Economic Characteristics	N	Minimum	Maximum	Mean	Std. Deviation
Age of the respondent (yrs)	91	20	70	41.76	12.117
Family size	95	1	12	5.33	2.482
Landholding (ha)	86	.125	3.000	.82703	.543061
Experience in beekeeping (yrs)	93	1	40	10.25	9.613

Table 3 Educational level and marital status of the respondents

Socio-economic characters	Frequency	Percent
Educational level	7	7.3
Illiterate	30	31.3
Read And Write	26	27.1
Other	33	34.4
Marital Status		
Single	1	1.0
Married	93	96.9
Divorced	1	1.0

Table 4 honeybee colony ownership

Hive type	Minimum	Maximum	Mean	Std. deviation
Frame hive	0	7	2.42	1.816
Transitional hive	2	4	3.00	1.414
Traditional hive	1	30	5.02	5.444

From the respondents 97.9% were smoke the hive during honey harvesting and 53.1 said it has no effect on honey quality. *Olea*, *Vernonia spp*, cow dung, *Juniperus procera* and *Carissa spinarum* were the major smoking material in the study area.

Honey storage

79.2 of the total respondents stored honey for medicine, strong market value and private consumption. Most of the responders only keep their honey in storage for one year(Table 5).

Table 5 honey storage

Respondent	Frequency	Percent
Yes	100.0	79.2
No	18	18.8
Total	96	100.0

Storage material

The most popular container used by beekeepers to store their honey is a plastic bucket (46.4%), followed by a plastic bucket and "qil" (29.8%), a plastic bucket, "qil," and "Tanika" (11.9%), and "Tanika" and a plastic bucket (6%).

Table 6 storage material

storage material	Frequency	Percent
Plastic bucket,"qil" and "Tanika"	10	11.9
Plastic bucket	39	46.4
Qil and "Tanika"	2	2.4
"Tanika" and plastic bucket	5	6.0
Plastic bucket and qil	25	29.8
Stainless steel	3	3.6

Physicochemical property of honey samples

Table 7 mean value of physicochemical property of honey at different storage period

Time (months)	PH value	Moisture (%)	HMF (mg/kg)	Free acidity (mequiv/kg)	Glucose (%)	Fructose (%)	Sucrose (%)
3	3.9	20.2	7.1	36.0	27.0	37.5	0.5
6	5.7	21.9	9.7	25.2	26.4	34.4	2.6
9	4.0	20.8	8.6	42.0	38.9	28.3	5.8
Over all mean	**4.6**	**20.6**	**8.5**	**34.4**	**30.8**	**33.4**	**3.0**
Ethiopian standard	**3.2-4.5**	**<20**	**<40**	**<40**	**-**	**-**	**<5**

The pH

Gemechis district showed significant increase in the pH, from 4.1-5.8 in the last six months of the storage and the pH declined to 4.2 during nine month storage. After six months of the storage, honey samples of the all sites experienced increased in the pH, whereas each sample of the honey from all site showed decrease in pH after nine months of storage. On the whole, Gemachis honey samples showed fluctuation in the pH during storage. The pH increased to 5.8 during the initial six months and decreased to 4.2 during the subsequent three month of storage (Fig. 1). The pH increased during the initial three months of storage which was 3.4-5.4 in Daro Lebu sample of the honey. Instead of further increase, pH of Daro Lebu honey samples showed a decrease, becoming lower than the one recorded at six month storage. The pH increased from 4.2 to 5.9 in oda bultum's honey during the initial six months. Instead of further increase, pH of the honey samples decreased to 4.2 at the end of nine months of storage. The pH of honeys samples from West hararghe zone showed significant difference (p=0.002) among the sample districts.

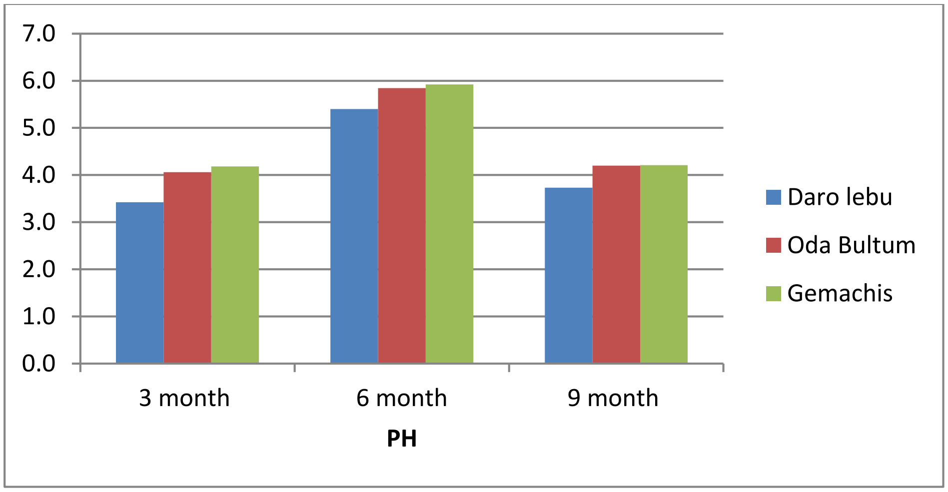

Figure 1 the effect of storage time on pH of honey samples

Moisture content

The moisture content is an important criterion for evaluating the grade of ripeness of the honey and its shelf-life. In general high amount of water causes the honey to ferment, to spoil and to lose flavor, with ensuing honey quality loss. Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year. High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. During storage of honey, the undesirable fermentation is caused by the action of osmotolerant yeasts resulting in formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water resulting in a sour taste. The moisture content of honey increased significantly with the increase of storage period. However, the values of moisture content of honey after 3, 6, and 9 months, of storage varied critically and were found to be non-significant. The mean value of honey at nine month storage was 20.6 %. The results of the present study of moisture content are in agreement with the findings of Fasasi (2012) who reported that the moisture content in ranges of 17.5-18.4%. Also supports the findings of Chakraborti and Bhattacharya, (2014) and Evahelda *et al.*, (2017) who reported increase in moisture content from 20.13 to 21.06% and 24.25 to 26.30 respectively with storage. Results clearly indicate that moisture content increased with increasing storage period of honey and decreased.

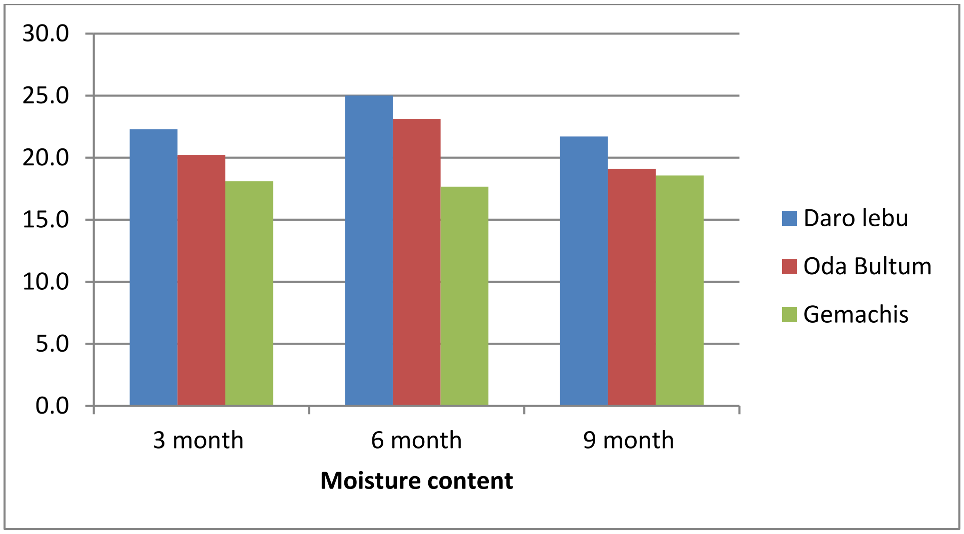

Figure 2 the effect of storage time on moisture content of honey

Acidity

On the whole, at the end of nine months of storage, the free acidity value in all Gemechis, Daro Lebu and Oda Bultum honey samples showed 37.84 meq/kg, 30.84 meq/kg, 34.5 meq/kg), value respectively. When stored, Gemechis honey samples had high free acidity values, which according to the international standards was close to the upper permissible, limit (50meq/kg). These samples showed (35.8- 30.5 meq/kg) decrease in the free acidity during initial six months of storage time and in the later three months free acidity of the honey increased to 47.3meq/kg (Fig. 3). Ageing increased the free acidity value of every independent sample during nine months storage. The free acidity of all honey samples from West Hararghe zone were less than 50 meq/kg, a maximum limit for acidity prescribed by International Honey commission (2009).

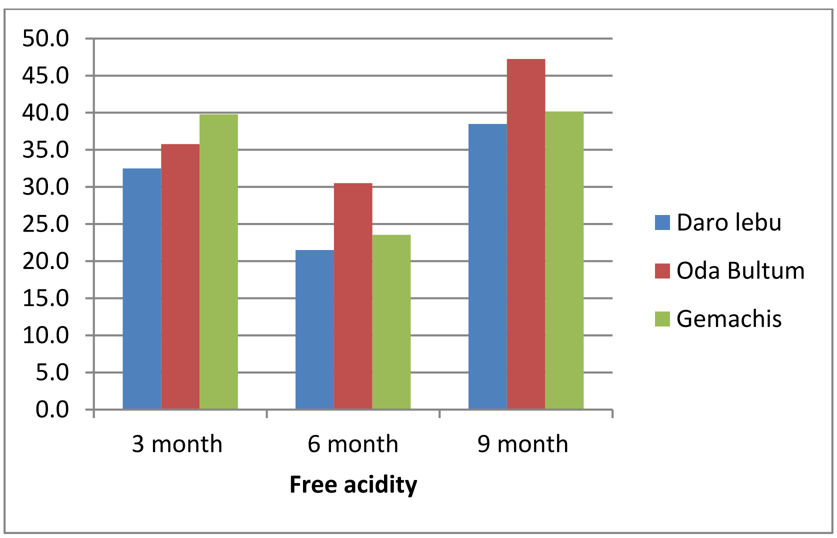

Figure 3 the effect of storage time on free acidity of sampled honey

Hydroxyl-methyl-furfural (HMF)

The HMF content in the samples of honey from Gemechis district decreased from 12.1 to 9.3 during three month to six month storage time. After six months of storage HMF content was less than permissible limit of 40 mg/kg in the samples. Daro Lebu honey samples showed significant increase in HMF content 2.8-9.2mg/kg during the first six months and decreased to 6.8 by the end of nine months (Fig. 4). The HMF content at Oda Bultum showed continuous increase during the six month storage time 6.2-10.7 mg/kg and decreased to 6.2 mg/kg after nine month of storage. The results are in line with Tafinine *et al*., (2018) who reported that the HMF content in honey increased with increase in the storage duration.

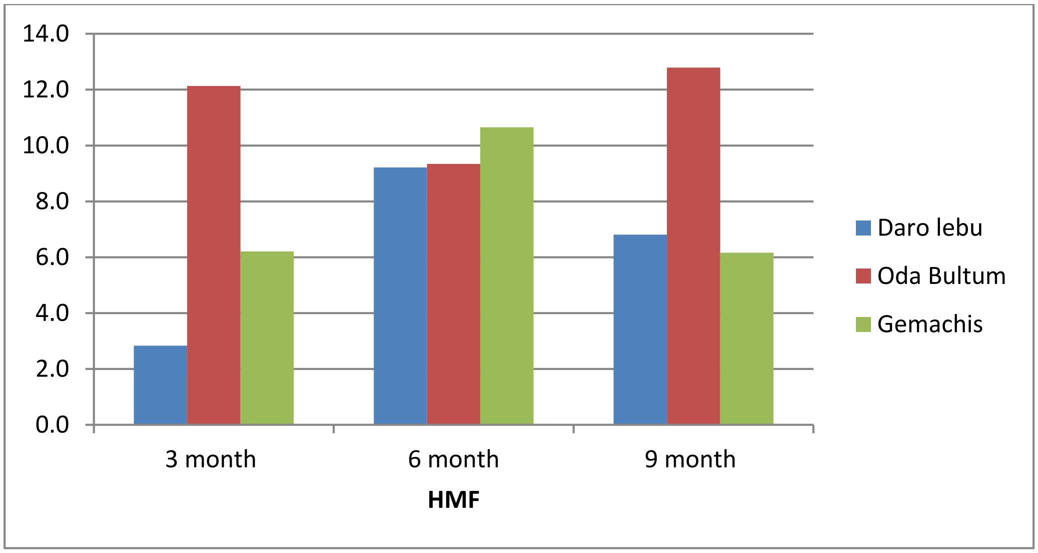

Figure 4 the effect of storage time on HMF

Fructose

The fructose content of honey sample of the study area was 33.4 g/100g. A significant difference was declared between districts (p=0.000) in terms of fructose content of the honey. The current finding is in line with Aregay *et al*., (2018) who reported the mean fructose content of 38.64± 0.61 g/100g from the Godere district.

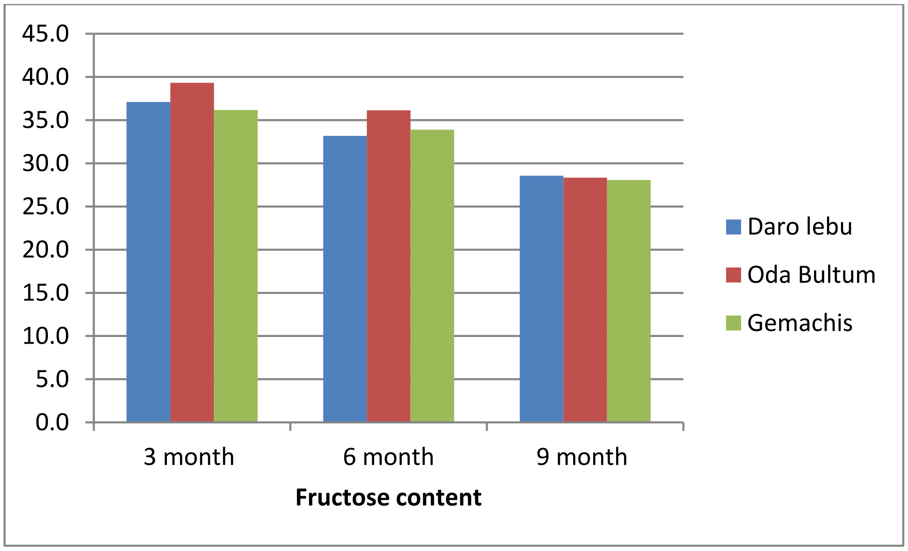

Figure 5 the effect of storage time on fructose content

Glucose content

The glucose content of the evaluated honey samples of the study area was 30.8 g/100g. There was a significant difference between districts (p=0.001) in terms of the honeys glucose content. The difference in the glucose content between the sources of the honey collection might be due to the difference in the flora of honeybee and Agro ecology. Aregay *et al*. (2018) reported similar finding of 36.37± 2.14g/100g glucose content from Godere district. The sugars of honey are responsible for several of the physicochemical properties such as viscosity, hygroscopic and granulation characteristics of honey.

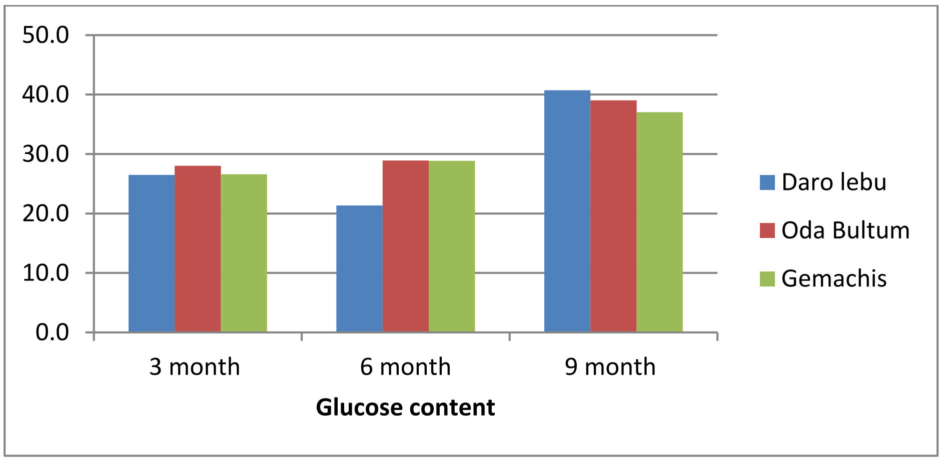

Figure 6 the effect of storage time on glucose content

Sucrose

The mean sucrose content of the sampled study area honey was 3 g/100g. The result revealed that the samples were within the standard range of QSAE (2005). A significant difference was not noticed between districts (P=0.044) in sucrose content of honey. The higher sucrose content of honey from shop as opposed to the farm-gate source of honey might be due to the adulteration of honey by the addition of commercial sugar in honey to increase the volume of honey. Additionally, it might be due to the early harvest of honey before sucrose is converted into fructose and glucose (shop traders might purchase unripen honey from their customers). In line with this result, Eyobel and Miresa (2017) reported the mean sucrose content of 2.60±0.51 g/100g from the Adaberga district of West Shewa zone. Nevertheless, higher than the current finding Alemayehu and Nuru (2011), Chala *et al*. (2011), Awraris *et al*. (2014), Addis and Malede (2014) and Abebe (2017) reported the mean of 4.1±1.2 g/100g, 7.55±4.03 g/100g, 4.46±2.59g/100g, 7.55g/100g, and 4.04g/100g sucrose content, respectively, from the different locations of the country. The variation in sucrose content from different parts might be due to harvesting, handling practices and flora sources. The low sucrose content of the studied honey samples indicated that honey produced from the study areas (farm gate) was natural and free of any adulteration. . International Regulatory Standards restricted sucrose content not to be greater than 5g/100g of honey.

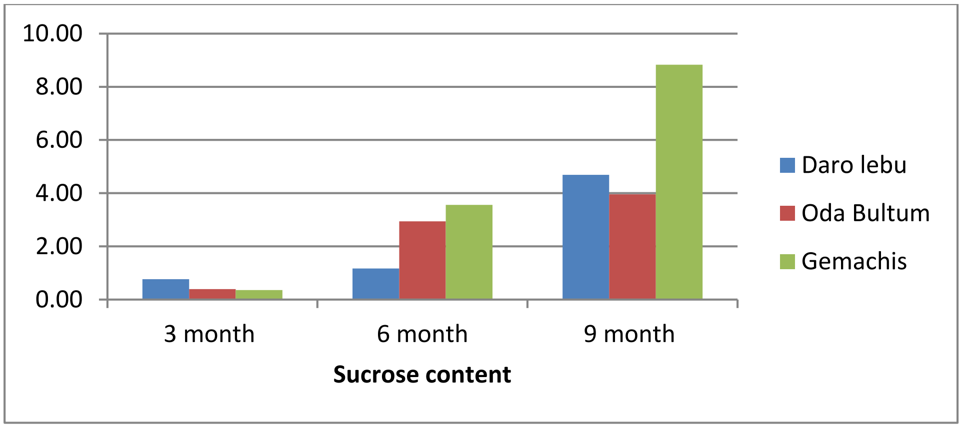

Figure 7 the of storage time on sucrose content

Correlation

As shown in Table 8 below, pH is strongly positively correlated with moisture and HMF; moisture content and HMF are also positively correlated. Sucrose and glucose are also highly positively correlated.

Table 8 Correlation between physicochemical properties of honey

Time	pH	Free acidity	Moisture	HMF	Fructose	Glucose	Sucrose
pH	1						
Free acidity	-0.97	1					
Moisture	1.00	-0.99	1				
HMF	1.00	-0.94	0.99	1			
Fructose	0.18	-0.44	0.27	0.11	1		
Glucose	-0.39	0.61	-0.47	-0.32	-0.98	1	
Sucrose	-0.12	0.37	-0.21	-0.04	-1.00	0.96	1

Conclusion and Recommendation

Beekeeping is practiced as an integral part of other agricultural activities mainly livestock and crop production. The majority (95.5%) of the beekeepers own traditional hive.

From the total number of respondents 79.2 % were store honey for different purposes. Majority of the respondents store their honey for only one year in different sizes and types of materials available in the area with the most common being Plastic bucket (46.4%), followed by Plastic bucket and “qil” (29.8%), Plastic bucket,”qil” and “Tanika” (11.9 %) and “Tanika” and plastic bucket (6 %). The results of the physico-chemical analysis indicated that except the pH all the samples are within the acceptable range of world and Ethiopian honey quality standards with moisture (20.6%), free acidity (34.4meq/kg), pH (4.6), glucose (30.8%), fructose (33.4%), sucrose (3%) and HMF (8.5 mg/kg). Honey collected from the study area is in good quality standard during storage, but further analysis is important to determine the effect storage time on honey quality in relation to different storage materials that farmers use and with different agro ecology.

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Diagnostic survey of Honeybee Diseases, Pests and Predators in Bale zone Southeast Oromia Region, Ethiopia

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Abstract

A cross-sectional study was conducted on parasitic honeybee diseases, pests and predators in Bale Zone southeastern Oromia. It was conducted in three selected districts with the objective to determine prevalence of honeybee diseases, pests and predators and to identify potential risk factors. For the study questionnaires and diagnostic survey were employed. A total of 81 beekeepers were interviewed for questionnaire survey. For major honeybee diseases and pests examination a total of 72 suspected bee colonies from 25 apiary sites were sampled. A sample of adult worker bees and brood for major honeybee diseases were taken and analyzed in veterinary laboratory of respective districts of the study. 200-300 adult worker bees were used for varroa mites and bee lice diagnosis by washing methods. SPSS version 20 was used for data analysis using descriptive statistics like, frequency, percentage and chi-square tests for associated risk factors and ranking index for ranking. From the present study, the major challenge of beekeeping activities declared by beekeepers includes pests and predators, lack of bee forage, absconding and mass death of bee colonies. Regarding to honeybee pests and predators, the most important identified were honey badger, spiders ant, wax moth, bee-eater birds, small hive beetles and monkeys. Majority of the respondents replied that honeybee pests and predators are the serious problems of honeybee colonies responsible for absconding and infestation. About 38.12 %, 37.67 %, 37.66 %, 19.96 %, 17.1 % and 6.81 % of the honeybee colonies absconded were due to honey badger, spiders, ants, wax moth, bird eater bee and small hive beetle respectively. Around, 12.2 %, 11.49%, 11.21 %, 8.65 %, 7.51 %, and 5.53 % of the honeybee colonies infested/attacked were due to wax moth, ants, honey badger, bird eater bee, spiders and small hive beetle respectively. The total honeybee colonies absconded and infested due to the major honeybee pests and predators estimated to be 388 (55.04 %) and 399 (56.67%) respectively. The finding of the study indicated the overall prevalence of Nosema apis, Amoeba mellifera, Varroa mites, bee lice, wax moth, small hives beetles in adult worker bees were 79.17 %, 79.16 %, 72.22 %, 18.06 %, 40.27 % and 54.2 % respectively. However, the current diagnosis of honeybee diseases were not detected American foul brood diseases, European foul brood diseases, and trachea mites. Agro ecology and hive types were identified as risk factors for prevalence of honey bee diseases and pests. To make benefit of the beekeeping sector, it should be very important to create awareness on technical bases for beekeepers in the study areas. Further study on prevalence of seasonal honeybee diseases and outbreak of honeybee diseases and pests is could be very important.

Key words: *Honeybee, Nosema apis, Malpighamoeba mellificae, diagnostic, disease, pests*

Introduction

Honeybees are essential pollinators of wild and cultivated plants and are vital to food production and biodiversity conservations. Honeybees provide a vast range of products for humans from honey to other bee products, such as pollen, beeswax, royal jelly, venom, etc. *A. mellifera* are commercially valuable as essential plant pollinators, and for the high demand for their products, like honey and wax. Honeybee colonies existing in the wild away from man's control produce a small surplus of honey above their requirements signifying beekeeping is much more productive and profitable if they only managed properly (Moeller, 1982).

Like other all living organisms, honeybees can be infected and infested with harmful diseases and pests respectively. The decline of the honeybee population due to agricultural chemicals, pests, predators, and diseases is of great concern to many countries around the world, including those in Asia (Kajobe *et al*., 2016; Abdulhay & Yonius, 2020). According to Kajobe *et al*. (2016) and Abdulhay and Yonius (2020) in tropical regions, beekeeping is threatened by various pests, predators, and diseases, which often lead to economic losses. Most of the time pests, predators, and diseases interact with each other and affect the health performance of the honeybee colonies, and reduces the yield of bee products (Forfeit *et al*., 2015). Hence, it is vital to maintain a healthy honeybee population to ensure the supply of honey and other bee products to adequately meet the domestic and international market demand. The recent high honeybee colony losses in many parts of the world are associated to the vulnerability of honeybees to parasitic mites, fungi, viruses, and bacteria (Bradbury, 2009). These pathogens and parasites can have harmful effects on honeybee health and the services they offer, which in turn can lead to severe economic losses (Genersch, 2010).

Even though, the majority of pathogens and parasites affecting honeybees have almost worldwide distribution (Ellis and Munn, 2005). The most commonly known honeybee diseases reported to exist in Ethiopia are Nosema and amoeba (Gezahegn T and Amssalu Bezabeh, 1991; Amssalu Bezabeh and Desalegn Begna, 2005, Desalegn Begna, 2005) and chalk brood (Aster et al, 2007; Amssalu et al, 2009; Aster et al, 2010; Amssalu et al, 2010).

Although research on diagnosing honeybee diseases and pests was done in Ethiopia at various times by various researchers, there were several areas that had not yet been well covered. Bale zone of Oromia regional State is noteworthy among the areas that have not yet been thoroughly researched since it has a significant potential for beekeeping. To fully take advantage of the beekeeping resources available in the Bale zone, it is prudent to conduct honeybee disease and pest diagnostic studies in this area. The goal of the current study was to identify the prevalent diseases, predators and pests in their effect in Bale zone as well as the risk factors that go along with them.

Materials and methods

Description of the study areas

The study was conducted in Bale zone Southeast Oromia Regional state, Ethiopia. Honeybee pathogens and pests were diagnosed and assessed from 2020-2021 in three selected districts namely Dinsho, Dello-Menna, and Goro districts. The study was conducted in Bale zone Southeast Oromia Regional state, Ethiopia. Honeybee pathogens and pests were diagnosed and assessed from 2020-2021 in three selected districts namely Dinsho, Dello Menna, and Goro districts. Dinsho district is found 30 km from zone town Robe, and 400 km from from the capital city of Ethiopia, Addis Ababa. Dello Menna district is found 125 km from the zonal town, Robe, and 555 km from Addis Ababa. Goro district is found about 60 km from zonal town, Robe, and 490 km from Addis Ababa. Geographically, Bale zone is located at below Figure shown.

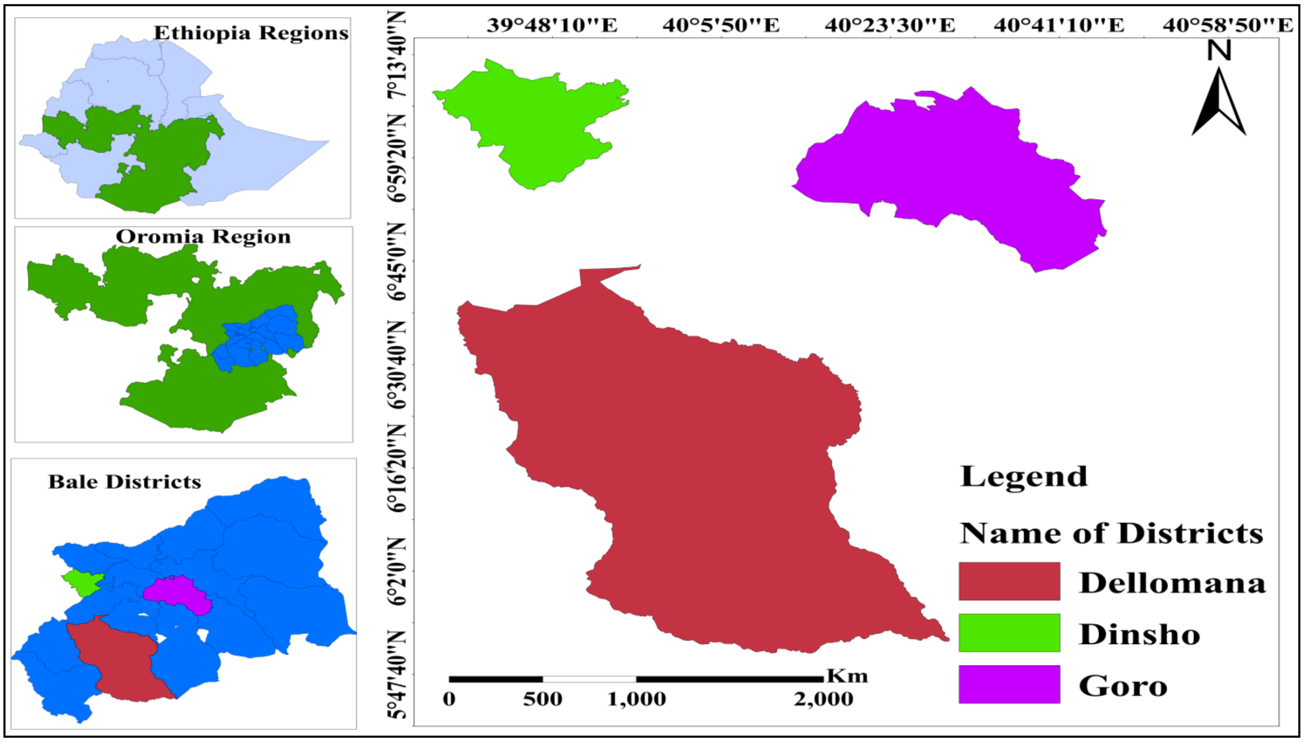

Figure 1.Map of the study areas

Study design

A cross-sectional study was carried out from 2020 to 2021 in three selected districts in Bale Zone to identify the major honeybee diseases, Pests and predators through inspection and examination of collecting samples from the colonies. Questionnaire survey was carried out during diagnostic study to determine honey production system and constraint caused due to pests and predators. Types of hive and agro-ecology were considered as explanatory variables (risk factors), and tested whether they have an impact on occurrence of honey bee disease and pests. Honeybee hive was categorized as Traditional, Transitional and Modern hives. Three altitude categories were considered: highland (>2400 meters), Midland (1800 to 2400 meters) and lowland (less than -1800 meters above sea level). Finally, prevalence for apiary and colony levels was calculated following the protocols of Vanenglesdorp *et al.* (2013).

Sampling method and sample size determination

For this study, multi sampling techniques were employed to collect sample of honeybee diseases and pests from suspected bee colonies in the study areas. Districts were selected using purposive sampling method based on their potential of beekeeping. Three rural kebeles from each district Abakera, Mio and Dinsho 02 from Dinsho district; Gomgoma, Cirri, and Erba from Delo Menna district and Balle Gadulla, Balle Anole and Dayu Abargada from Goro district were also selected purposively based on their beekeeping potential and representativeness of highland, midland and lowland agro-ecologies. 3 to 4 honeybee colonies were selected randomly from each apiary and inspected for pests and diseases at field. Brood and adult bees were also taken from the same colonies. In general 72 honey bee colonies were diagnoses in this study.

Sample Collection and Questionnaire survey

In this study, a semi structured questionnaire was prepared and administered to collect information from the beekeepers. Both primary and secondary sources of data were used. Secondary data were obtained from reports of zonal and districts of agricultural Office, and other published and unpublished materials. Primary data was collected using questionnaire survey. Information about trends of beekeeping practices and constraint caused due to pests and predators was collected through interviewing 81 beekeepers using a structured questionnaire.

Diagnosis for major honeybee diseases

Diagnosis of major honeybee diseases was conducted at field through inspection and examination of samples at laboratory of perspective districts Veterinary for Chalk brood, American foul brood, European foul brood, Nosema, Amoeba using their respective protocols.

Diagnosis for Chalk Brood (CBD)

Both external and internal inspection was conducted for the presence of chalk brood clinical symptoms. A dry scale with white to dark color molds and chalk brood mummies was carefully inspected in the comb cells and on the bottom boards of the hives. From each suspected colonies samples were collected from the comb cells and brought to lab for examination. The suspected samples placed on the slid and then moistened with a drop of distilled water. The suspension was examined for spores and/or, spore ball and/or spore cysts of *Ascosphaera apis* using light microscope under magnification power of 40 x.

American Foul Brood (AFB) and European Foul brood (EFB)

For AFB and EFB diseases in selected apiaries 3 to 4 colonies were inspected internally for the major clinical symptoms. Typical clinical symptoms like irregular brood arrangement, sunken and dark capping with puncture holes, dead and decayed larvae with dark “scales” and slight odor suspected was examined for the occurrence of AFB. Similarly, twisted larvae with creamy-white guts visible through the body wall, melted and yellow white larvae with unpleasant sour odor and loosely-attached brown scales were directly observed for the infected colonies of EFB. Furthermore, match stick test (stretch test) were employed to observe the robby thread stretching for the typical clinical symptoms of bacterial diseases. For the suspected brood showing one of the above important clinical symptoms, brood samples were prepared on microscopic slide for further laboratory diagnosis. The samples was examined under microscope for the presence of *Paenibacillus larvae* and *Melissoccocus pluton* in positive samples AFB and EFB, respectively using Zeiss AxioVert A.1 light microscope under oil immersion (magnification power of 100X).

Protozoa diseases (*Nosema apis and Amoeba*)

For suspected colonies, a sample of 30-60 worker adult honeybees was collected from the hive entrance following Fries *et al.* (2013) procedure. The abdomens were placed and grounded by mortar containing 5-10 ml distilled water until an even suspension is formed using pestle. The mortar and pestle were thoroughly cleaned before being used again. A loop of suspension were placed on microscopic slid using the sterilized loop and covered with cover slid. The suspension was examined under microscope using 40-

magnification power for the presence of Nosema slippery and rod shaped spores and for Amoeba round cysts and spore balls.

Diagnosis for honeybee Ecto-parasites

Examination for Varroa mites

For diagnosis about 200-300 adult workers bees samples were collected from suspected bee colonies. The samples were examined using shaking method by adding detergent solution (10 ml of detergent is used to 1000ml of water solution) was poured into each of jar containing bees up to half of the jar get full. Then shaking for one minute until the varroa mites dislodged from adult honeybees. Straining the solution through a ladle (8-12 mesh) to remove the bees and then sieving the solution through tea strainer to collect Varroa mites. Finally, the presence of varroa mites was checked either by necked eye or by using hand lens count according to Cramp, 2008.

For examination of varroa mites in bee brood, samples were taken from the suspected 3-5 bee colonies of brood comb 5cm x 5cm size. About 100 pupae was removed from their cells using forceps or match stick test and checked for the presence of varroa mites. At the end, number of varroa mites per diagnosis samples was recorded and determined the infestation level per colony.

Bee lice: For the examination of bee lice, the same procedure with varroa mite was followed.

Examination for Tracheal mite

For diagnosis of tracheal mites, an average samples of 60 worker bees' were collected from suspected honeybee bee colonies in order to detect 5% of diseased bees with 95% confidence (Fries I, 1988) . The abdomen of bees was held between thumb & forefinger & push off the head with forelegs, cut parallel a small disc of the breast put all the discs in small bottle containing 10% Potassium and boil in water bath for 4-8 minutes put the discs on wire gauze. Then the discs was rinsed in tape water to clean all muscles dissolved, put on slide with few drops The trachea suspension was examined for infested part and *Acrapis woodi* under to examine through microscope using magnification of 40X.

Diagnosis of major honeybee pests

The occurrence of major honeybee pests in the all the study areas was determined through internal and external hive inspections. In addition, for clinical symptoms and infested combs, adult and larvae of small hive beetles and wax moth was detected in the hive through inspection of the beehives described by Neumann *et al.* (2016).

Infestation and prevalence

$$\textbf{Infestation}\ (\%) = \frac{\textbf{Positive number of bee colonies}}{\textbf{The total number of bee colonies examined}} \boldsymbol{X100}$$

$$\textbf{Prevalence}\ (\%) = \frac{\textbf{Number of sites diseases positive}}{\textbf{The total number of sites surveyed}} \boldsymbol{X100}$$

Ranking of the different types of beekeeping constraints obtained in the study were done by using the rank index formula as described by (Musa *et al.*, 2014).

Data Management and Statistical Analysis

The collected data were entered & stored into Microsoft Excel program for further analysis. SPSS version 20 statistical package was used to analyze the data. Summarized data was presented in the form of tables and figures. For the survey data, analysis was done by using descriptive statistics and the rank index formula as described by (Musa *et al*., 2014). Chi-square test was used to assess the association of the risk factors with the prevalence of the disease and pests. Statistical significance was set at $p \leq 0.05$ with 95 % CI

Result and Discussion

Socio-economic characteristics of the Respondents

Out of the total sample respondents about 96.3% were male headed household and only 3.7% were female headed household. This indicated that most of beekeeping in study area is male's job and it is probably because of traditional taboos that females are not allowed to carry beekeeping activities in the study areas. A similar issue was reported by Tesfaye *et al*. (2017) from the high lands of Bale and Workneh (2006) also reported beekeeping as male-headed households dominated activity in the Northern part of Ethiopia.

According to the current findings, beekeepers' ages range from 30 to 80 years old, with a mean age of 49.17 years (Table 1). This result demonstrated that beekeeping activities were carried out by different age groups and more actively by younger age groups. This result in line with the report of Tesfaye *et al*. (2017) and Chala *et al*. (2012) whose reported from high land of Bale and Jimma Zones respectively.

Table 1. Age of sample respondents in the study areas

Districts	Total sample size (N=81)		
	Maximum	Minimum	Mean ± SE
Dinsho	80.00	32.00	52.00±3.29
Goro	70.00	30.00	44.71±3.27
Dello Menna	72.00	30.00	50.80±3.18
Overall mean	**74.00**	**3.67**	**49.17 ±3.25**

Source: Survey result, 2022

Practices of beekeeping time in the study areas

Beekeeping in the study districts was exclusively traditional between 1966 and 1996. Between 1997 and 2020, enhanced modern beekeeping practices began in the study areas. Transitional beekeeping and frame box hive beekeeping practices were respectively begun in the same year by 35.9% and 55.6% of the respondents in the study areas in Table 2.

Table 2. Practices of beekeeping time in study areas of Bale zone

	Years	Traditional beehives		Transitional beehives		Modern beehives	
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Yes	1966-1996	36	44.44	0	0	0	0
	1997-2020	42	51.85	29	35.19	45	55.60
No	1966-1996	3	3.7	53	64.81	0	0.00
	1997-2020	0	3.7	0	0	36	44.4
Total		81	100	81	100	81	100.00

Source: own survey 2022

Source of honeybee colonies and apiary site place

The strategies used by beekeepers to start their beekeeping businesses varied depending on where they live. As beekeepers reported about 82.72% of them began their beekeeping careers by catching swarms, while 7.4% of the sample respondents started by getting gift from their parents and 9.88% of respondents started by getting colonies gift from their parents and caught their own swarm. This indicated that most of the respondents engaged in beekeeping by getting colonies from their locality. This result showed that swarm catching was the main source of colonies in the study area. Similarly, Tesfaye *et al.* (2017) and Chala *et al.* (2012) reports that beekeepers started beekeeping in Bale and Jimma zones by swarm capturing 98.3% and 87.8% respectively. The majority of respondents (92.6%) stated that they were started beekeeping as sideline activities, whereas only 7.4% of beekeepers used beekeeping as their primary source of income. This finding demonstrated that beekeeping can typically integrate with other farming activities.

Of all the respondents about 39.51% keep their colonies in their backyards, whereas 24.93% keep in forest by hanging on long trees, 16.05% in apiary sites, 18.52% of beekeepers install their honeybee colonies in their backyards or hang on trees in forest (Table 3). Earlier research found that 50% of beekeepers in the Bale zone set up honeybee colonies in their backyards (Tesfaye *et al.,* 2017). Many scholars reported that the majority of beekeepers in various regions of Ethiopia set up their honeybee colonies in their backyard (Tessega, 2009; Gidey *et al*., 2012; Nebiyu and Messele, 2013).

Table 3. Location of honeybee colonies placed as declared by respondents

Place beekeeping	Total sample size (N=81)	
	Frequency	Percentages
Backyard	32	39.50
hanging on trees in forest	21	25.93
Backyard and hanging on trees	15	18.52
Apiary sites	13	16.05
Total	**81**	**100.01**

Source: Own survey result, 2022

Trends of beekeeping in the study areas

As the sample respondent mentioned about 69.14% beekeeping trend was decline in the number of honeybee colonies, while 19.75% of respondent said that the population of honeybee colonies had not changed and only 11.11% of the respondents stated that honeybee colony populations were upward trending. In addition, about 74.07% of the respondents claimed that honey yield was decline, while 16.05% said there were no changes in honey production and the remaining 9.89% said that honey yield in the research areas was increasing (Table 4). This result is agreement with report of Tesfaye *et al*. (2017) who reported that honey bee population and honey yield were decreasing from time to time in the Bale Zone.

Table 4. Trends of honeybee colonies in the study areas

Trends of categories	Total sample size (N=81)			
	Honeybees colony		Honey yield	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Decreasing	56	69.14	60	74.07
Increasing	9	11.11	8	9.89
Unchanged	16	19.75	13	16.05
Total	**81**	**100.00**	**81**	**100.00**

Source: Own survey result, 2022

Constraints of beekeeping in the study areas

The respondent reason out that many factors are responsible for the declining of honeybee production and they have mentioned that caused by climate change (drought, shortage of feeds, lack of water), pesticides, or unwise use of agrochemical, pests and predators and diseases the detail reason presented in Table 5. This result agrees with reports by Shunkute *et al.* (2012), Haftu and Gezu (2014) and Tesfaye *et al.* (2017) in Southern Nation and Nationalities People (Keffa, Sheka and Bench- Maji Zones), Tigray region and Bale zone southeast Oromia region respectively.

Table 5. Major causes of honeybee colonies and honey yield declining

Major causes	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	Rank index	Rank
Pests and predators	18	54	72	54	0	126	90	0.698	1
Lack of bee forage	84	56	14	56	28	84	56	0.519	2
Absconding	18	24	30	6	24	60	6	0.265	3
Mass death of bee colonies	55	11	22	11	33	11	55	0.252	4
Pesticides	36	18	45	18	0	36	18	0.238	5
Diseases	26	26	26	0	39	39	0	0.229	6
Drought	16	40	32	24	0	16	0	0.198	7

Source: own survey result, 2022

*Index = sum of (7*ranked 1st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5 th+2* ranked 6th+1* ranked 7th) for individual and predators divided by the sum of (7*ranked 1 st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2*ranked 6th+1* ranked 7 th) for over all Constraints*

Categories of Honeybees Behaviour in Study areas

According to the response of sample respondents, honeybees behaviour in the study areas were categorized as 25.93% aggressive, 18.52% medium, 11.11% docile, 25.93% medium and aggressive, 11.10 docile and aggressive, 7.41% docile, medium and aggressive. Similarly, Dubale BT (2017) reported that honeybee's behaviour as very aggressive (27.2%), aggressive (51.1%) and docile (21.7%) and depending on their stinging behavior.

Table 6. Honeybees' behaviour in the study areas

Honeybees Behaviour	Total sample size (N=81)	
	Frequency	Percentage (%)
Aggressive	21	25.93
Medium & aggressive	21	25.93
Medium	10	18.52
Docile	15	11.11
Docile & aggressive	15	11.10
Docile, medium & aggressive	6	7.41
Total	**81**	**100.00**

Source: Own survey result, 2022

Inspection of Honeybee colonies

Sample respondents were requested to describe the frequency of inspection of their apiaries and colony status. The majority of respondents (83.3%) were inspected their apiary sites, while 16.7% did not. It was mentioned that both internal and external hive inspections were practiced by beekeepers in the study areas. However, the majority of beekeepers in the study areas inspected their honeybee colonies externally (72.22%), internally (18.52%) and both internal and external inspections (9.26%) with frequencies of 51.89%, 33.33% and 14.85% some times, frequently and rarely respectively. This result in line with the findings of Tessega (2009) who studied in Burie district of Amhara region.

Major Honeybees Pests and Predators

Presence of pests and predators were reported by sample respondents in the study areas. The major honeybee pets and predators mentioned were honey badgers (*Mellivora capensis*), spiders (*Arachnids*), ants (*Dorylus fulvus*), wax moths (*Acheroea grisella*), bee-eater birds (*Meropidae*), small hive beetles (*Aethina tumida*), and Monkeys. When they were requested to rank these pests and predators according to their economic importance ranked as in Table 7. According to the ranking index honey badgers were took the most problematic and dangerous predator of honeybees and followed by spiders in Table 7 whereas monkey has the least impact on honeybees. Different authors have reported similar finding (Shunkute *et al.*, 2012; Chala *et al.*, 2012; Tesfaye *et al.*, 2017; Guesh Godifey, 2019).

Table 7. The major pests and predators of honeybees in the study areas

Major pets and predators	Relative level of importance							Index	Ranks
	1st	2nd	3rd	4th	5th	6th	7th		
Honey badger (*Mellivora capensis*)	98	60	45	28	18	10	3	0.693	1st
Spiders (*Arachnids*)	91	60	35	40	15	12	3	0.677	2nd
Ant(*Dorylus fulvus*)	77	54	60	24	24	12	2	0.669	3rd
Wax moth (*Acheroea grisella*)	91	60	40	24	18	14	4	0.664	4th
Bee-eater birds (*Meropidae*)	63	42	65	32	21	10	5	0.630	5th
Small hive beetles(*Aethina tumida*)	56	30	50	40	27	16	4	0.590	6th
Monkey	49	36	40	36	15	16	11	0.537	7th

Source: own survey result, 2022

*Index = sum of (7*ranked 1st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5 th+2* ranked 6th+1* ranked 7th) for individual and predators divided by the sum of (7*ranked 1 st+ 6* ranked 2nd+5* ranked 3rd+4* ranked 4th+3* ranked 5th+2*ranked 6th+1* ranked 7 th) for over all Constraints*

Effect of pests and predators on honeybee colonies and honey yield

According to respondents, honey badgers have been linked to absconding honeybees in 43.21% of cases, bee colony shrinking/dwindle in 25.93% of cases, and honey yield loss in 28.40% of cases. The majority of respondents claimed that honey badgers pose the greatest threat to honeybee colonies and bee-related products. In the study areas, natural forests may contain this predator. In the study areas, predators may also have an effect on beekeeping practices and the economy of beekeeping activities. Tesfaye et al. (2017) reported a similar finding in Bale.

A spider is the second most common pest of a honeybee colony, after the honey badger. According to respondents, spiders have caused 19.75% in honeybee colonies dwindling, 19.75% loss in honey yield, and 17.28% colony absconding. In the study areas, respondents claimed that ants are the most common pest of honeybee colonies, followed by spiders. A 19.75% colony absconding rate, a 16.05% honeybee colony decline, and a 16.05% loss in honey yield were stated as the effects of ants on honeybees by respondents. The previous studied that economical effect of ant accounted that 44.2% of honeybee colonies are yearly attacked by Ants of which 24% absconds and 4.2% dies (Desalegn 2007) in West and South west Shoa zones.

Majority of respondents replied that wax moths are the primary pests of honeybee colonies in the study areas. According to respondents, the wax moth has contributed to 12.35% colony absconding, 13.58% honey output loss, and 13.58% honey bee colony reduction. Similar result was reported severe problems of to wax moth in the country, on its special effects on honeybees and their products (Amssalu and Desalegn 2007). Bee-eating birds are causing an 11.11% decrease in honeybee colony size, an 8.64% loss in honey yield, and a 3.70% colony abscond in the study areas.

Sample respondents identified small hive beetles as the main pests of honeybee hives. According to respondents, small hive beetles are responsible for 8.64% of honeybee colony decline, 6.17% of honey output loss, and 2.47% of colony absconding in the study areas. The previous studied shows that the pest is found widely distributed in maize and coffee growing areas of the country (Amssalu et al 2009 and Amssalu et al 2010). Moreover, respondents stated that monkeys had the least substantial influence on honeybee colonies in the study areas, accounting for 7.41% honey yield loss, 4.94% honeybee colony reductions, and 1.23% colony absconding.

Table 8. Frequency of respondents declared the effect of pests and predators on bee colonies & honey yield

	(n= 81)					
	Honeybee colony dwindling		Loss of honey yield		Colony absconding	
Pests and predators	No. of declared effect	%	No. of declared effect	%	No. of declared effect	%
Honey badger	21	25.93	23	28.40	35	43.21
Spiders	16	19.75	16	19.75	14	17.28
Ant	13	16.05	13	16.05	16	19.75
Wax moth	11	13.58	11	13.58	10	12.35
Bee-eater birds	9	11.11	7	8.64	3	3.70
Small hive beetles	7	8.64	5	6.17	2	2.47
Monkey	4	4.94	6	7.41	1	1.24
Overall	81	100	81	100	81	100.00

Source: Own survey result 2022

Infestation and absconding of honeybee colonies by pests and predators

Majority of the respondents replied that honeybee pests and predators are the serious problems of honeybee colonies responsible for absconding, dwindling and honey yield loss. The numbers of honeybee colonies were infested or attacked by pests and predators were identified by the sample respondents. In the study areas, accounting 11.21% and 38.22 % by honey badger attack and absconded honeybee colonies respectively. Honey badger attacks have a distribution rate that range from 6.35% to 14.01%, with an average distribution rate of 11.21%, as shown below in Table 9. And also honey badger absconded honeybee colonies have a distribution rate that spans from 8.99 % to 18.5%, with an average distribution rate of 38.22%, as shown below in Table 9. According to the current findings, this predator may reduce the number of honeybee colonies, and their beehive products may result in a decline in beekeeping activities in the study areas. A similar result reported by Tesfaye et al, (2017) and Guesh Godifey (2019) in Bale and Tigray region respectively.

The numbers of bee colonies infested and absconded by spiders were 7.51% and 37.67% reported by the respondents of the sample in the surveyed areas. Spider infestation rates range from 6.61% to 8.61%, with an average distribution rate of 7.51%, as shown below in Table 9. Also, spider escaped colonies have distribution rates that range from 12.11% to 13.23% with an average distribution rate of 37.67% as shown below in Table 8. According to the present findings, spiders can reduce the number of bee colonies and their hive products can lead to a decrease in beekeeping activities in the study areas. A similar result is reported by Tesfaye et al, (2017) and Guesh Godifey (2019) in Bale and Tigray region respectively

The numbers of honeybee colonies were attacked and absconded bee colonies by ants 11.49 % and 37.66 % were declared by the sample respondents in the study areas. Ants of infestation have a distribution rate that spans from 10.57 % to 13.76 %, with an average distribution rate of 11.49 %, as shown below on Table 8. And also ants absconded honeybee colonies have a distribution rate that spans from 7.05 % to 13.35% with an average distribution rate of 37.66%, as shown below in Table 9. According to the current findings, Ants may reduce the number of honeybee colonies, and their beehive products may result in a decline in beekeeping activities in the study areas. A similar result reported by Tesfaye et al, (2017) in Bale zone and 44.2% of honeybee colonies are yearly attacked by Ants of which 24% absconds and 4.2% dies (Desalegn 2007) in West and South west Shoa zones.

The numbers of honeybee colonies were infested and absconded bee colonies by wax moth 12.2 % and 19.96 % were declared by the sample respondents in the study areas. Wax moths of infestation have a distribution rate that spans from 7.94 % to 18.94%, with an average distribution rate of 12.2 %, as shown below in Table 9. A similar result was reported by (Amssalu and Desalegn, 2007) having high infestation rate 26.66%, 22.85% and 26.66%, South west shoa, West and East Shoa zones respectively. And also wax moth absconded honeybee colonies have a distribution rate that spans from 4.29 % to 10.13% with an average distribution rate of 19.96%, as shown below in Table 9. Also, the same study indicated that about 56%-75% of the wax moth infected honeybee colonies absconded (Amssalu and Desalegn, 2007). According to the current findings, wax moth may reduce the number of honeybee colonies, and their beehive products may result in a decline in beekeeping activities in the study areas. A similar result reported by Tesfaye et al, (2017) and Guesh Godifey (2019) in Bale zone and Tigray region respectively.

The number of colonies that were attacked and absconded by bird-eater bees was 8.65% and 17.01% was declared by the respondents in the study areas. Attacking bird-eater bees have a distribution rate that ranges from 7.96% to 9.25%, with an average distribution rate of 8.65%, as shown below in Table 9. Honeybee colonies that have absconded bees also have a distribution rate ranges from 6.88% to 10.13% with an average distribution rate of 17.01% as shown below in Table 9. According to the present findings, birds-eater bees can eat colony numbers and their hive products can lead to a decrease in beekeeping activities in the study areas. A similar result was reported by Tesfaye et al, (2017) and Guesh Godifey (2019) in Bale and Tigray region respectively.

The numbers of honeybee colonies were attack and absconded by small hive beetles 5.53 % and 6.81 % were declared by the sample respondents in the study areas. Small hive beetles of attack have a distribution rate that spans from 5.28 % to 5.82 %, with an average distribution rate of 5.53 %, as shown below in Table 9. And also small hives beetle absconded honeybee colonies have a distribution rate that spans from 5.73 % to 12.8 % with an average distribution rate of 6.81%, as shown below on Table 9. According to the current findings, small hives beetle may reduce the number of honeybee colonies, and their beehive products may result in a decline in beekeeping activities in the study areas. The previous studied shows that the pest is distribution rate that ranging from 21 % -66 % (Desalegn and Amssalu 2006) and found widely distributed in maize and coffee growing areas of the country (Amssalu et al 2009 and Amssalu et al 2010). A similar result reported by Tesfaye et al, (2017) in Bale. Generally, the total honeybee colonies absconded and infested due to the major honeybee pests and predators about 388(55.04 %) and 399(56.67%) respectively. similar result was reported by Guesh Godifey (2019) in Tigray region.

Table 9. Honeybee colonies infested and absconded due to pests and predators declared by respondents

Honeybee colonies infested and absconded	Districts			
	Dinsho	Goro	Delo mena	Total
Total colony owned by respondents	227	289	189	705
Number of colonies attack by honeybee badger	32(14.01%)	35(12.11%)	12(6.35%)	79(11.21%)
Number of colonies absconded by honey badger	42(18.5%)	31(10.73 %)	17 (8.99%)	90(38.22%)
Number of colonies infested by spiders	15(6.61%)	25(8.65%)	13(6.88%)	53(7.51%)
Number of colonies absconded by spiders	28(12.33%)	35(12.11%)	25(13.23%)	88 (37.67%)
Number of colonies infested by ant	24(10.57 %)	31(10.72%	26(13.76%)	81(11.49%
Number of colonies absconding by ant	16(7.05%)	29(9.97 %)	14(13.5%)	59(37.66%)
Number of colonies infested by wax moth	43(18.94%)	28(9.69%)	15(7.94%)	86(12.20%)
Number of colonies absconding by wax moth	23(10.13%)	16(5.54%)	27(4.29%)	66(19.96%)
Number of colonies attack by birds	21(9.25%)	23(7.96%)	17(8.99%)	61(8.65%)
Number of colonies absconded by birds	23(10.13%)	0	13(6.88%)	36(17.01%)
Number of colonies infestation by small hive beetle	12 (5.28%)	16(5.53 %)	11(5.82%)	39(5.53%)
Number of colonies absconded by small hive beetle	13(5.73 %)	22(12.80%)	13(6.88%)	48(6.81%)
Total bee colonies infestation	147(64.76%)	158(54.67%)	94(49.74%)	399(56.6%)
Total bee colonies absconded	146(64.32%)	133(46.02%)	109(57.7%)	388(55.04%)

Source: own survey result 2022

Traditional strategies for controlling of honeybee pests and predators

During study period, several techniques were stated by sample respondents to control pests and predators in the study locations. When they were asked how to control pests and predators in your area, the respondents were mentioned using ash around hives stands for the most common pests, attaching smooth iron sheets to the trunks of trees where hives are hanging, hanging hives on long trees with very smooth bark so that honey badgers cannot climb them, using dogs for back yard, and killing honey badgers by using *wotmed* (Table 10). Different authors have reported that beekeepers in different part of the country were used several defense strategies to keep their honeybees from pests and predators (Chala *et al*., 2012; Dabessa and Belay, 2015; Tesfaye *et al*., 2017).

Table 10.Traditional practices of controlling major honeybee pests and predators by beekeepers

Pests and predators	**Traditional controlling mechanisms /practices**
Honey badger	Use of dog for chasing, use of "*wotmed*" to kill, fencing the apiary site with strong fence, hanging hives by rope on long trees
Spiders	Cleaning apiary site always, removing the spider webs, putting ash under the hive stand
Ants	Applying ash under hive stand, cleaning apiary site
Wax moth	Supply supplementary feeding and water to the colonies to be strong, fumigating the hive, removing the old comb from hives, and cleaning beehives
Birds	Frightening birds from the area, putting like tallow, mastic, and plastic on hive entrance, placing wheat seed or barely, putting an image of a human near the hives using cloth
Small hive beetles	strengthening the colony or keeping strong colonies, removing weak colonies, cleaning apiary, smoking/fumigating the hive
Monkey	Hanging beehives on a branch of long trees by ropes, keeping beehives near home steady
Bee lice	Fumigate the hive with materials like tobacco, dung and grass, making colonies strong, supplying additional food for weak colonies

Source: own survey result, 2022

Laboratory Diagnosis of Honeybee Diseases and Pests

Honeybee diseases

However, current results of laboratory diagnosis of honeybee diseases such as American brood disease, European brood disease, chalk brood disease, and tracheal mite were not found in the study areas. However, a nationwide diagnostic survey widened the prevalence of chalk brood disease in the country (Amssalu *et al.*, 2009 and Amssalu *et al*., 2010). Chalk brood diseases have identified disease ecological suitability zones in which dry and wet run areas are not suitable and areas with wet Dega, wet weinadega, and wet weina dega very high and excellent suitability of climatic zones (Aster *et al.*, 2007 and Aster *et al*., 2010).

Prevalence of *Nosema apis* and its associated risk factors

The present study revealed that the overall prevalence of *Nosema apis* in Bale Zone was 79.17% and ranges from 68.18% to 86.96% (Table 11). There was statistically significant different ($p<0.05$) between agro-ecologies. The highest prevalence of *Nosema apis* was observed 86.96 % highland followed by 81.48 % in midland and the least was observed 68.18 % in lowland. The result showed that honeybee colonies infected by *Nosema apis* was found healthy and active in their production performance. The same result was reported by Semere *et al*. (2021) in Kaffa zone. The current finding is also in agreement with the finding of (Amssalu , 2012; Nega *et al.*, 2019) who stated that increase in humidity and rainfall limit honeybees to fly out for cleansing, which in turn enhances spread of the disease among the members and autoinfection.

There was a significant difference on prevalence of *N.apis* honeybees colonies kept in different beehive types. The highest prevalence was observed 85.71% in modern beehives, followed by 72.22 % traditional beehives while the lowest was found 66.67% (Table 11) transitional beehives during the study period. This variation might be related to various operations on beehives management practices; exchange of bee equipment, placement of beehives may conducted by beekeepers. Desalegn and Kebede (2005) stated that *Nosema apis* disease was more prevalent in the modem beehives (72.2%) than in traditional beehives (41.3%) and transitional beehives (35.3%) systems. Similarly, Amsalu (2012) reported that *N. Apis* infection rate in the central Ethiopian highlands had reached up to 82%.

Table 11: Prevalence of *Nosema apis* and Associated risk factors in the study areas

Variables	**Category**	**No. of sampled colonies**	**Prevalence (%)**	**x^2**	**p-values**
Beehive types	Movable frame	42	36(85.71%)		
	Transitional	12	8(66.67%)	2.76	0.252
	Traditional	18	13(72.22%)		
Agro-ecology	Highland	23	20(86.96%)		
	Midland	27	22(81.48%)	2.54	0.28
	Lowland	22	15(68.18%)		
Overall prevalence		**72**	**57(79.17)**		

Prevalence of *Malpighamoeba mellificae* and Associated Risk Factors

In the current study, the overall prevalence of amoeba (*M. mellificae*) disease was 79.16% (Table12). The result indicated that amoeba (*M. mellificae*) disease was higher in movable frame hives (83.33%) followed by transitional hives (75.00%) and traditional hives (72.22%). Furthermore, there was no a statistically significant difference between these hives ($p > 0.05$). This might be due to several in management practices like exchange of old combs easy operation bee equipment and use of traditional systems. The current result agrees with the finding of Begna and Kebede (2005) who reported the prevalence of amoeba was high in the modern beehives (88.9%) than in the traditional beehives (61.9%) and transitional beehives (47.1%).

The present investigation had shown that the prevalence of Amoeba was higher in the highland (82.61 %) than midland land (77.78%) and lowland (77.27%) agro-ecologies (Table 12). There was no a significant difference on the prevalence of amoeba disease among agro-ecologies ($p > 0.05$). Similar result was reported by Amssalu B. (2012) stated that amoeba diseases was widely distributed and identified in the most places of the country throughout the year. This result was in line with the previous studies reported in different part of the country for prevalence of amoeba 88 % Oromia region, 95 % Amhara region and 60 % Benishangul-Gumuz region (Yohannes A, 2009).

Table12: Prevalence of *Malpighamoeba mellificae* and associated risk factors

Variables	Category	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
Beehive types	Movable frame	42	35(83.33%)			
	Transitional	12	9(75.00 %)	2	1.095	0.578
	Traditional	18	13(72.22%)			
Agro-ecology	Highland	23	19(82.61%)			
	Midland	27	21(77.78%)	2	0.245	0.885
	Lowland	22	17(77.28%)			
Over all prevalence		**72**	**52(79.16 %)**			

Prevalence of honeybee pests

Prevalence of *varroa* mites and associated risk factors

The study result showed that the prevalence of *varroa* mites was observed 95.45 % lowland followed by 66.67 % midland while the lowest was found 56.2 % highland (Table 13). The prevalence of varroa mite in Bale zone ranges from 56.52% to 95.45% with an average of 72.22%. The overall prevalence of bee varroa mites has a significant variation between agro-ecologies ($p > 0.01$) and it was associated risk factors. Moreover, there was statistically significance difference ($p<0.05$) among agro-ecologies. This indicated that hot environment was very conducive for the spread of varroa mite's populations. This result is higher than the earlier research finding of 82% varroa mite prevalence in the Tigray Region (Desalegn Begna, 2015). Thus with this prevalence rate, varroa mite is less likely to impact honeybee health and its honey production in the study areas.

The present result revealed that the prevalence of *varroa* mites was higher in transitional beehives (83.33 %), than modern beehives (73.81 %) and traditional beehives (61.11 %) (Table13). The overall prevalence of bee varroa mites has no a significant variation between hive types ($p > 0.387$) and it was no associated risk factors. The prevalence of varroa mites high in transitional and modern beehives might be due to transmission of the pest during exchange of bee equipment's and management of bee colonies like feeding. Similar finding was reported by Tsegaye (2015) noted that about 94.2%, 84.8%, and 79.85% in movable frame hives, intermediate hives, and traditional hives, respectively, in the eastern of the Amhara region.

Table13. Prevalence of Adult varroa mites and associated risk factor

Variables	Category	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
	Movable frame	42	31 (73.81%)			
Beehives types	Transitional	12	10(83.33%)	2	1.899	0.387
	Traditional	18	11(61.11%)			
Agro-ecology	Highland	23	13 (56.52 %)			
	Midland	27	18(66.67%)	2	9.16	0.01
	Lowland	22	21(95.45%)			
Over all prevalence		**72**	**52(72.22 %)**			

Prevalence of varroa mites on Brood and associated risk factor

The current result showed that the overall prevalence of v*arroa* mites in brood was 18.06%. The highest varroa mites in brood was observed 27.78% in traditional beehives and followed by 25.00 % in transitional beehives and 11.90 % in modern beehives. The overall prevalence of bee varroa mites has no a significant variation between hive types ($p > 0.270$) and it was no associated risk factors. Guesh (2015) reported that traditional hives had a higher prevalence of varroa mites than movable frame hives.

Concerning agro-ecology, the highest varroa mite's population in brood was found in midland (33.30) and followed by lowland (9.09%) and the least populations found in high land (8.69%) agro-ecology (Table 14). The overall prevalence of bee varroa mites has a significant variation between agro-ecologies ($p <0.033$). The present finding showed varroa mites found in all agro-ecologies throughout the year and higher during brood rearing season. Begna *et al.* (2016) stated that the population of varroa mites recovered was low during the dry season and low brooding tendency and growth of brood population mites depend on honeybee brood production.

Table14. Prevalence of varroa mites in brood and associated risk factor

Variables	Category	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
	Movable frame	42	5(11.90%)			
Beehives types	Transitional	12	3(25.00%)	2	2.615	0.270
	Traditional	18	5(27.78%)			
Agro-ecology	Highland	23	2 (8.69 %)			
	Midland	27	9(33.30%)	2	6.816	0.033
	Lowland	22	2(9.09%)			
Over all Prevalence		**72**	**13(18.06%)**			

Infestation of varroa mite

The current result indicated that varroa mite infestation in workers honeybees' were higher in transitional beehives (91.67%) followed by modern beehives (77.5%) and the least infestation was found in traditional beehive (47.37%) with overall mean (71.83%). The result indicates that infestation of varroa mites is less likely to impact honeybee health and its production in the study areas. Similarly, Begna *et al.* (2016) state that the level of honeybee population and their activities in nectar collections did not affected by the varroa mites infestation. The result also indicated that varroa mites' infestation was more common in lowland (81.81 %) followed by mid highland (76.92%) while the lowest was found in highland agro ecology (56.52 %) (Table15).

The overall varroa mite infestation in honeybee broods was observed 21.78%. However, the distribution was ranges from 9.09% to 50% in brood. The present result indicated that varroa mite infestation in honeybee broods was higher in traditional beehives (35.71%) followed by transitional beehives (25.00%) and modern beehive (14.29%). The result also indicated that varroa mites' infestation in honeybee broods' were more common on midland (50 %) followed by lowland (10.0%) while the lowest was found in highland agro ecology (9.09 %) %) (Table15).

Table15. Infestation of adult and varroa mite brood

Variable	Category	Adult sample colonies	infestation (%)	brood samples	infestation (%)
Bee hive types					
	Movable frame	42	31(77.5%)	28	4(14.29 %)
	Transitional	12	11(91.67%)	4	1(25 %)
	Traditional	19	9(47.37%)	14	5(35.71 %)
Agro-ecology					
	Highland	23	13 (56.52 %)	22	2(9.09%)
	Midland	26	20(76.92%)	14	7(50 %)
	Lowland	22	18(81.81%)	10	1(10 %)
Over all infestation		**72**	**51(71.83 %)**	**46**	**10 (21.74%**

Prevalence of *Bee lice* and Associated Risk Factors

The presented result indicated that the prevalence of bee lice was higher in transitional beehives (16.67%) followed by traditional beehives (33.33%) and modern beehives (26.19%) (Table15). The study result showed that the prevalence of *bee lice* was observed 31.81 % in lowland followed by 25.96 % in midland while the lowest was found 21.74 % in highland. The overall prevalence of bee lice has no a significant variation between hive types ($p > 0.597$), and agro-ecologies ($p > 0.743$) it was no associated risk factors. The current study agrees with report by Nega *et al.* (2019) in Amhara region northern Gondar zone.

Table15. Prevalence of bee lice and associated risk factor

Variables	Category	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
Beehives types	Movable frame	42	11(26.19%)	2	1.032	0.597
	Transitional	12	2(16.67%)			
	Traditional	18	6(33.33%)			
Agro-ecology	Highland	23	5(21.74 %)	2	0.593	0.743
	Midland	27	7(25.96%)			
	Lowland	22	7(31.81%)			
Over all prevalence		**72**	**19(26.39%)**			

Prevalence of Wax moth and Associated Risk Factors

The current result showed that prevalence of Wax moths was highest in modern beehives (45.24%), followed transitional beehives (41.67%) and the lowest prevalence was observed in traditional beehives (38.89%). The overall prevalence of bee wax moth has no a significant variation between hives ($p > 0.897$) and it was no associated risk factors. This might be because of poor management practices by beekeepers in modern beehives and transitional beehives like no replace of old combs and no remove of queen excluder throughout the year as we had observed during sample collections. Moreover, concerning agro-ecologies high prevalence of *wax moth* was observed %) in the highland (48.83 %) followed midland (40.74%) by while the lowest was observed in lowland 31.81%) (Table16). The overall prevalence of bee wax moth has no a significant variation between agro-ecologies ($p > 0.855$) and it was no associated risk factors. Also, the same result reveled that widely scale distribution of the pest in the country (Amssalu et al 2009 and Amssalu et al 2010).

Table 16: Prevalence of Wax moth and Associated Risk Factors

Variables	Categor	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
Beehives types	Movable frame	42	19(45.24%)	2	0.219	0.897
	Transitional	12	5(41.67%)			
	Traditional	18	7(38.89%)			
Agro-ecology	Highland	23	11(48.83 %)	2	0.314	0.855
	Midland	27	11(40.74%)			
	Lowland	22	7(31.81%)			
Over all prevalence		**72**	**29(40.27%)**			

Prevalence of Small Hive Beetles and Associated Risk Factors

Small hive beetle (SHB) is a nest parasite of honey bees (*Aphis mellifera* L.) colonies native to Sub-Saharan Africa and is considered a minor pest of honeybees. The overall prevalence of small hive beetles was observed 54.22% across agro-ecologies. The highest prevalence of small hive beetles was detected in the lowland (63.6%) followed by the midland (51.90%) while the lowest was observed in the highland (48.80%) in the study districts (Table 17). The overall prevalence of bee Small hive beetles has no a significant variation between hive types ($p > 0.248$). This might be due to this pest favors to hot temperature and low relative humidity along maize and sorghum cultivated land and livestock rearing area. This finding agreement with Nega *et al*. (2019) who stated that variation in agro-ecologies may be attributed to different factors such as ecological variability, season, and management aspects. Also similar finding was reported by (Amssalu and Desalegn 2008, Amssalu et al 2009 and Amssalu et al 2010) studies show that the pest is found widely distributed in maize and coffee growing areas of the country.

Regarding hive types, the result indicated that high prevalence of Small hive beetles was observed in modern beehives (61.90%) followed by transitional beehives (50.00%) while the lowest prevalence was observed in traditional beehives (38.90%). The overall prevalence of bee Small hive beetles has no a significant variation between agro-ecologies ($p > 0.542$) and it was no associated risk factors related to agro-ecologies. this is probably due to poor management practices of beekeepers had on modern and transitional hives in the study districts during the study period.

Table 17: Prevalence of Small hive beetles and Associated Risk Factors

Variables	Category	No. of sampled colonies	Prevalence (%)	df	x^2	p-values
	Movable frame	42	26(61.90%)			
Beehives types	Transitional	12	6(50.00%)	2	2.789	0.248
	Traditional	18	7(38.90%)			
Agro-ecology	Highland	23	11(47.80 %)			
	Midland	27	14(51.90%)	2	1.225	0.542
	Lowland	22	14(63.6%)			

Conclusions and Recommendations

The present study identified major challenges of beekeeping practices in the study areas were pests and predators, shortage of bee forage, absconding, pesticides, diseases, and mass death of bee colonies during the study period. Majority of the beekeepers practicing traditional beekeeping which are more face for these problems. Furthermore, there is lack of knowledge and skill gap about modern beekeeping equipment how to use and practices in the study areas. The study finding revealed that the major honeybee pest and predators according to important order were ranked. These pests and predators were honey badger, spider, ant, wax moth, bee-eater birds and small hive beetles and monkeys were declared by respondents. Majority of the respondents replied that honeybee pests and predators are the serious problems of honeybee colonies responsible for absconding, infestation, loss of honey yield and colony dwindling in the study areas. Honey badger is the most dangerous predator for honeybee colonies that cause absconding and colony losses in the study areas. The total honeybee colonies absconded and infested due to the major honeybee pests and predators estimated to be 388 (55.04 %) and 399 (56.67%) respectively. The finding was identified honeybee diseases and pests in the study areas were Nosema apis, Amoeba, varroa mites, bee lice, wax moth, and small hives beetle. However, current studies were not detected American foul brood disease, European foul brood disease, and trachea mites during laboratory analysis. In conclusion, honeybee colonies absconded and infested due to the major honeybee pests and predators, across agro-ecologies and beehive types were identified as risk factors for prevalence of honey bee diseases and pests in the study areas.

Based on the above conclusion the following recommendations are forwarded;

- Awareness creation should be needed in terms of technical knowledge and skills that could capacitate the knowledge of beekeepers to benefit them from the apiculture sub-sector,
- Beekeepers should be maintain strong and healthy honeybee colonies via proper seasonal colony management practices from diseases, pests and predators,
- Beekeepers should avoid contamination of bee equipment, avoid transfer of infected combs to healthy colony, avoid common feeding of honeybee colonies which spread diseases and pests transmission,
- There is a great need to develop scientifically mechanisms for controlling of pests and predators,
- Further, study on seasonal prevalence and outbreak of honeybee diseases and pests could be very important.

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Diagnostic survey of honey bee disease, Pests and predators in East Wollega Zone, Oromia National Regional State, Ethiopia

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ABSTRACT

The study was conducted to assess the honey bee pests, predators, and diseases in the selected districts of the East Wollega Zone. A cross-sectional study design was used to determine honeybee disease and pests. A questionnaire survey and laboratory diagnostic methods were also used. The questionnaire was administered to 104 beekeepers of which 97.1% were males. Fifty two beekeeper's sites were selected and two honeybee colony samples were considered from each site. Therefore, a total of 104 honeybee colonies were collected from frame box hives for laboratory diagnosis. The major causes of decrease in honey bee colony and honey yield, as ranked by respondents, were pests and predators, honeybee diseases and inappropriate agrochemical application. Of the total honey bee colonies the respondents said to have, the majority (538 or 21.3%, were infested by ants, by beetles (378 or14.9%) and by wax moth (315 or 12.5%), in all districts. According to the respondents, among all pests and predators listed, the major cause of absconding of honeybees were ants (213 or 8.4%), wax moths (194 or 7.7%), and beetles (193 or 7.6%). Ants, beetles, Waxmoth, and honey badgers were ranked 1st to 4th economically important pests. From 52 beekeeping sites and 104 honeybee colonies examined for honeybee parasites (Braulacoeca and Varroa destructors) and honeybee pathogens (Nosema apis, Malpighamoeba mellificae, and Ascosphaera apis) were confirmed. However, American Foulbrood, European Foulbrood, Stone brood, and tracheal mites were not confirmed during the study. The prevalence of honeybee parasites were 23.1% for Braulacoeca, and 94.2% for Varroa destructor whereas for honeybee pathogens the prevalence of Nosema apis Malpighamoeba mellificae and Ascosphaera apis were 50.0, 46.2 and 19.2%, respectively.

Key words: *Honeybees, Disease, Pests, Prevalence, Infestation, Oromia,*

Introduction

Ethiopia is endowed with diverse agro climatic conditions which favor for the growth of diverse natural and cultivated floral species supporting huge number of bee colonies (Yibrah T 2008; Abadi B, *et al*, 2016; Haftey S. *et al.,* 2018). Beekeeping is an economic activity of the agricultural sector and an environmentally sustainable production model, crucial for biodiversity and agriculture development. Beekeeping provides additional income for many poor communities, creating new opportunities in rural areas, and improves the living conditions of many families. In addition, the pollination service provided by honeybees enhances crop yields and contributes to the balance of the ecosystem and biodiversity.

Like all other insects, honeybees (*Apismellifera*) are susceptible to pests and diseases. Honey bees suffer from a range of bacterial, fungal, microsporidian and viral pathogens, as well as ectoparasitic mites, which can all lead to poor colony health and colony loss. It is important for beekeepers to be aware of these disorders, to identify them and effectively manage disorders to maintain healthy colonies. This is particularly important because the health of one beekeeper's colony can impact another beekeeper's colony in the surrounding area. The honeybee population and its products decline from time to time by some factors like, honey bee disease, pests, predators, pesticide, environmental stress and genetic disorder (IISl, 2013).

In the present time the major honeybee diseases, pests and predators and their prevalence rates are reported in Ethiopia (Amssalu B, *et al*, 2010, Haylegebriel T, 2014, Amsalu A, 2020). The local honeybees are challenged by most recognized honeybee pests (Kerealem Ejigu, 2005; Desalegn Begna, 2012) and some disease present in the world (Desalegn Begna, 2012). The most commonly known honeybee diseases reported in Ethiopia are *Nosema apis* and *Melpighamoeba mellificae* (Gezahegn T and Amsalu B, 1991; Amssalu B and Desalegn B, 2006); Chalk brood (Desalegn B , 2006); Some major types of honeybee pests and predators (Desalegn B, 2001;Desalegn B and Amssalu B,2001); Small hive beetle (*Aethina tumida Murray*; *Coleoptera: Nitidulidae*) (Desalegn B and Amssalu B ,2006); different Ants (*Dorylus fulvus*) (Desalegn B ,2007); the bee lice (*Braula coeca*) (Adeday *et al*., 2012; Gemechu G *et al*., 2013). Honeybee parasites, Varroa and bee lice, from pathogens Nosema, Amoeba and chalk brood disease were confirmed

However, there should be regular and wide-scale diagnostic surveys to monitor the occurrence of new pests and diseases affecting honeybees' health. There is still insufficient evidence on the effects of pests and diseases. Very importantly, a comprehensive strategic response to the recently occurred varroa mite threat in determining its thresholds, economic damages, and behavioral attributes with devising control options are very important (Desalegn B, 2015). Inspection for bee diseases is an important part of beekeeping. Apiary inspectors and beekeepers must be able to recognize bee diseases and parasites and to differentiate the serious diseases from the less important ones. Thus the present study is initiated to assess the current status of honeybee colony health problems in the East Wollega Zone with special emphasis to determine the occurrence, and prevalence of pests, predators, and diseases with their effects on honeybee health and their products.

Materials and methods

Description of the Study Area

The study was conducted in East Wollega Zone, Oromia Regional state at about 332 km away from Addis Ababa, the capital city of Ethiopia. The zone is located at 36 ^{0} 30'00" to 36 ^{0} 45'00''E longitude and 9 ^{0} 05'00'' to 9 ^{0} 15'00''N latitude with elevation ranging from 1000 m to 3207m. The annual rainfall of the zone ranged from 1500mm to 2200mm and the mean annual temperature ranges from 15-20^{0}C (CSA 2005). The study districts covered in this study were; Diga , Gida Ayana, Gobu Sayyo and Wayu Tuka.

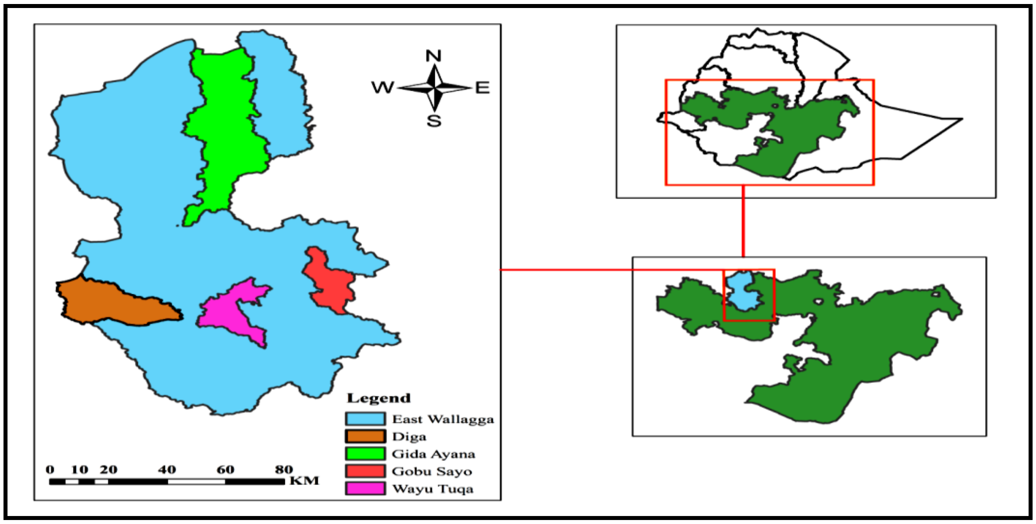

Figure 1. Map of the study area

Study design and sampling procedure

A cross-sectional study was conducted in Diga, Gida Ayana, Gobu Sayo and Wayu Tuka districts of East Wollega Zone.to assess the prevalence of common honeybee diseases, pests and predators. Honeybee diseases and parasitic mites were examined in laboratory using respective protocols while pests such as Ants, Wax moth, Small hive beetles and the likes were diagnosed at the field via observation and pre tested questionnaire.

Adult honeybee and brood sampling

Samples of Adult honey bees and brood were collected from 104 honeybee colonies from 52 apiaries, 2km-5km distant from each other. The samples were brought to the laboratory for examination for pests and diseases. Prevalence of the pests and diseases was calculated using the formula:

$$\text{prevalence} = \frac{\textbf{number positive}}{\textbf{total number samples/population examined}} \textbf{*100}$$

Diagnosis for major honeybee diseases and pests

Laboratory tests for Nosema, Amoeba, Chalk brood, American foul brood, European foul brood where suspected clinical symptoms, Varroa and tracheal mites following the respected standard procedures

Laboratory examination of *Varroa destructor*

Laboratory examination of varroa mites was conducted using the standard methods for Varroa detection (Dietemann *et al*., 2013). From each sample collected from honeybee colonies, 250 adult honeybees were added into a wide mouth plastic container with 10 ml of 1% detergent-water solution and then vigorously shaken for 1 minute to dislodge mites. The mites were collected by filtering the solution through a ladle (8- to 12-mesh) that hold the bees back and let out the mites with the solutions. Then, wire gauze was used to hold the mites back and discharge the solutions. The wire gauze was turned down to white paper on which the presence/absence of the mite was examined and counted brood examinations were done by cutting off 5*5cm brood comb areas from drone and worker pupae broods. About 100 pupae were removed from their cells using forceps and checked for the presence of Varroa mites on the worker and/ or drone pupae. At the end, number of Varroa mites per diagnosed sample was recorded.

Laboratory examination of tracheal mite

Samples of 20-30 adult honeybees collected from colonies at random and preserved by adding 70% alcohol. The head and first pair of legs of honeybees were removed using scissors. Transverse-section thoracic disks were sliced and placed directly in a small bottle containing 10-percent potassium hydroxide (KOH). The sliced thoracic disks in KOH were heated and stirred gently near to the boiling point for approximately 10 minutes until the soft internal tissues dissolved to expose tracheal rings. The disk-trachea suspension was examined for mite infestation under light microscope (Sammataro *et al*., 2013).

Laboratory examination of Nosema and Amoeba diseases

As these two diseases affect the abdominal contents of adult honeybees, their sampling and diagnostic techniques are almost the same. The abdomen of 20 honeybees from each sample was cut and grounded in a mortar containing 20ml distilled water. The mortar and pestle were thoroughly cleaned before being used again. A loop of suspension were placed on microscopic slide using the sterilized loop and covered with cover slid. Then the suspension was examined under a light microscope.

Laboratory examination of chalk brood disease (CBD)

Both external and internal inspections were conducted for the presence of chalk brood clinical symptoms. Presence of dry scales with white to dark color molds and chalk brood mummies were carefully observed in the comb cells and on the bottom boards of the hives. Then mummies were moistened with distilled water and the supernatant was placed on a microscope slide and covered with coverslip. Then the prepared slide was examined under the light microscope with magnification of 40X for either spores or/and spore balls or/and cysts of *Ascosphera apis* (Annette *et al.*, 2013).

Examination of American Foulbrood (AFB) and European Foulbrood (EFB)

In randomly selected apiaries at least three colonies were inspected internally for major clinical symptoms of bacterial diseases with emphasis to AFB and EFB. Typical clinical symptoms such as irregular brood arrangement, sunken and dark capping with puncture holes, dead and decayed larvae with dark “scales” and slight to pronounced odor was examined for AFB while, twisted larvae with creamy-white guts visible through the body wall, melted and yellow white larvae with unpleasant sour odour and loosely-attached brown scales EFB. Furthermore, match stick test (stretch test) was employed to detect AFB.

From suspected brood showing one of the above important clinical symptoms, smear was prepared on frosted end microscopic slide and legibly labeled for further laboratory diagnosis according to Primefact, 2009 (www.dpi.nsw.gov.au/primefacts). The prepared slides examined under microscope for the presence of *Paenibacillus* larvae and *Melissoccocus pluton* using Zeiss AxioVert A.1 light microscope under oil immersion (magnification power of 100X).

Diagnosis for major honeybee pests

Pests and predators of honeybees were cause devastating damage on honeybee colonies and at most time cause absconding The occurrence and economic importance of major honeybee pests (including: *Wax moth, small hive beetle, ants, spiders, bee-eater birds, honey badger, bee lice, lizards, Dead hawks moth etc*) in the study areas were determined through beekeepers interview using semi-structured questionnaires and internal and external hive inspections. Moreover, clinical symptoms and infested combs, adult and larvae of small hive beetles and wax moth and other decayed materials were observed in the hive through inspection of the bee hives described by Neumann *et al.* (2013).

The presence of small hive beetle infestation (*Aethinatumida)* was identified through detection of adult, larvae or pupae. The larvae of SHB have pairs of prominent brownish dorsal spines on each segments with 3 pairs of anterior prologs (Ellis *et al*. 2013) while the larvae of wax moth has no spines, but has number of setae(hairs) on each segments with 8 pairs of prologs. (Unlike Small hive beetles, it produces silken galleries.

Data management and statistical analysis

The collected data were stored in Microsoft Excel and SPSS software programs (SPSS @, version 20) for analysis. The statistical analysis used in the study was varied depending on the type of variable and information obtained. Summarized data was presented in the form of tables and figures. The data collected through semi-structured questionnaires were analyzed using descriptive statistics and the beekeeping constraints, causes of honeybee colony and yield decline, prevention method of bees from agrochemicals and economic importance of pest and predators were ranked using the rank index formula as described by (Musa *et al.,* 2006). The formula was:

Rank index*=sum of (5 X number of household ranked first + 4 X number of household ranked second + 3 X number of household ranked third + 2 X number of household ranked fourth + 1 X number of household ranked fifth) for an individual reason divided by the sum of (5 X number of household ranked first + 4 X number of household ranked second + 3 X number of household ranked third + 2 X number of household ranked fourth + 1 X number of household ranked fifth) for overall reasons.*

Results and discussions

Socio-demographic characteristics of the respondent

From 104 sample households, about 2.9% female and 97.1% male headed. About 75% of respondent's age ranges from 18 to 42. Concerning to occupational status of beekeepers, 99% were farmers. Most of the beekeepers practice beekeeping as a side of crop production in the study area. This result shows that beekeepers in the study areas were in productive age. Based on the education status of respondents, about 40.4%, 34.6% and 23.1% of respondent beekeepers have attended elementary, secondary school and read and write respectively. Beekeeping activities in the study area were practiced by literate beekeepers. Beekeepers with the better educational backgrounds were more productive since they adopted efficient beekeeping technologies of beekeeping.

Table 5. Socio- demographic characteristics of households

Socioeconomic Variables	**Category**	**N**	**Percentage (%)**
Sex	Female	3	2.9
	Male	101	97.1
age	18-30	31	29.8
	31-42	47	45.2
	43-45	17	16.3
	56-68	6	5.8
	>69	3	2.9
Occupation	Farmer	103	99
	Merchant	1	1
Education level	Can Write And Read	24	23.1
	Elementary	42	40.4
	Secondary	36	34.6
	Diploma	2	1.9

Beekeeping activities and potentials

This study indicated that the beekeeping activities in all districts are practiced sideline with other agricultural activities (100%). There were no any respondent who depend solely on beekeeping. Most beekeepers (92.3%) started beekeeping before 1994 and they keep their colonies in traditional beehives. Between the year 1994 and 2004, most beekeepers showed a shift from traditional way of beekeeping to transitional and movable frame have beekeeping (Table 2). About 49%, 29.8% and 21.2% of the beekeepers used traditional, Transitional and movable frame hives, respectively in the same year. Similarly between the years 2005and beyond the proportion of beekeepers using movable frame hive increased to 40.4 % and those using traditional hives dropped to 31.7%. This implies that the use of improved beekeeping technologies is in progress in the study areas. The beekeeping experience has positive correlation with the use of improved beekeeping technologies ($P<0.01$).

Table 2, Beekeeping activities and potentials

Time category	Hive type	Frequency (%)
<1994	Traditional	92.3
	Transitional	5.8
	Moveable frame box	1.9
1994-2004	Traditional	49
	Transitional	29.8
	Moveable frame box	21.2
2005-2015	Traditional	35.6
	Transitional	27.8
	Moveable frame box	35.6
>2015	Traditional	31.7
	Transitional	28.9
	Moveable frame box	40.4

Sources of Honeybee Colony

The result indicated that sources of honeybee colonies for starting beekeeping and increasing stocks were mainly swarms catching and gifts from parents (Table 3). This indicates that there were no colony transportation from one place to other and beekeepers can get honeybee colony from their area. This is an opportunities for future honey production improvement in the study areas

Table 3. Sources of honeybee colonies

Source of colony	Frequency	Percent (%)
From parents	41	23.2
By catching swarms	85	56.8
By Buying colonies	13	9.4
From parents and catching swarm	27	19.1
From parents and buying colonies	7	2.4
By catching swarms and by buying colonies	6	4.1

Apiary site Inspection by Beekeepers

Respondents were interviewed to describe the frequency of inspecting their apiary and honeybee colonies. Majority of the beekeepers inspected their apiary and colonies every day. 70.2% of the respondents carried out external inspection of honeybee colonies every day while 28.8% of the beekeepers did once in a week (Table 4).

Table 4, Apiary site Inspection Schedule by Beekeepers

Inspection Schedule	N	Frequency (%)
Everyday	73	70.2
Per week	30	28.8
Per month	1	1.0
Rarely	0	0.0

The trend of honeybee colony population and products producting

About 50% of the respondents declared that honeybee colony population and production of honeybee products were decreasing, while less than 40% of these respondents had the perception that honeybee colonies and the honey production were in increasing trend (Figure 2). According to the respondents, honeybee colonies and honey production decline attributed to shortage of bee forage and honeybee health problems.

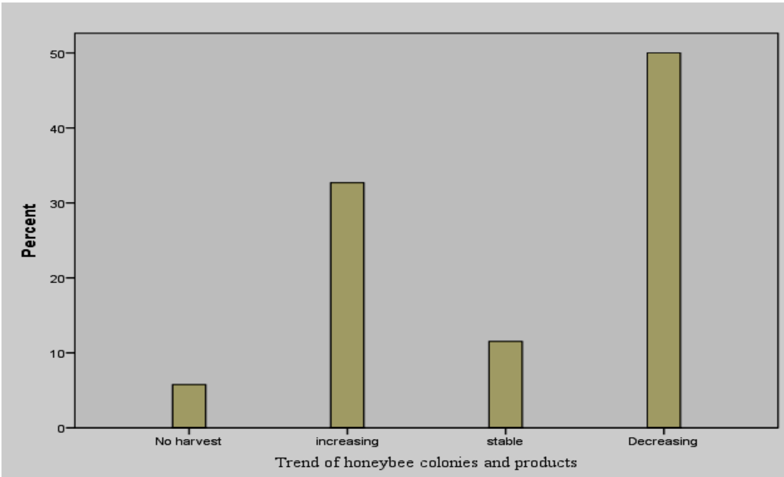

Figure 2, the trend of honeybee colony and products

Causes of Honeybee Colony and Yield Decrease

Beekeepers reported different problems affecting the beekeeping activities in their areas. The cause of honey bee colony and yield decreases were listed by respondents, and they also ranked these constraints as the most important factors affecting beekeeping. These constraints were ranked in terms of their relative degree of importance as 1st pets and predators, 2nd honeybee diseases, 3rd inappropriate agrochemical applications, 4th high price of bee equipment's, and 5th low price of honey (Table 5). All of these factors resulted in a decrease in productivity and the honeybee colony population. These results are in agreement with (Amsalu A, *et al*, 2020; Kerealem *et al*., 2009; Mulisa F and Fekadu B, 2017). Shortage of bee forages is also critical in the study areas due to population pressure, lack of land use policy, and the, expansion of farmlands put pressures on mountainous grazing areas which in turn resulted in deforestation, soil erosion and, irreversible ecological degradation (Kidane Mollaw, 2014).

Table 5. Cause of honeybee colony and yield decrease

Common problems	Relative degree of importance									Index	Rank
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th		
Lack of bee forage	13	18	28	6	1	1	1	1	0	0.113	6
Lack of water	0	0	0	1	1	1	1	5	18	0.044	8
Drought	0	0	0	0	0	0	3	5	10	0.030	9
Absconding	2	11	14	8	1	0	17	5	5	0.103	7
Unwise use of agrochemicals	15	8	23	24	4	5	4	0	0	0.136	3
Pests and predators	55	14	22	5	2	1	1	0	0	0.164	1
Low price of honey	9	11	10	13	10	15	5	2	2	0.126	5
Honeybee disease	32	45	8	2	1	1	2	1	0	0.151	2
High price of bee equipment	13	18	28	10	3	5	2	1	0	0.131	4

*Index = sum of (9*ranked 1st + 8* ranked 2nd +7* ranked 3rd+6* ranked 4th+5* ranked 5th+4* ranked 6th+3* ranked 7th +2* ranked 8th +1* ranked 9th) for individual reasons divided by the sum of (9*ranked 1st+ 8* ranked 2nd+7* ranked 3rd+6* ranked 4th+5* ranked 5th+4*ranked 6th+3* ranked 7th +2*ranked 8th +1*ranked 9th) for overall reasons.*

The result is also similar to the finding of (Amsalu A, 2020). The causes of decrease of honey bee colony and yield, in order of importance, were lack of bee forage, pest and predators and Honeybee diseases. All these factors lead to decrease in productivity and honeybee colony population in Diga and Wayu Tuka districts. According to Segni Sh, (2017), in Ejere District the major problem affecting beekeeping development in decreasing order of importance were also pests and predators, high cost of modern hives and accessories, agro-chemical application, honey bee disease, shortage of bee forage, and absconding. Similarly Amssalu B *et al.*, (2010) reported the presence of honeybee pests and pathogens, prevailing bad weather, Lack of knowledge and skill of honeybee pest and diseases control, application of agrochemicals, shortage of bee forage, poor or absence of practice of hive shading, Lack of practice of hive inspection and shortage of improved hive types to affect development of beekeeping in a decreasing order of importance.

Honeybee Pests and Predators

Among all constraints of beekeeping, these natural bee enemies were known to cause great damage to honeybee colonies and their products. After identifying the major pests, beekeepers were requested to rank them in order of their importance and the result indicated that Ants ranked 1st, beetles 2^{nd}, Wax moth 3^{rd} and honey badgers 4^{th}. The were the most harmful pests (Table 6). Similarly Ants are most disturbing to honey bees affecting bee keeping sector thus causing severe economic loss in honey production by killing bees, robbing their products, initiating aggressiveness in bees, leading to absconding and destroying the entire colony of honey bees (Amssalu, and Desalegn, 1999). Also in Tigray, Amhara and SNNP regional states and Gomma district in Jimma zone bee keepers ranked ants as first honeybee pest in honey bees (Amsalu *et al*., 2010). Bees are the first and most victim of the attack with ants followed by honey budger (Desalegn, 2007).

Table 6, Honeybee pests and predators

Factors	The relative degree of pests and predators effects									Index	Rank
Pest and predators	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
Ants	63	11	5	3	1	1	1	1	1	0	0.171	1
Wax moth	1	4	25	32	2	3	2	1	0	0	0.138	3
Bee lice	0	0	0	0	2	3	6	17	1	0	0.057	8
Beetles	22	28	11	7	6	2	2	0	0	0	0.154	2
Dead head hawks moth	1	2	7	11	12	15	1	0	1	1	0.100	6
Spiders	0	0	0	0	1	5	17	8	0	0	0.061	7
Wasps	0	0	0	0	0	0	0	1	12	15	0.055	9
Bee eater birds	0	1	3	9	16	8	7	5	2	3	0.106	5
Lizard	0	0	1	4	12	0	0	1	4	12	0.033	10
Honey badgers	2	9	12	23	6	2	4	2	2	1	0.124	4

*Index = sum of (10*ranked 1^{st} + 9* ranked 2^{nd} +8* ranked 3^{rd}+7* ranked 4^{th}+6* ranked 5^{th}+5* ranked 6^{th}+4* ranked 7^{th} +3* ranked 8^{th} +2* ranked 9^{th} +1* ranked 10^{th}) for individual reasons divided by the sum of (10*ranked 1^{st} + 9* ranked 2^{nd} +8* ranked 3^{rd}+7* ranked 4^{th}+6* ranked 5^{th}+5* ranked 6^{th}+4* ranked 7^{th} +3* ranked 8^{th} +2* ranked 9^{th} +1* ranked 10^{th}) for overall reasons.*

According to Yetimwork *et al.* (2015) and Adeday *et al.* (2012) honey badger, ants, wax moth, spider, birds, lizard and snake were identified as pests and predator to the bees in eastern part of Tigray. Similar honeybee pests and predators were reported from other parts of the country such as Amhara region Tessega (2009), Gomma district of Jimma zone, South-west Ethiopia (Chala *et al.*, 2012), Gamo Gofa zone of southern Ethiopia, (Nubiyu and Messele, 2013), Sidama Zone, Southern Ethiopia (Tariku and Mechthild 2013) and Ada'a district of east Shoa Oromia region (Tesfaw, 2012). Similarly, reports from Eastern Tigray (Yetimwork G,*et al*,. 2015) indicated the most important pests and predators in honey production are honey badger (*Mellivora capensis*), ants, wax moth, spider, birds, lizard, and snake in that order. Also in Ugand (Kajobe R 2016) it is documented that the eleven honeybee pests and predators that affect beekeeping and are of greatest economic importance were black ants (Monomorium minimum), small hive beetles (Aethina tumida), wax moths (Galleria mellonella), bee hornets (Vespa ssp.).

Honeybee colonies infested and absconded by pests and predators

According to beekeepers report, the honeybee colonies in all districts were infested by pests and predators (by ants 538 colonies or 21.3%, by beetles 378 colonies or 14.9%, and by wax moth 315 colonies or 2.5%). In all of the study districts, beetles, wax moth and ants, among other pests and predators, were responsible for absconding of 193 honeybee colonies or 7.6%, 194 or 8.4%, and 213 or 8.4%, , respectively (Table 7). This finding is similar with that of Tiblets M, (2017) in which the major cause of an enormous damage to the bee colonies (adult and brood) and their products, among the most frequent pests and predators, were reported to be ants. Despite being more prevalent than wax moth and more attackers of honeybee colonies, beetle infestation was still high. In Ethiopia ants are series problems in bee keeping as reported by Awraris *et al*. (2012) in Keffa, Shako and Bench- Maji zone; and by Tesfaye (2007) in Adami Tulu and by Etsay and Ayalew (2001) in Eastern Tigray.

Table 7. honeybee colonies infested by and absconded due to pests and predators

Honeybee colonies infested and absconded	Study districts				Colonies in All Districts
	Diga	Gida Ayana	Gobu Sayo	Wayu Tuka	
Total colony owned by respondents	674	693	584	579	2530
Number of colonies infested by wax moth	77(14.8%)	90(13.9%)	63(11.9%)	85(15.0%)	315(12.5%)
Number of colonies absconded by wax moth	43(8.3%)	58(8.9%)	42(7.9%)	51(9.0%)	194(7.7%)
Number of colonies attacked by bird	71(13.6%)	103(15.9%)	64(12.1%)	64(12.2%)	307(12.1%)
Number of colonies absconded by birds	19(3.7%)	24(3.7%)	21(3.9%)	21(4.6%)	90(3.6%)
Number of colonies infested by beetles	102(19.6%)	107(16.6%)	90(17%)	79(13.9%)	378(14.9%)
Number of colonies absconded by beetles	43(8.3%)	58(8.9%)	42(7.9%)	50(8.9%)	193(7.6%)
Number of colonies attacked by honey badger	73(14.0%)	89(13.8%)	73(13.8%)	80(14.2%)	315(12.5%)
Number of colonies absconded by honey badger	29(5.7%)	44(6.8%)	29(5.5%)	36(6.4%)	138(5.5%)
Number of colonies infested by ants	139(26.7%)	143(22.1%)	149(28.1%)	107(18.9%)	538(21.3%)
Number of colonies absconded by ants	49(9.4%)	64(9.9%)	54(10.2%)	46(8.1%)	213(8.4%)
Number of colonies infested by head hawks moth	59(11.3%)	74(11.5%)	39(7.4%)	66(11.7%)	238(9.4%)
Number of colonies absconded by death head hawks moth	20(3.8%)	32(5.0%)	24(4.5%)	23(4.1%)	99(3.9%)
Total colonies attacked/infested by predator/pests	521(77.3%)	606(87.5%)	478(81.9%)	486(83.9%)	2091(82.6%)
Total honeybee colonies absconded	203(30.1%)	280(40.4%)	212(36.3%)	232(40.1%)	927(36.6%)

Local control practices of honeybee pests an3d predators

Beekeepers use different methods to control honeybee pests and predators. 98.7% of the beekeepers reported that they try to minimize the risk by different indigenous practices (Table 8). Moreover, frequent visiting and cleaning of an apiary were indicated to be part of the solution.

Table 8, local control practices of honeybee pests and predators

Pests and predators	Local Control method
Ant	Cleaning the apiary, Spreading fresh ash beneath hive stands, painting the legs of hive stands with old motor oil, sandwiching smooth iron sheets between the hive and hive stands, plastering the hive stands' legs with mud or cement, and taking daily inspection.
Honey Badger	Chasing and Killing, Fencing of the apiary, using dog to keep it away during the night
Bee-Eater Birds	Placing the seeming image of human made of clothes and plastic sheets near the hives
Wax moth	Cleaning apiary, removing old comb, and strengthening the colony, removing empty hives from the apiary, carrying out seasonal management, supervising the apiary daily s
Lizard	Removing their nesting
Death's-Head Hawkmoth	watching and killing
Beetle	Cleaning hives, narrowing the hive entrance, hand picking and killing.

The prevalence of honeybee disease and Pests

In this study, 52 beekeeping sites and 104 honeybee colonies were examined for major honeybee parasites (Varroa mites, tracheal mites and bee lice), for adult honeybee diseases (Nosema and Amoeba) and for brood diseases (Chalk brood disease- CBD, American foulbrood -AFB and European Foul brood -EFB). No AFB, EFB, and tracheal mite were found positive during the study time. Field data collection and laboratory diagnosis during the study is shown in Figure 3.

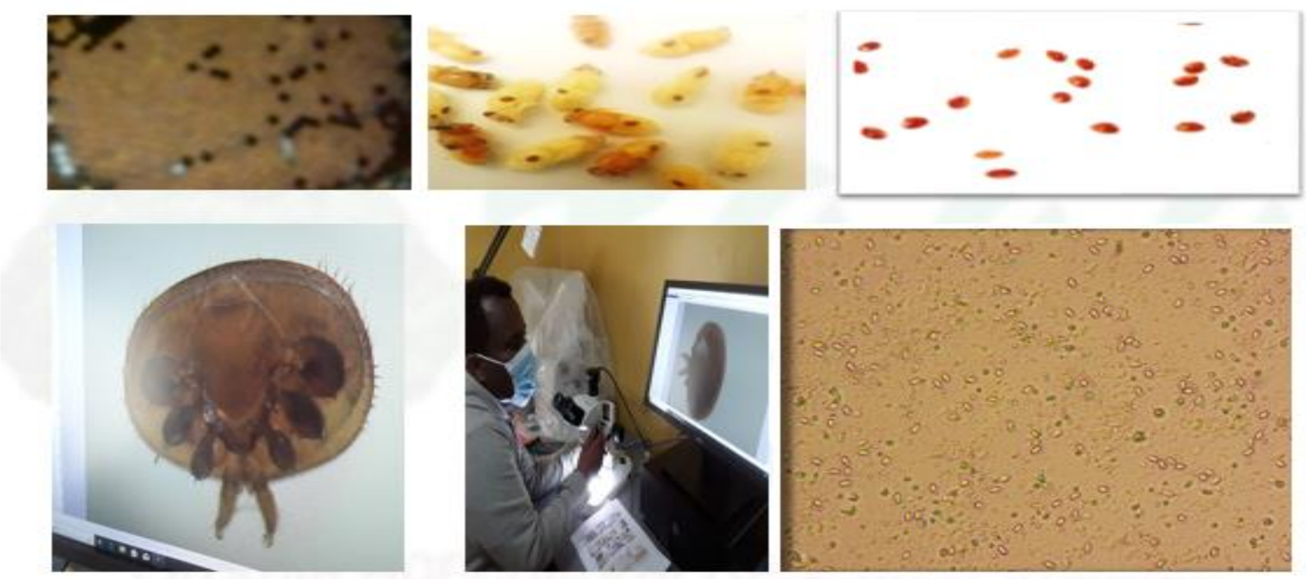

Figure 3. Field and laboratory diagnosis carried out.

Prevalence of chalk brood disease

Out of 52 apiary sites 10 (19.2%) and out of the 104 colonies examined 21 (20.2%) were found to be infected with chalk brood disease (*Ascosphaera apis)*. The chalk brood prevalence in East wollega Zone ranged from 10 to 31.8 % with an average of 20.2%. Highest prevalence (31.8%) was recorded in Diga district and the lowest (10%) in Gobu Sayo district (Table 9). With regard to apiary sites, the distribution rate of chalk brood ranged from 6.7% to 30.8% with mean of 19.7%. Relatively the highest distribution rate was recorded at Wayu Tuka (30.8%) while the lowest was recorded at Gobu sayo (6.7%) (Table 9).

The current result was less than earlier reports made at various times and in various parts of Ethiopia. When the Ascosphaera apis was initially discovered in Ethiopia (Desalegn, 2000; Amsalu, 2020), it had infected 61.5% of apiaries and 17.4% of honeybee colonies. After that, 56.5% chalk brood prevalence was reported in the Shoa and Arsi zones of the Oromia area (Desalegn 2001). Desalegn (2006) also reported a prevalence of 43% from Addis-Abeba. According to diagnostic survey of conducted by Aster *et al*. (2010), the Chalk brood prevalence rates of 37.12, 19.9 and 17.9% were reported for Amhara, Oromia, and Benshangul-Gumuz regional states, respectively.

Table 9. Prevalence of Chalk brood disease

Districts	Distribution rate			Prevalence		
	Examined sites	Positive	Distribution (%)	Examined colonies	Infected colony	Infection (%)
Diga	11	3	27.3	22	7	31.8
Gida Ayana	13	2	15.4	26	5	19.2
Gobu Sayo	15	1	6.7	30	3	10.0
Wayu Tuka	13	4	30.8	26	6	23.1
overall	**52**	**10**	**19.2**	**104**	**21**	**20.2**
	Chi square = 3.57		**P-value = 0.31.**			

Prevalence of Amoeba disease

From investigation of the 52 beekeeping sites for *M. mellificae,* 24 (46.2%) were found to be infested and out of the 104 honeybee colonies, 52 (50.0%) were found positive for the Amoeba (Table 10). Prevalence and distribution levels of amoeba disease was not significantly different among study areas (X^2 = 5.85, $P>0.05$). Amoeba became the most common infection of adult honey bees in the study districts, as they are common in most parts of the country as mentioned by (Amsalu B, and Desalegn B, 2005). Amoeba (*M. mellificae*) diseases is widely distributed and identified in most places of the country (Bezabeh A., 2012). From assessment of 146 beekeeping sites and 292 honeybee colonies in Diga and Wayu Tuka districts, Amsalu A, (2020) found the infestation of Amoeba (*Malpighamoeba mellificae*) disease to be 113 (77.4%) for apiary sites and 119(79.7%) for colonies

Table 10. The Prevalence of *Malpighamoeba mellificae*

Study Districts	N (Apiary))	Distribution of Amoeba		N (colony)	prevalence of Amoeba	
		+Ve	%		+Ve	%
Diga	11	5	45.5	22	12	54.5
Gida Ayana	13	4	30.8	26	13	50.0
Gobu Sayo	15	7	46.7	30	14	46.7
Wayu Tuka	13	8	61.5	26	13	50.0
overall	**52**	**24**	**46.2**	**104**	**52**	**50.0**
X^2 for prevalence and distribution	**Chi square = 5.85.**		**P-value = 0.12**			

N=Number of beekeeping sites and honeybee colonies examined, +Ve= Number of honey bee colonies found positive,

Results of the current study indicated that the distribution of amoeba was higher than what was reported by Bezabeh A. (2012). From 384 honeybee colonies he examined for the presence of amoeba disease, an overall prevalence of 159 colonies or 41.4% was obtained. However the result of the current study is lower than those of previous studies in different parts of the country such as in the Oromia region 88% prevalence rate, the Amhara region 95%, and the Benishangul-Gumuz 60% (Yohanis A. et al, 2009).

Prevalence of Nosema disease

Examination of the 52 beekeeping sites and 104 honeybee colonies for the prevalence of *Nosema apis* indicated that 26 sites (50%) and 53 colonies (51%) were infected (Table 11). There was no significant difference in the prevalence and distribution levels among the study areas. As mentioned by Amsalu B. and Desalegn B. (2005), *Nosema apis* was the most common infection of adult honey bees in the study districts, as they are common in most parts of the country

Table 11**,** Prevalence and distribution of *Nosema apis* in the study areas

Study Districts	N=52 Apiary	Prevalence Of Nosema apis		N=104 colony	Infectious of Nosema apis	
		+Ve	%		+Ve	%
Diga	11	5	45.5	22	12	54.5
G/Ayana	13	6	46.2	26	14	53.8
G/Sayo	15	8	53.3	30	14	46.7
W/Tuka	13	7	53.8	26	13	50.0
Overall	52	26	50.0	104	53	51.0
X^2 for prevalence and Distributionn		Chi square = 1.87, P value = 0.6				

N=Number of beekeeping sites and honeybee colonies examined, +Ve= Number of cases found positive

The overall prevalence of Nosema disease was 50.0% for the sites, which is in agreement with the study conducted by Yohannes *et al*. (2009) in which 47% prevalence was reported in the Amhara region. Furthermore, finding from the current study is lower than those from other studies conducted by Begna and Kebede, (2005) a prevalence of 53.3% in Addis Ababa, by Yohanis A. et al, (2009) a prevalence of

58% in Oromia and 60% in Benishangul Gumuz. The 51.0% colony infection by *N. apis,* found in the current study was higher than the 40.5% reported by Amssalu and Desalegn (2005) in Southern Nations, Nationalities Region..

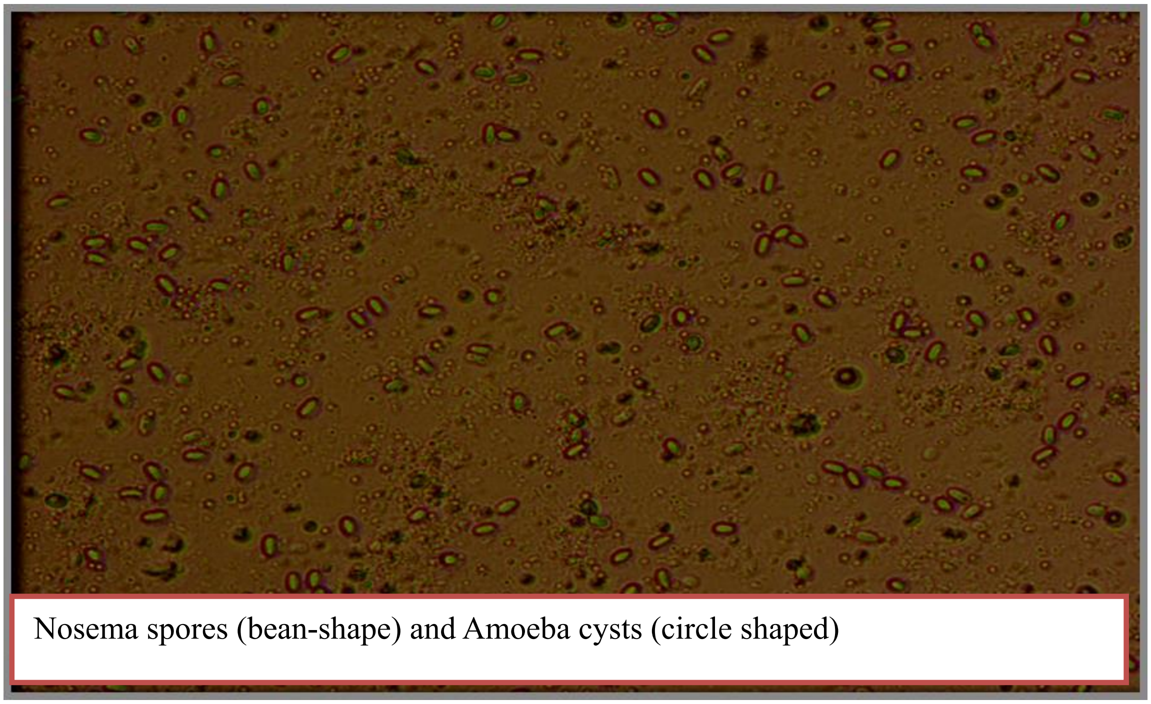

Figure 4 *Nosema apis sporses and and Malpighamoeba mellificae cysts under 40X of light microscope***.**

Prevalence and infestation of Varroa mites

The Asian honey bee (*Apis cerana*), is where Varroa first developed. Since then, it has spread to most of the world's populations of western honey bees (*Apis mellifera*). (Paul 2012) claims that Varroa is currently present in almost all honey bee colonies at varying levels of infestation and that if left untreated, the infestation would continue to grow. Varroa can devastate heavily infested colonies due to its effects of parasitism on both adult and brood honeybees. It has a devastating impact on apiculture industry worldwide due to its major role in the colony collapse (Zattara, E.E. and M.A. Aizen, 2021). With a nearly global distribution, this parasitic mite will severely weaken or cause the collapse of most honeybee colonies if it is left uncontrolled or untreated in a sustainable manner (Traynor, K, 2020).

In this study, Varroa mite was detected in both the brood and adult honey bee samples in all districts. Results from samples of adult honeybees and broods collected from 52 apiary sites showed that, 94.2% of adult honeybees and 93.0% of broods were varroa positive. This indicated that Varroa distribution in the study areas is 94.2% in adult bee samples and 92.3% in sealed broods. Forty nine of the 104 honeybee colonies diagnosed for varroa also tested positive for the parasite. The Varroa infestation ranged from 90% to 96.2% in the colonies with an average of 93.3%. There were no significant variations among the study areas in distribution and prevalence of varroa. The reason for similarity and difference in prevalence and infestation of varroa mite is based on the presence and absence of brood in the hive. The infestation of varroa mite is based on access to brood where female varroa mite lay its eggs in larval cell of bees to complete its stage of development. Since this study is based on cross sectional study as indicated above there is no difference in prevalence and infestation of varroa mite in the study area.

The result was much higher than the findings by Amsalu A, (2016) in Diga and Wayu Tuka districts, in which 69.6% of the apiary sites and 78.5% of the colonies were found to be infested by Varroa Mites.

Table 12, Prevalence and distribution levels of Varro *mites in the study areas*

Study Districts	N=52	Varroa Mite In Apiaries		Varroa Mite In Sealed Brood		N=104	Infestation of varroa	
		+Ve	%	+Ve	%		+Ve	%
Diga	11	11	100	10	90.9	22	21	95.5
G/Ayana	13	11	84.6	12	92.3	26	25	96.2
G/Sayo	15	15	100	14	93.3	30	27	90
W/Tuka	13	12	92.3	12	92.3	26	24	92.3
Overall	52	49	94.2	48	92.3	104	97	93.3
X^2 for prevalence and distribution levels	Chi square = 1.36 P-value = 0.71							

N=Number of apiary sites examined, +Ve= Number of sites found positive

The prevalence and infestation of bee lice

Twelve (23.1%) of the total 52 apiary locations tested and 25 (24.0%) of the total 104 honeybee colonies tested for the presence of Braula coeca were found positive. Thus these results indicated that the distribution and prevalence levels of bee lice were 23.1% and 24%, respectively. There was no significant variation among locations in both prevalence and distribution of bee lice, (Table 13).

Table 13, the prevalence and infestation of bee lice

Study districts	**N=52**	**prevalence of bee lice**		**N=104**	**Infestation of bee lice**	
		+ve	%		+Ve	%
Diga	11	2	18.2	22	6	27.3
G/Ayana	13	4	30.8	26	5	19.2
G/Sayo	15	5	33.3	30	6	20
W/Tuka	13	1	7.7	26	8	30.8
overall	52	12	23.1	104	25	24
X^2 for prevalence and distribution	Chi-square = 21.44. P-value =0.15.					

N=Number of apiary sites examined, +Ve= Number of sites found positive

In contrast to past findings in Ethiopia, the total prevalence of bee lice (23.1%) in the current study was higher than the 4% prevalence rate report by Gidey *et al*. (2012) in Tigray. Also 42% lice prevalence was noted around Holata by Gemechis et al. (2013). The finding of this study is similar with that of Gemechu G, (2013) in whih from a total of 385 bee colonies examined for the presence of bee lice, 162 (42%) were found infested with lice.

Conclusion and Recommendations

Beekeeping plays a significant role in rural communities by supplying a range of goods, in particular, honey, wax, and pollen, and enhancing the ecology through pollination. Beekeeping activities in all districts are practiced as sideline with other agricultural activities. The sources of honeybee colonies to start beekeeping and increasing stocks in the study area were swarm catching and gifts from parents. Honeybee colony population and honeybee products were decreasing, due to pets, predators, honeybee diseases, and inappropriate agrochemical applications. Among all restraints of beekeeping identified as the major pests, Ants, beetles, Wax moths, and honey badgers were the most harmful ones. The honeybee colonies in all districts were infested by ants, beetles, and wax moths that were responsible for absconding honeybees and their yield decrease. Despite being more prevalent than wax moths in attacking honeybee colonies, beetle infestation was high. The identified majority of honeybee pests were ants, beetles, and wax moths, and the majority of honeybee parasites were varroa mites and **bee lice**. Based on laboratory findings, the most prevalent parasites and pathogens, including Braulacoeca, Varroa destructor, Nosema apis, Malpighamoeba mellificae, and Ascosphaera apis, were confirmed in the area. AFB, EFB, Stone brood diseases, and tracheal mites were not confirmed. To determine the economic threshold of honeybee disease and pests, regular evaluation of honeybee diseases and pests is required.

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Assessment of beekeeping practices and honey quality analysis in Borana Zone, Southern Ethiopia

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Abstract

The current study was conducted at Elewaya and Yabello districts of southern rangelands of Borana zone with the objective of assessing beekeeping practices and honey quality parameters. Structured questionnaire for assessing the beekeeping production practices. A total of 164 beekeepers were selected for the household field survey. The laboratory analysis of honey was examined to verify quality states of honey in the area. The honey quality parameters considered were, Moisture content, Ash, HMF, Free acidity, pH, Electrical conductivity and reducing sugars were analyzed following the standard procedure. Descriptive statistics and GLM procedure of SAS were employed to summarize traditional honey production practice and identify the quality status of honey samples collected from various localities in the study area. Inclusively, honey production practice of the area is traditional with 96.91% of beekeepers hanging the log hives on selected trees nearby rangelands. The type of honey produced in the area was classified based on color as Black, Red and White and the white one was ranked first in terms of quality and price. There were decreasing trend of number of honeybee colonies and yields as stated, by (25.5%) and (35.8%) of the respondent respectively. The decreasing trend was associated with declining of bee forage which is consistent ($\chi^2 > 0.05$) across the study area. Annual honey production of the household (HH)⁻ significantly varied ($P<0.05$) across the study PAs with an overall 117.01kg per annum and about 95.06% produced from two honey flow seasons. Laboratory analysis of honey sample indicated that the reducing sugar (RS), sucrose content (SC), moisture content (MC), pH, free acidity (FA), Electrical conductivity (EC), ash percentage (Ash) and Hydroximethylfurfural (HMF) are significantly varied ($p<0.05$) between the districts which was associated with agro-ecolgies and post-harvest handling of honey. Pure honey samples analysis for comparison after addition of 1:1 table sugar significantly increased SC, pH and HMF value towards 3.6, 1.2 and 52.69 respectively. It can be concluded that the honey production system is predominately traditional and honey quality analysis in the area has a good quality within the range of Ethiopian and International standards. To improve the existing traditional beekeeping system, introduction of improved beekeeping technologies and improving the pre- and post-harvest management of honey in the area are recommended.

Key words: ***Beekeeping practice, Borana zone, Honey Adulterant, Honey qualitiy***

Introduction

Beekeeping has been practiced in Ethiopia for centuries and currently the country is the largest honey producer in Africa. According to Ayalew and Gezahegn (2002), Ethiopia is the leading honey producer in Africa and one of the ten largest honey and beeswax producing countries in the world. However, the majority of beekeepers in the country still use traditional beekeeping (MOARD, 2007) and hence honey is almost exclusively used for local consumption, and to a very large extent (80%) for brewing of honey mead (Tej). Ethiopian produces 129,000 tons of honey and 30% from its potential (CSA, 2020/2021).

Honey is natural complex food product produced by bees from nectar of flower or from secretions of living parts of plants, which honey bees collect, transforms and combines with specific substances of their own, store and leave in the honey comb to ripen and mature". It is a unique sweetening agent that can be used by humans without processing, (Ahmed et al., 2007). Honey is a widely consumed, not only for its taste and nutritional value, but also for its health benefits. Honey is associated with its medicinal properties and traditionally used for healing wounds and for the treatment of colds and sore throats. Regarding its nutritional value, honey is essentially composed of water and sugars (mainly fructose and glucose). Honey also constitutes other valuable substances, such as vitamins, minerals, enzymes, flavors, free amino acids, and numerous volatile compounds (Schievano et *al*., 2013).

During the last several decades, documents have been developed aiming at proposing tools to assess the quality and authenticity of honey, satisfying the consumers demand and promoting fair competition among producers. The quality of honey is mainly determined by its sensory, chemical, physical and microbiological characteristics (Khalil et al., 2012), with the required composition criteria (sugar content, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, and hydroxyl methyl furfural HMF content) being described in honey quality standards and legislation.

The purpose of the honey quality analysis is to verify the authenticity of the product and to reveal the possible presence of artificial components or adulterants, as well as to address processing and market needs (Krell, 1996). For honey quality analysis, physicochemical properties of honeys have been helpful for comparison of natural honey samples from different locations and also serve as important indicators that can help to distinguish natural honey from adulterated honey (Krell, 1996). Honey adulterated by sugar addition can bring, changes in some physical and biochemical parameters, such as enzymatic activity, electrical conductivity, HMF, sugars and ash) as compared to a natural one.

Regardless of the large number of honey bee colonies and enormous honey bee flora in the Ethiopia in general and Borana Rangelands in particular, production and productivity of beekeeping is below its potential. The postharvest handlings of bee products are comparatively low to maintain the quality of the honey. Furthermore the adoptions of improved honey harvesting, processing and storage technologies are limited to improve the quality and to produce marketable bee products.

Despite the fact that Borana zone is believed to have diversified type of vegetation and cultivated honeybee flora creating conducive environment for beekeeping activities, so far little is known about the existing beekeeping production practices, quality of honey produced, and constraints and opportunities of beekeeping in the zone. This is a paramount importance for producers and other stakeholders involved in the business so as to promote beekeeping production efficiently. Therefore, the main objective of study was to assess the honey production practice, analyze the quality state of honey and to evaluate the effect of sugar adulteration on honey quality in Borana Zone.

Materials and Methods

Description of the Study Area

The Borana zone is one of 13 administrative zones within Ethiopia's Oromia Regional state. It is located in the Southern part of the Oromia at 3°36'– 6°38'N and3°43'-39°30' E. Kenya. The altitude of the Borena zone ranges from 1000-1650m (Figure, 1). Yabello is the capital town of the Borana zone and lies at 570 km south of Addis Ababa. According to the information obtained from livestock resource development office, the agricultural activities in the zone are pastoral and agro pastoral production system. The areas exhibit bimodal pattern of precipitation with long rainfall between March–May and the short rains between September and November. The mean annual rainfall ranges from 400mm to 700mm and the average temperature varies from annual minimum of 24 ^{0}C to annual maximum temperature of 30^{0}C.

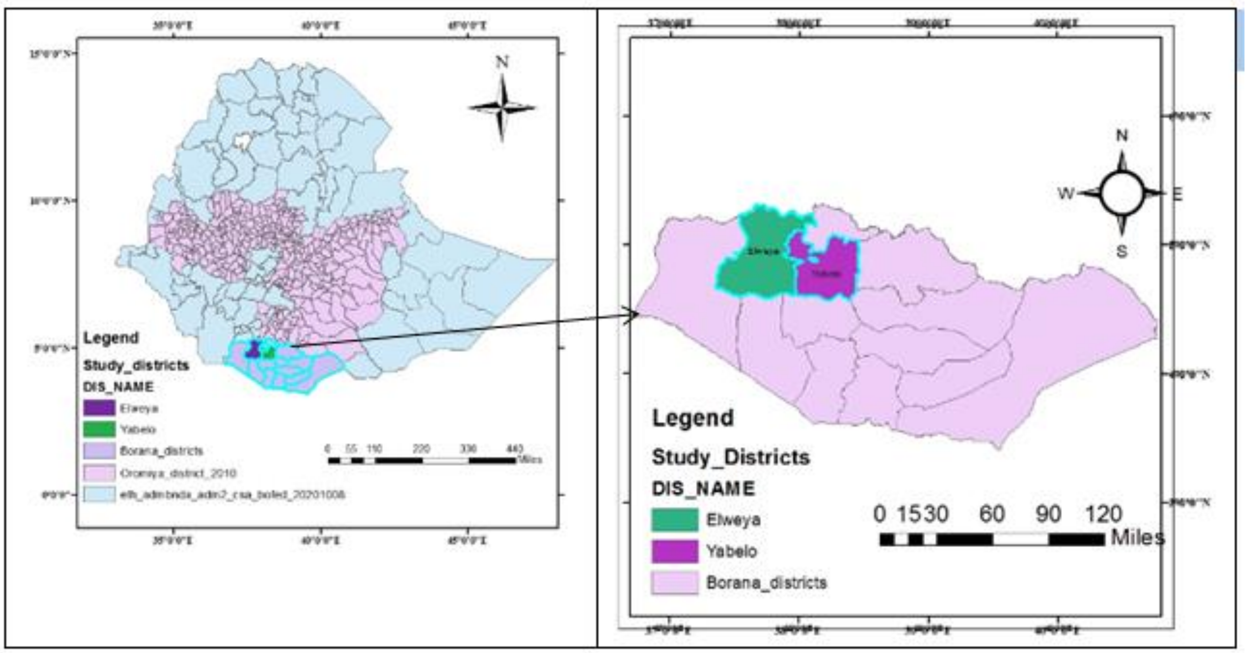

Figure 3. Map of the study area

Sampling techniques and procedures

A multistage sampling technique was employed to collect the data from beekeeping households (HHs). The selection of the districts was made based on the potentiality of the area for beekeeping. Accordingly two (2) kebeles from each study districts were selected purposively based on honey production potential, in consultation with the Office of Livestock and Fishery Resources of the districts. A total of 164 respondent households were sampled using the formula developed by Yamane (1967).

$$\mathbf{n = \frac{N}{1+N(e)^2} = 846/1+846(0.0049) = 164}$$

Where n is required sample size, N is the population size, and e- is the level of precision.

Table 6: Sampling of Households (HHs)

District	Kebele	Total house holds[1]	Total beekeepers	HHs > = 2 bee hives	Proportion (%)	Sample HHs
Elwaya	Hiddi	630	280	180	0.19	34
	Bidha	571	271	165	0.19	31
	Obda	730	380	211	0.19	42
Yabello	Yubdo	894	407	290	0.19	55
	Total	**2825**	**1338**	**846**		**164**

Source: District livestock resource development office, 2019.

Data collection

Relevant data for the survey work was gathered from the selected HHs using the pretested structured questionnaire. Moreover Focused group discussions and key informant interview were conducted using a prepared checklist that would help to confirm the responses from the HHs. The Focused group discussions was held with selected beekeepers having a representation of both gender while the key informants' interview was made with bee experts and model beekeepers who have long experience in beekeeping in the districts. The data collected include: Socio-economic characteristics of HHs, land holding, livestock holding, practices of honey production, types of bee forages, honey yield, contribution of honey to the HH income, constraints and opportunities of beekeeping etc.

Collection of honey Samples and Laboratory Analysis

A total of 96 honey samples were collected randomly from farm get and local market surrounding Yabello and Eleweya districts of Borana zone. The collected samples were taken to Yabello Pastoral and Dry land Agriculture Research Center (YPDARC) Laboratory following the required sample handling procedures. The honey samples to be analyzed were homogenized by stirring thoroughly to prepare representative samples. Crystallized honey samples in a hard and compact mass was softened by heating using hot plate at temperature less than 40 ^{0}C for analysis of all test parameters except for determination of HMF content.

Honey quality analysis

The collected honey samples from bee keeping households, and local markets were analyzed for their physico-chemical properties including honey samples with deliberate addition of some susceptible adulterants. All collected honey samples were analyzed during the same time period to ensure uniform conditions and comparability. The honey quality parameters were analyzed following the procedure of International Honey Commission (IHC).

Free Acidity and pH

Free acidity of honey was determined by dissolving 10 grams of sample in 75 mL of distilled water with the help of magnetic stirrer. Free acidity ((meq/kg)) was calculated using the formula: Free acidity (meq/kg) = Volume of 0.10M NaOH (mL) x 10. For the measurement of pH the Electrode of calibrated pH meter was immersed into the solution and the pH value was recorded.

Determination Ash Content

To determine the mineral (ash) content of honey, 5g honey samples were transferred into ignited and pre-weighed platinum crucibles. The contents were charred on Bunsen burner until the sample is dry and smokeless. The sample is then ignited in a muffle furnace at 600^0C for about 4 hours. After complete ignition to constant weight, the sample was cooled in a desiccator and weighed immediately. Percent ash content was calculated using the following formula (QSAE, 2009):

$$\textbf{Ash content (\%)} = ((\underline{w2 - w1})\ x100)/M$$

Where, W1 = weight of empty crucible; W2 = weight of the ash + crucible after ignition; M = mass of the sample taken for test.

Moisture Content

The moisture content of collected honey samples was determined by smearing drops of homogenized honey on the surface of Abbé-refractometer, prism and allowing refractive index for 2 minutes. Here, the refractive index was then adjusted to a standard 20^0C temperature and mean refractive index readings were used to calculate the moisture content of the samples using the following formula.

$$\textbf{Moisture (\%)} = (-\log 10\ (\text{Corrected Refractive Index}-1) -0.2681)/0.002243$$

Electrical Conductivity

To measure electrical conductivity of honey, about 20g of honey was weigh and dis-solved in 100 ml distilled water. Electrical conductance of the honey solution was measured using digital conductivity meter.

Hydroximethylfurfural (HMF) Content

The analysis of the Hydroximethylfurfural (HMF) content was done based on the determination of UV absorbance at 284 nm. In order to avoid the interference of other components at that wavelength, the difference between the absorbance of a clear aqueous honey solution and the same solution after addition of bisulphite was determined. The HMF content was calculate after subtraction of the background absorbance at 336 nm .The HMF content of the sample was then calculated by the following formula.

$$\textbf{HMF (mg/Kg)} = (A284 - A336)\ x149.7x5/W$$

Where, A284 = absorbance at 284 nm; A336 = absorbance at 336 nm; W= Weight of sample taken

Reducing Sugars and Sucrose

Reduction of sugar was carried out using the Lane-Enyon method. About 2.6 g of honey sample was weighed and then transferred to a 500 mL volumetric flask. Five milliliters of standardized Fehling A and B solutions was transferred to a 250 ml Erlenmeyer, with 7 mL of water and 15 mL of honey solution. The Erlenmeyer was heat and 1 mL methylene blue 0.2 % was added. Titration was carried out adding the diluted honey solution until the indicator was decolorized. Determining sucrose content was carried out by inversion, adding 10 mL of diluted HCl, 50 mL diluted honey solution and water to a 100 mL volumetric flask, heating in water bath, then cooling and diluting to mark. Finally the Lane-Enyon method was applied and sucrose content was obtained by difference.

Data management and Statistical analysis

The collected data was summarized and presented using simple descriptive statistics in. Statistical Package of Social Science (SPSS) version 23. Analysis of variance (ANOVA) following the General Linear Model (GLM) procedure of SAS, version 9.1.3was used to analyze the honey sample data.

The model fitted for the experiment was:

$$\mathbf{Y_{ij} = \mu + HS_i + E_{ij}}$$

Where; Y_{ij} = response variable, μ = overall mean effect, HS_j = the effect i^t source of honey sample on honey quality parameters, E_{ij}=random error

Result and Discussions

Socio-Economic Characteristics

The socio-economic characteristic of respondents in the study area was depicted in Table 2. The analysis of the result showed that the mean age of the traditional beekeepers was 32.62 years with a range of 18 to 76 years. The results also further revealed that the respondents' average beekeeping experience was 11.42 years which ranged from 1 to 35 years. The higher the numbers of years spend in beekeeping activity by beekeepers, the more they become aware of new production techniques (Iheanacho, 2000) there by increasing the level of productivity. Information pertaining to the average number of colonies owned was 18.25 ranging from 3 to 70 colonies in the study area. With an estimated low cost, of traditional practice, beekeepers have been generating substantial annual income earning an average of 12, 319.14 ETB from the honey sale ranging from 1,000.00 to 27, 000.00 ETB per annum.

Regarding the age of beekeeper households, most of the beekeepers are in their younger age. This shows that, beekeeping activity needs intensive labor work including cutting of big trees for bee hives preparation, climbing and hanging the hives on the trees etc. Consequently in the current study the average age of beekeepers was **32.62** years old. This indicates that practicing forest beekeeping is strongly associated with younger age and excludes the involvement of women and older people. This is in agreement with previous findings by Tijani et *al*. (2010) and Mollaw (2014) who stated that traditional beekeeping practice is hardly possible for women and old people as it mostly associated with climbing a tree.

Table 7: Socio-economic characteristics of the sample households in the study area

Element	Mean ± STD	Minimum	Maximum
Age ($Years^{-1}$)	32.62 ± 13.45	18.00	76.00
Experience ($Years^{-1}$)	11.42 ± 7.91	1.00	35.00
Colony ($Num.^{-1}$)	18.25 ± 9.86	3.00	70.00
Income (ETB^{-1})	12,319.14 ± 6,718.32	1,000.00	27,000

Educational Level and religion of the respondents

The educational status of respondents showed that 46.91% were illiterate and 27.78% attained junior school and the remaining also completed primary and secondary school Table 3. The educational status of the respondent is a great important for any beekeeping production intervention. According to Workneh (2006) indicated that education is very crucial to extend beekeeping production, improving knowledge which confers with the current results of the study. From this study, any beekeeping production development can substantially be installed as long as appropriate extension is intervened to the traditional beekeepers of the area. It was also illustrated that about 46.91% and 44.44 % of the respondents were Wakefatas and Protestant religion followers, respectively (Table 3).

Table 8: Educational level and religion of the sample households in the study area (n=162)

Educational Level	Frequency	Percent	Religion	Frequency	Percent
Illiterate	76	46.91	Muslim	14	8.64
Read and write	7	4.32	Protestant	72	44.44
Primary	16	9.88	Wakefata	76	46.91
Junior	45	27.78			
Secondary	12	7.40			
College graduate	6	3.70			

The grouping order of number of beehives owned by beekeepers

According to the respondents, all of the beekeepers have traditional type of beehive, and the numbers of beehives owned ranged between 3 to 7 (9.8% of HHs), 8 to 12 (19.14% of HHs) 13 to 17 (21.60% of HHs) and the rest owned 18 and above which accounts for 49.38% of HHs (Table 4). Traditional log hive is the common one that is made either by drilling or cutting logs into two halves then perforating of a piece of tree trunk. Noticeably, there has been no size standard for locally made traditional log hives; rather the size of a particular log hive is dependent up on the tree trunk width. The predominance of traditional hive (100%) in the study area is similar with other findings conducted in the Northern, South Western and Central parts of Ethiopia and only 2% of the beekeepers owned modern hives Nuru, (2002).

Table 9: Grouped order of number of beehives owned by beekeepers

Number hives Owned	Frequency	Percent
3-7	16	9.88
8-12	31	19.14
13-17	35	21.60
18 and above	80	49.38
Total	**162**	**100.00**

Major Beekeeping Activities

Placement of beehives

In the study district, about 157 (96.91%) of beekeepers placed their beehives hanging on tree branches inside the rangelands far away from their residential houses, whereas the remaining 5 (3.09%) of respondent beekeepers keep their bee colonies in back yard and hang on the trees near homestead. This implies that the beekeeping practice entirely depends on forest but only few beekeepers have concentrated beekeeping near their residential areas.

Perception of beekeepers on honey color for consumption

Perception on honey quality in-terms of consumption preference is presented in Figure 2. The normalized rank result of this study showed that the quality of honey which is perceived as best quality for consumption is white in color for both districts. In the contrary, black type of honey is reported as the least in quality and less preferable for consumption in the area.

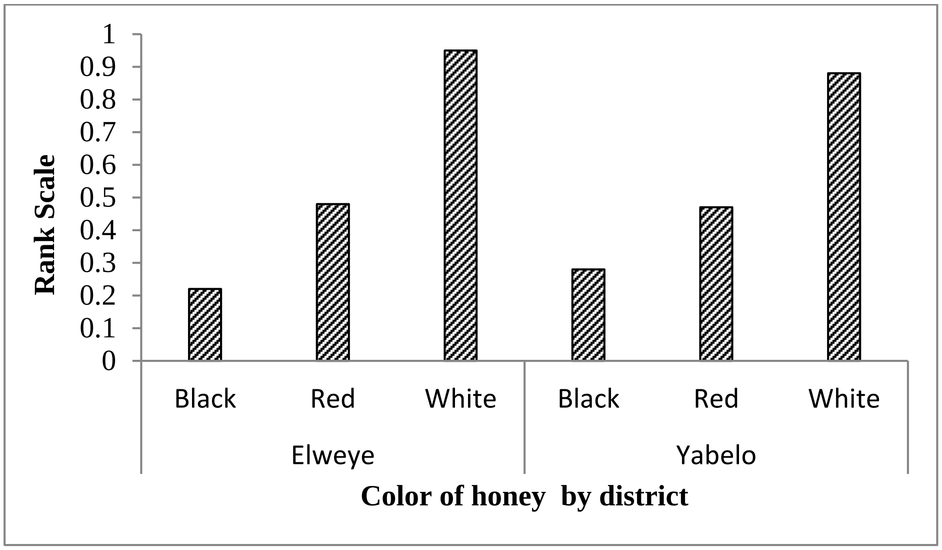

Figure 4. Perception of beekeepers on honey color for consumption

Price of honey in the area

The price for various types of honey per Kg is presented in Table 5. In reference to respondents, three type of honey colors (Black, Red and White) were observed in the study area and the price vary based on color of honey. Accordingly, white honey is the most expensive as compared to black and red honey in Elweye and Yabello districts respectively. The price of white honey is found to be ranging from 9.23-17.05 ETB kg $^{-1}$ while 5.5 ETB kg $^{-1}$ for black honey. However, the price of white and red honey is not significantly different in Yabello district.

Table 5. The price for various types of honey per “Kg in the area

District	Type of Honey	Mean ± STD (ETB)$^{-1}$	MIN. (ETB)$^{-1}$	Max (ETB)$^{-1}$
	Black kg^{-1}	99.38 ± 32.17	30	175
Elweye	Red kg^{-1}	91.56 ± 22.89	30	175
	White kg^{-1}	108.61 ± 27.15	30	150
	Black kg^{-1}	100.45 ± 28.93	37.5	175
Yabello	Red kg^{-1}	105.65 ± 27.38	37.5	175
	White kg^{-1}	105.95 ± 28.52	37.5	175
Overall	**Kg^{-1}**	**103.24 ± 27.66**	**30**	**175**

Trends of honey yield and honeybee colony population

The trend of honeybee colony population and honey yield over the last four gada system (8-32 years) in the study sites are presented in Table 6. From, the current study result showed that, the overall trend of honeybee colonies reported to be increasing (54.3%) , decreasing (30.20%) and stable (15.40%) of the respondents. The respondents also revealed that the trend of honey yield is increasing (42.60%), decreasing (35.80%) and stable (21.60%).

Table 10: The trend of colony number and honey yield as replied by beekeepers of the study sites.

Variables	Pastoral Association	Stable		Decreasing		Increasing		χ^2	df	*P<0.05*
		Frequency	Mean (%)	Frequency	Mean (%)	Frequency	Mean (%)			
Honey Bee population	Bidha	5	16.10	11	35.50	15	48.40	2.125	6	0.908
	Hidi	6	17.60	12	35.30	16	47.10			
	Obda	6	14.30	12	28.60	24	57.10			
	Yubdo	8	14.5	14	25.50	33	60.00			
	Total	**25**	**15.42**	**49**	**31.34**	**88**	**53.24**			
Honey Yield	Bidha	6	19.4	12	38.70	13	41.90	1.868	6	0.931
	Hidi	10	29.4	10	29.40	14	41.20			
	Obda	8	19.0	15	35.70	19	45.20			
	Yubdo	11	20.0	21	38.20	23	41.80			
	Total	**35**	**21.6**	**58**	**35.50**	**69**	**42.52**			

Reasons for the decreasing trend of honeybee colony population and honey yield

The reasons for honeybee colony and honey yield reduction in the study area are due to shortage of bee forage, pests and predators and drought are some of the major problems of beekeeping in the area as it is presented in Table 7. Shortage of bee forage causes the honeybee colony to abscond to areas where resources are available for their survival. The respondents also reported the occurrence of sever feed shortage usually happened after the harvesting time since there is no provisions of supplementary feeds. The existence of honey bee pests, predators and drought are factors which ultimately resulted in frequent absconding of colonies and high migratory tendencies. Some pests like ants and honey badger can cause serious damage to the bee hives which might be the main cause of the current tremendous decline of the bee population in the study districts.

Table 11: Reasons for decreasing colony population and honey yield as ranked by percent respondents

Pastoral Association	Constraints	1	2	3	4	5	6	Index	Rank
Bidha	Shortage of Bee Forage	17.4	11.6	2.9	.	.	.	0.319	1
	Shortage of Water	13	5.8	2.9	.	.	.	0.217	2
	Drought	8.7	11.6	0	.	.	.	0.203	3
	Pesticides and Herbicides	8.7	0	0	.	.	.	0.087	4
	Pests and Predators	8.7	0	0	.	.	.	0.087	4
	Lack of Bee Hive	4.4	0	2.9	.	.	.	0.072	6
	Deforestation	0	0	1.5	.	.	.	0.014	7

Hidi	Pests and Predators	17	3.5	2.6	0	0	.	0.233	1
	Shortage of Bee Forage	0	17.2	2.6	3.45	0	.	0.233	1
	Drought	12.9	6.9	0	0	0	.	0.198	3
	Shortage of Water	0	0	12.9	0	0.86	.	0.138	4
	Pesticides and Herbicides	8.6	3.5	0	0	0	.	0.121	5
	Absconding	0	3.5	0	1.72	0	.	0.052	6
	Migration	0	0	2.6	0	0	.	0.026	7
	Lack of Bee Hive	0	0	0	0	0	.	0	8
Obda	Shortage of Bee Forage	18.18	5.68	1.52	1.14	0	0	0.265	1
	Drought	6.82	9.47	3.03	1.14	0	0	0.205	2
	Shortage of Water	0	5.68	12.12	0	1.52	0	0.193	3
	Absconding	2.27	1.89	0	3.41	4.55	0	0.121	4
	Migration	0	1.89	1.52	4.55	2.27	0.76	0.11	5
	Pests and Predators	0	3.79	0	2.27	0	1.14	0.072	6
	Pesticides and Herbicides	0	1.89	0	0	0	0	0.019	7
	Lack of Bee Hive	0	0	1.52	0	0	0	0.015	8
Yubdo	Shortage of Bee Forage	14.88	6.89	1.1	0	1.1	0	0.24	1
	Drought	8.26	8.26	2.2	0.83	0	0	0.196	2
	Pests and Predators	6.61	0	0	3.31	0	2.48	0.124	3
	Absconding	1.65	2.75	0	3.31	4.41	0	0.121	4
	Migration	0	1.38	2.2	3.31	2.2	1.1	0.102	5
	Pesticides and Herbicides	0	2.75	0	0	0	0	0.028	6
	Lack of Bee Hive	0	0	1.1	0	0	0	0.011	7

Crude Honey Production from Traditional Hive

The productivity of traditional hives per year as reported by sample households is presented in Table 8. The results showed that 29.7 percent of the respondents harvest between 4 to 8 kg of honey, while 36.3 % and 34 % of the respondents harvest between 9 to13 kg and 14 to18 kg of honey per hive per year, respectively. It was found that the average honey yield obtained from traditional hive per year was 12 kg, the maximum yield being 21 kg.

Table 12: Annual average crude honey production (kg) per HHof respondent from traditional hive

PAS	N	Mean± Std	Confidence Interval (95%)		F.Value*(P<0.05)*
			Lower	Upper	
Bidha	31	158.19 ± 118.82	123.10	194.66	
Hidi	34	108.00 ± 68.05	86.94	131.48	
Obda	42	107.62 ± 64.77	86.71	128.82	3.541 (0.016)
Yubdo	55	106.55 ± 63.77	89.41	123.88	
Total	162	117.01 ± 80.10	104.70	129.19	

Inspection of honeybee colonies

With regards to collecting information on the inspection of bee colonies by the beekeepers, 92% of the respondents do undertake inspection of their bee colonies, while only 8% do not practice the inspection. This indicates that most of beekeepers visit and inspect the traditional beehives externally at varied frequency to check either the hive was safe or filled with honey (Table 9). However, internal hive inspection was limited to those honeybee colonies placed at backyard and under the eaves of the house. Different authors (Kerealem, 2005; Tesfaye, 2007; Chala, 2010) have reported that beekeepers in Ethiopia do not commonly practice internal hive inspection due to the difficulty of the traditional hives for internal inspection because fixed combs are attached to the body of traditional beehive.

Table 13: Inspection of honeybee colonies

Inspection Frequency	**Colony Inspection Type**	**Pastoral Associations**				χ^2	**df**	***P<0.05***
		Bidha	Hidi	Obda	Yubdo			
Every day	External	15.38	12.82	28.21	35.90	1.44	3	0.6952
	Internal	0	0	2.56	5.13			
Every week	External	13.33	21.67	26.67	33.33	1.91	3	0.5917
	Internal	0	0	1.67	3.33			
Every two week	External	25.40	23.81	20.63	26.00	1.88	3	0.5974
	Internal	1.59	1.59	0	0			

Honey harvesting season, and frequency of harvest

According to the survey results 40% of the respondents revealed, the major honey flow and harvesting season is in July (Obora Guda) and 36% of them reported that it is in April and 24% of respondents reported as it is in the August, September and November, which is associated with the major cropping season of the area. On the other hand, 31.5% of the respondents reported July as minor honey flow and harvesting season of the year Figure3 a and b. However, larger proportion (48.8%) of the respondents stated that the minor honey flow and harvesting season extends from September to November. The rest 19.7%Respondents explained that honey flow season has been associated with erratic and unevenly distributed type of rainfall, which is a key factor that determines a particular season and flowering of a specific plant as well.

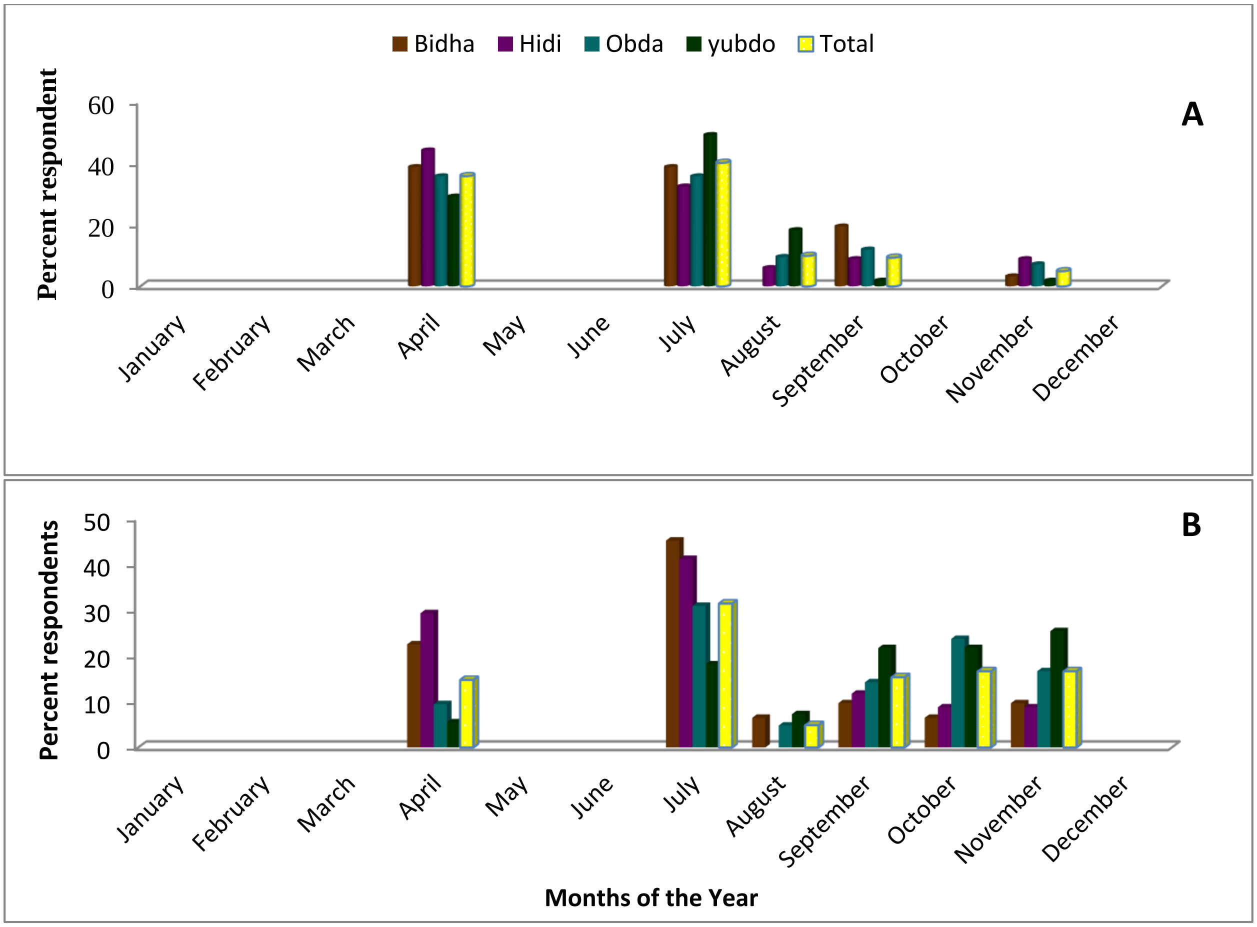

Figure 5: Major (a) and minor (b) honey flow and harvesting season of the study area

Regarding the frequency of honey harvesting in the study area revealed that honey harvested either one or two times per annum (Table 10). About 95.06% of respondent beekeepers are harvesting honey twice per annum in response to the bimodal rainfall characteristic which is coupled with d seasonal flowering pattern of various flowering plants in the area. The current result corresponds to Shenkute et al. (2012) who indicated twice harvesting frequency of honey in Kaffa, Sheka, and Bench-Maji Zones of Ethiopia.

Table 14: Frequency of harvesting honey in a Year by Respondents

Harvesting Frequency	Bidha		Hidi		Obda		Yubdo		Total		χ^2	df	*P<0.05*
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%			
One time	5	16.13	0	0.00	1	2.38	2	3.64	8	4.94	15.32	3	0.0179
Two times	26	83.87	34	100.00	41	97.62	53	96.36	154	95.06			

Dearth Period

Season or months of the year which is regarded as dearth period (a period where there is no any type of flower) Figure 4. Accordingly, the largest (67.3%) of respondent household beekeepers of the area expressed that December is the where bees face extreme scarcity of flower. Moreover, January (18.2%) and February (14.5%) have been identified as second and third months of year where honeybees face feed shortage in the study area. Generally, December has been considered as on set for long dry period of the year where large proportions of honeybee colonies usually migrate from the study area to other area in search for flowers.

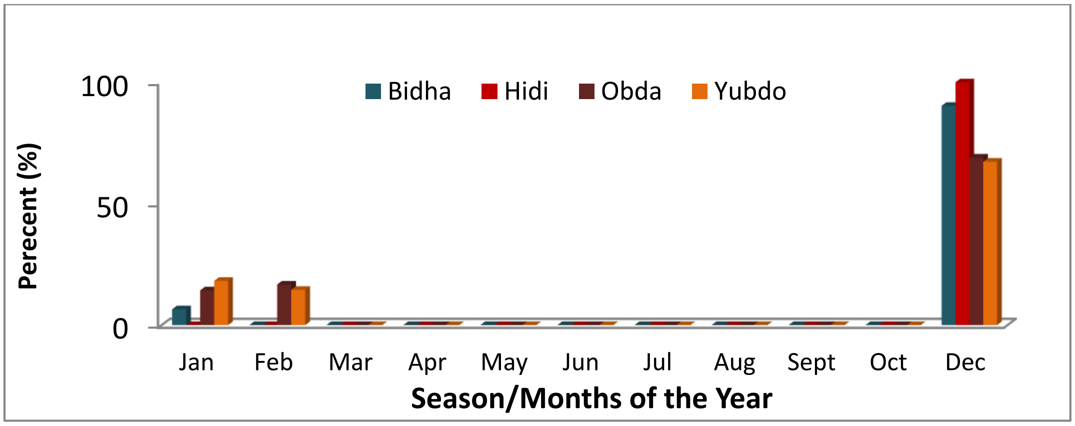

Figure 6: Dearth Period of the year in the study area

Feeding honeybees

Practice of feeding, time of feeding and types of feeding for honeybee colonies in the study area are presented in Table 11. Of the total (164) interviewed respondent households, only 26.5% of them confirmed to supplement their honeybee colonies while 73.5% do not practice feeding. Consequently, during the months of January (46.5%) of HHs) followed by December (37.2% of HHs) and February (16.3%) which are a particular dearth and supplementing periods. Majority (65.1%) of beekeeper households only provide water and the remaining (34.9%) provide flour of various sources. On the other hand, larger proportion (70.1%) of household beekeepers leave small amount of honey in the hive.

Table 11: Practice of supplementing, supplementing period, types of supplementation and proportion of honey harvested in the area

Activities	Response	Bidha	Hidi	Obda	Yubdo	Total
Practice of Supplementing	No	74.2 (23)	76.5 (26)	76.2 (32)	69.1(38)	73.5 (139)
	Yes	25.8 (8)	23.5 (8)	23.8 (10)	30.9 (7)	26.5 (23)
Supplementing Period	Jan	25.0 (2)	50.0 (4)	50.0 (5)	52.9 (9)	46.5 (20)
	Feb	0	12.5 (1)	20.0 (2)	23.5 (4)	16.3 (7)
	Dec	75.0 (6)	37.5 (3)	30.0 (3)	23.5 (4)	37.2 (16)
Types of Supplementation	Various Flour	0	0	10.0 (1)	11.8 (2)	7.0 (3)
	Spice Flour	0	0	10.0 (1)	11.8 (2)	7.0 (3)
	Maize Flour	0	12.5 (1)	10.0 (1)	11.8 (2)	9.3 (4)
	Beans/Peas Flour	0	0	10.0 (1)	11.8 (2)	7.0 (3)
	Water	87.5 (7)	75.0 (6)	60.0 (6)	52.9 (9)	65.1 (28)
	Wheat Flour	12.5 (1)	12.5 (1)	0	0	4.7 (2)
Honey harvest Proportion	Keep some for bee	87.1 (27)	97.1 (33)	73.8 (31)	78.2 (41)	82.7 (134)
	Whole	12.9 (4)	2.9 (1)	26.2 (11)	21.8 (12)	17.3 (28)
Proportion of honey remained un harvested	Half	3.7 (1)	3.0 (1)	3.2 (1)	2.3 (1)	3.0 (4)
	One fourth	3.7 (1)	3.0 (1)	0	0	1.5 (2)
	One third	18.55	21.2 (7)	29.0 (9)	30.2 (13)	25.4 (34)
	Small amount	74.1 (20)	72.7 (24)	67.721	67.4 (29)	70.1 (94)

Various Flour = *burki,barbare,boshe;* Spice Flour= *Irid;* Maize Flour = *boshe;* Beans & Peas Flour =*shiro*; Wheat Flour = *burki. Values outside* and *in the parenthesis representing the percentage* and *the numbers of respondents, respectively.*

Brood rearing and swarming season

According to respondents (40%) beekeepers stated that the major brood-rearing and swarming season of colony takes place in the main rainy season (March to May) when flowering plants get bloomed as indicated in Figure 5. The second substantial brood-rearing and swarming season is during September and November. However, the substantial proportion of respondents considered December as one of the swarming period of the study sites. The mentioned month of the year is principally known as a period where migration of honeybee colony intensively takes place as per the response to onset of long dry season ("Bona").

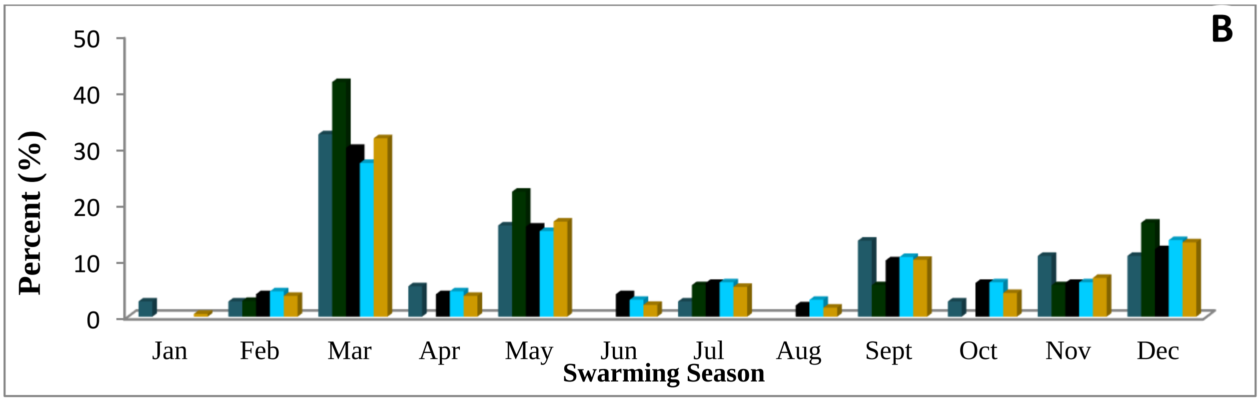

Figure 7: Brood-rearing (a) and swarming (b) season of the study sites

Honeybee Colony migration

The migration of honeybee colonies occurred in different months of the year predominantly g in December (75.9%) followed by January (11.7%) and February (6.8%) as stated by respondents (Table 12). The result obtained was found to be consistent across all the study kebeles. Exceptionally higher proportion of migration phenomenon takes place in December, due to seasonal variation of weather and scarcity of flowers. The result is in agreement with previous results reported by Nuru,(2002) that described migration occurred immediately following the main honey flow season and continued throughout the dry season up to the next active period in mid rift valley of Ethiopia.

Table 15: Migration incidence of honeybee colonies as replied by respondents of study sites

Item	Parameters	Bidha	Hidi	Obda	Yubdo	Total
Jan	Freq.	3	0	6	10	19
	Perc.(%)	9.7	0	14.3	18.2	11.7
Feb	Freq.	1	0	5	5	11
	Perc.(%)	3.2	0	11.9	9.1	6.8
Mar	Freq.	1	0	0	0	1
	Perc.(%)	3.2	0	0	0	0.6
Sept	Freq.	0	1	0	0	1
	Perc.(%)	0	2.9	0	0	0.6
Nov	Freq.	0	0	1	2	3
	Perc.(%)	0	0	2.4	3.6	1.9
Dec	Freq.	26	32	29	36	123
	Perc.(%)	83.9	94.1	69.0	65.5	75.9
Unknown	Freq.	0	1	1	2	4
	Perc. (%)	0	2.9	2.4	3.6	2.5

Freq. = Frequency; Perc. (%) = Percent

Honeybee Colony Absconding

According to the response of respondent household beekeepers, absconding may occur in several months of the year, however, the incidence of absconding is being sever in some particular months such as December (58% of HHs), July (14.8% of HHs) and January (11.1% of HHs).The incidence of absconding exclusively consistent across the considered kebeles of the current study.

Table 16: Absconding incidence of honeybee colonies as reported by respondents

Item	Bidha		Hidi		Obda		Yubdo		Total	
	Freq.	Perc.(%)	Freq.	Perc. (%)	Freq.	Perc. (%)	Freq.	Perc.(%)	Freq.	Perc. (%)
All season	0	0	1	2.9	1	2.4	2	3.6	4	2.5
Jan	2	6.5	2	5.9	5	11.9	9	16.4	18	11.1
Feb	1	3.2	0	0	1	2.4	1	1.8	3	1.9
Mar	1	3.2	0	0	0		1	1.8	2	1.2
May	2	6.5	0	0	1	2.4	2	3.6	5	3.1
Jun	0	0	0	0	2	4.8	1	1.8	3	1.9
Jul	0	0	3	8.8	10	23.8	11	20	24	14.8
Aug	0	0	0	0	0	0	1	1.8	1	0.6
Sept	1	3.2	0	0	0	0	0	0	1	0.6
Nov	0	0	0	0	2	4.8	2	3.6	4	2.5
Dec	24	77.4	28	82.4	19	45.2	23	41.8	94	58
Unknown	0	0	0	0	1	2.4	2	3.6	3	1.9

Freq. = Frequency; Perc.(%)= Percent

Predator and Pests of honeybee colony

Accordingly, beekeepers of Bidha and Hidi revealed ants are the most honey production challenges with respective rank index values of 0.375 and 0.475. The second the most honey production challenges of study sites was honey badger Bidha (Rank Index= 0.372) and Hidi (Rank Index = 0.341. On the other hand, household beekeepers of Obda (Rank Index = 0.389) and Yubdo (Rank Index= 0.387) revealed as H-Badger is the most honey production challenges followed by ants with a rank index values of 0.359 and 0.358, respectively (Table 14).

Table 17: Major honeybee enemies/predators and pests as ranked by respondents of study sites

Pastoral Association	**Constraints**	**R1**	**R2**	**R3**	**R4**	**R5**	**Index**	**Rank**
Bidha	Ants	45	72	6	2	0	0.375	1
	H-Badger	100	24	0	0	0	0.372	2
	Wasps	0	4	15	0	0	0.057	3
	Birds	0	4	9	2	0	0.045	4
	Spider	0	0	12	2	0	0.042	5
	Wax-Moth	5	0	6	0	0	0.033	6
	Lizard	0	0	6	2	0	0.024	7
	Bee-Lice	0	4	0	2	0	0.018	8
	Beetle	0	0	0	4	0	0.012	9
	Monkey	0	4	0	0	0	0.012	9
	Rat	0	0	0	2	1	0.009	11
Hidi	Ants	90	56	6	0	0	0.475	1
	H-Badger	70	36	3	0	0	0.341	2
	Monkey	5	12	3	0	0	0.063	3
	Birds	0	0	15	0	0	0.047	4
	Spider	0	0	6	0	1	0.022	5
	Wax-Moth	5	0	0	2	0	0.022	5
	Bee-Lice	0	4	0	2	0	0.019	7
	Beetle	0	0	0	4	0	0.013	8
Obda	H-Badger	125	24	6	0	0	0.389	1
	Ants	45	92	6	0	0	0.359	2
	Birds	5	8	15	0	0	0.07	3
	Monkey	5	12	3	4	0	0.06	4
	Spider	0	0	9	4	1	0.035	5
	Bee-Lice	0	0	9	4	0	0.033	6
	Wax-Moth	0	0	9	2	2	0.033	6
	Beetle	0	0	0	4	1	0.013	8
	Lizard	0	0	3	0	0	0.008	9
Yubdo	H-Badger	165	28	9	0	0	0.387	1
	Ants	55	120	12	0	0	0.358	2
	Monkey	10	24	3	4	0	0.079	3
	Birds	5	8	15	0	0	0.054	4
	Bee-Lice	0	0	12	6	0	0.034	5
	Spider	0	0	12	4	1	0.033	6
	Beetle	0	0	0	8	2	0.019	8
	Lizard	0	0	3	0	0	0.006	9
	Wax-Moth	0	0	12	2	2	0.031	7

Honeybee enemies and pests control

Practice of customary knowledge by the respondent household beekeepers to prevent pests and bee enemies in the study area is given in (Figure 6). The overall proportions of household beekeepers that are practicing traditional controlling methods are substantially greater (66%) than from those that are not using it. The traditional means of pest and predators controlling is, putting ash around hive stand to prevent the damage of ants and fixing smooth iron sheet at trunks of a trees to prevent the up climbing of honey badger are the most commonly practiced techniques in the area.

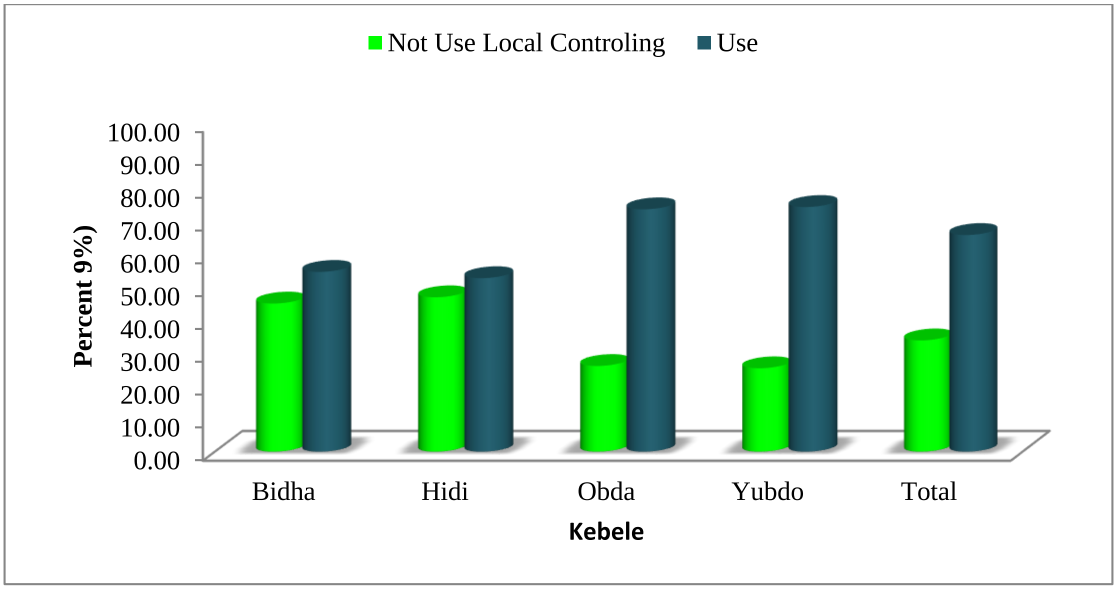

Figure 6. Practice of honey enemies and pests controlling technique in study area (N=162)

Honeybee floras

The results of the survey, honeybee plants of the study area comprise trees, shrubs, Forbes, woody climber and cultivated crops. Lists of vernacular and botanical names of commonly foraged honey bee plants are presented in Table 15. Accordingly, 70 various types of nectar and pollen sources plants that are recognized as a major bee forages were identified in the area. Regardless of having ample nectar sources, scarcity of bee forages has been a common incidence which is directly associated with off flowering season of major honey bee forages. Out of the major honey plants, most of them densely flowered in July and August. Consequently, the mentioned months locally known as “OboraGuda” (July) and “OboraTika” (August) are considered as major and minor flowering months of the year.

Table 18: Common honeybee floras with their Vernacular names as responded by respondents of the study sites

No	Scientific Name	Vernacular name	Flowering period	Habit
1	*Coleus igniarius*	Abune	Mar-May	S
2	*Euphorbia candelabrum*	Adama	Oct-Jan	T
3	*Carissa edulis*	Agamsa	Mar-May	S
4	*Commiphora paolii*	Agarsu	Mar-May	T
5	*Acacia bussei*	Halo	Jan-April	T

6	*Acacia brevispica*	Hamaresa	Mar- May	S
7	*Euphorbia tirucalli*	Anno	Sep-Nov	Su
8	*Grewia bicolor*	Aroresa	Sep-Nov	S
9	*Zea mays*	Badala	Sep-Nov	H
10	*Balanites aegyptica*	Badana	Mar-May	T
11	*Delonix baccal*	Balanji	Mar-May	T
12	*Plectranthu spp*	Baranbaresa	Jan-April	S
13	*Ipomea* sp	Baate	Jan-April	S
14	*Terminalia brownii*	Bidhesa	Oct-Dec	T
15	*Pappea capensis*	Biqa	Jan- April	T
16	*Acacia goetzei*	Bura	Mar-May	T
17	*Acacia nilotica*	Burquqe	Mar-May	T
18	*Sansevieria abyssinica*	Cake	Mar-May	S
19	*Haplocoelum foliolosum*	Cana	Jan-april	S
20	*Cyphostemma* spp.	Cophi	Sep-Oct	F
21	*Rhus natalensis*	Dabobesa	Mar-May	S
22	*Boswellia hildebrandtii*	Dakara	Mar-May	T
23	*Helichrysum glumaceum*		After rainy season	F
		Dargu		
24	*Acacia tortolis*	Dhadacha	Jan- May	T
25	*Grewia fembensis*	Dheka	Oct-Dec	S
26	*Phyilanthus sepialis*	Dhiri	Oct-Dec	S
27	*Maerua triphylla*	Dhumayo	Mar-May	S
28	*Veronia amygdalina*	Ebicha	Dec-Feb	S
29	*Olea africana*	Ejersa	April-Jun	T
30	*Canthium schimperianum*	Gaale	July-Aug	S
31	*Dobera glabra*	Garse	Mar-May	T
32	*Capparis tomentosa*	Gora	Sept	S
33	*Indigofera volkensii*	Gurbi	July-Aug	F
34	*Osteospermum vailliantii*	Hada	Sep-Nov	F
35	*Acacia etbaica*	Halqabesa	Dec-Feb	T
36	*Lannea floccosa*	Handaraka	April-Jun	T
37	*Phaseolus vulgaris*	Halquqa	Nov-Dec	**H**
38	*Sarcostemma viminale*	Hangaya	Jan-Feb	
39	*Otostegia erlangeri*	Harcaha	Jan-fEB	F
40	*Aloe* spp	Hargesa	Nov-Dec	F
41	*Euphorbia schizacantha*.	Harkena	Nov-Dec	F
42	*Acacia senegal*	Hidhadho	Dec-Feb	T
43	*Solanum somalense*	Hidi	Sept-Nov	S
44	*Dichrostachys cinerea*	Jirime	Oct-Dec	T
45	*Maytenus senegalensis*	Kompholcha	Oct-Dec	T
46	*Maeru atriphylla*	Dhumasho	Jan-Mar	T
47	Grass spp.	Marga	Aug	H

48	*Chlorophytum gallabatense*	Mirtu	Aug	H
49	*Euclea schimperi*	Miyesa	Oct-Mar	S
50	*Croton macrostachys*	Mokonisa	April-July	T
51	*Grewia villosa*	Ogomdi	Oct-mar	S
52	*Capparis tomentos*a.	Ogora	sept-nov	S
53	*Ipomoea hildebrandtii*	Omboroke	Jan-Mar	T
54	*Pterolobiumstellatum*	Qajima	Jan -Mar	S
55	*Bosciaangustifolia*	Qalqalcha	Jan- Mar	T
56	*Sterculiarhynchocarfa*	Qararu	Jan-mar	T
57	*Vernoniacinerascens*	Qaxe	Sept-Nov	S
58	*Terminalia pruniodes*	Qorobo	Nov-Dec	S
59	*Indigoferasuaveolens*	Rigaa	Nov-Dec	H
60	*Combretummolle*	Rukesa	Oct-Dec	T
61	*Acacia mellifera*	Saphansa	Dec-Feb	T
62	*Asparagus racemosus*	Sareti	Nov-Feb	Wc
63	*Acacia reficiens*	Sigirso	Dec-Feb	T
64	*Dombeya kirkii*	Silaalcha	Jan-Mar	T
65	*Delonixelata*	Sukela	Jan-Mar	T
66	*Acacia seyal*	Wachu	Dec-Feb	T
67	*Cordia gharaf*	Wadesa	Sep -Nov	T
68	*Erythrina melanacantha*	Walensu	Nov-Feb	T
69	*Dalbergia microphylla*	Walchamala	Nov-Feb	T
70	*Acacia nubica*	Wanga	Dec-Feb	T

Growth form is indicated as S shrub), T (tree), Wc (woody climber). H (herbaceous) and Su (succulent).

Harvesting and Post-harvest handling of honey

Beekeepers use various honey harvesting procedures. About 59.9% harvesting procedure of honey from traditional hives was done by using smoke without displacing the hive from its position. The second (37.7%) harvesting procedure as reported by the respondent was dropped hives from the tree and uses smoke to calm dawn the bees from being aggressive. The remaining (2.5%) respondent beekeepers *revealed that they use only brush* to harvest honey. The most important (81.5%) smoking materials used as anti-aggressiveness are reported to be various types of dried wood (Table 17).

The survey result revealed the respondent in the area used various types of containers to handle honey (Table 16). The major containers used to handle honey in the area were tin (40.1%), plastic container and tin (34.3%) and plastic container only (25.3 %). The beekeepers in the study area used tin and poor quality plastic container that can be regarded as worn-out for honey storage which contributes to the deterioration of honey quality. The current study is in line with the result by Shenkute et al. (2012) who reported similar containers used for post handling of honey in Keffa, Skeka and Bench-Maji zone.

Table 19: Traditional harvesting procedures and post-harvest handling mechanisms of honey in the study area

Activity	Response		kebele				Total	χ^2	df	*P<0.05*
			Bidha	Hidi	Obda	Yubdo				
Harvesting Procedure	Brush	Freq.	0	0	2	2	4	2.924	6	0.818
		%	0	0	4.8	3.6	2.5			
	Drop hive and smoke	Freq.	12	13	16	20	61			
		%	38.7	38.2	38.1	36.4	37.7			
	Smoke	Freq.	19	21	24	33	97			
		%	61.3	61.8	57.1	60.0	59.9			
Smoking materials	Dung	Freq.	2	2	5	5	14	8.598	12	0.737
		%	6.5	5.9	11.9	9.1	8.6			
	Dung & Leaf	Freq.	0	1	3	3	7			
		%	0	2.9	7.1	5.5	4.3			
	Leaf	Freq.	0	2	2	3	7			
		%	0	5.9	4.8	5.5	4.3			
	Old Cloths	Freq.	1	1	0	0	2			
		%	3.2	2.9	0	0	1.2			
	Plants	Freq.	28	28	32	44	132			
		%	90.3	82.4	76.2	80.0	81.5			
Storing form	Alone in safe place	Freq.	31	32	39	50	152	3.065	6	0.801
		%	100.0	94.1	92.9	90.9	93.8			
	Not stored	Freq.	0	1	1	2	4			
		%	0	2.9	2.4	3.6	2.5			
	With other products	Freq.	0	1	2	3	6			
		%	0	2.9	4.8	5.5	3.7			
Honey storage materials used	Clay pot, Plastic and tin	Freq.	2	0	0	0	2	10.829	9	0.288
		%	6.5	0	0	0	1.2			
	Plastic container	Freq.	6	7	12	16	41			
		%	19.4	20.6	28.6	29.1	25.3			
	Plastic container and tin	Freq.	9	14	13	18	54			
		%	29.0	41.2	31.0	32.7	33.3			
	Tin	Freq.	14	13	17	21	65			
		%	45.2	38.2	40.5	38.2	40.1			

Producing quality honey and its importance

Based on current study, majority (70.4%) of respondent beekeepers disclosed that there is variation in honey quality produced in the study sites (Table 17). Accordingly quality of honey can be influenced by different management and environmental factors like flower type (53.3%), harvesting process (9.6%) and storage period (31.6%). The result is in line with previous findings (Krell et al. 1988; Krell 1991) who confirmed that different management; harvesting and processing techniques can influence the l quality of honey.

Table 20: quality variation and sources of variation for the honey produced in the study sites (n=162)

Activity	Response		Sites				Total	χ^2	df	*P<0.05*
			Bidha	Hidi	Obda	Yubdo				
Variation in quality	Not Existed	Freq.	6	7	16	19	48	4.984	3	0.173
		%	19.4	20.6	38.1	34.5	29.6			
	Existed	Freq.	25	27	26	36	114			
		%	80.6	79.4	61.9	65.5	70.4			
Sources of quality variation	Flower	Freq.	12	15	13	21	61	5.921	9	0.748
		%	48.0	55.6	50.0	58.3	53.5			
	Harvesting processing	Freq.	4	0	3	4	11			
		%	16.0	0.0	11.5	11.1	9.6			
	Longer storage	Freq.	8	11	8	9	36			
		%	32.0	40.7	30.8	25.0	31.6			
	Unknown	Freq.	1	1	2	2	6			
		%	4.0	3.7	7.7	5.6	5.3			

In view of the results obtained in this study, it seems that the quality of honey harvested from traditional hives has various social and commercial importance' (Figure 7).Accordingly, 32.7% of respondent beekeepers revealed, quality honey provides the benefit of enjoying healthy food and higher selling price. Similarly, 25.3% and 21.5% of respondent beekeepers disclosed as the paramount importance of quality honey respectively, in terms of its commercial and medicinal value. Moreover, small proportion of respondent beekeepers indicted that quality honey provides quality beverage ingredient and possess longer shelf

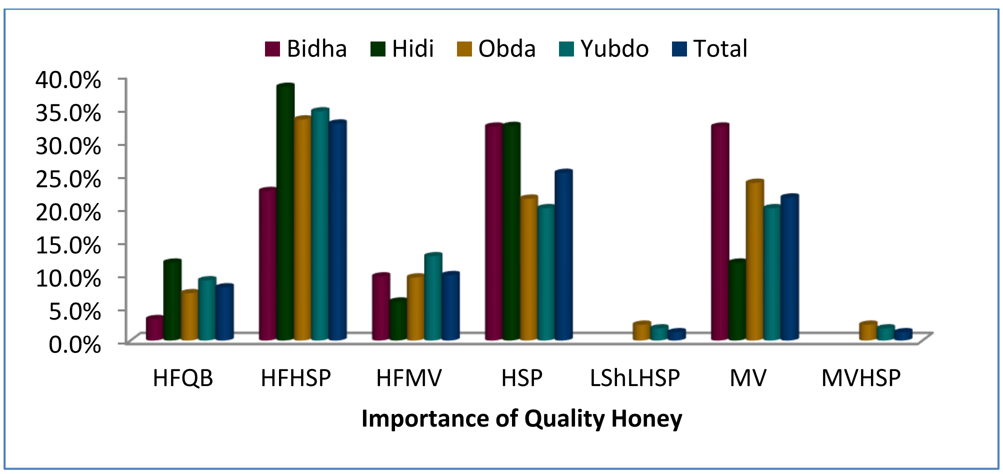

Figure 8: Importance of the produced quality as recognized by respondent of beekeepers of the study sites

Key: *Healthy food and used to prepare quality beverage (**HFQB**), Healthy food and Higher selling price (**HFHSP**), Healthy food and Medicinal Value(**HFMV**), Higher selling price (**HSP**), Long Shelf life and Higher selling price (**LShLHSP**), and Medicinal Value(**MV**)*

Perception of beekeepers on honey contamination

The response on contamination status, source of contamination, contaminant identification means, and impact of contaminant to human health and price of harvested honey is presented in Table 18. Out of the total 164 respondent beekeepers, 25.9% recognized that the produced honey is contaminated from various sources. Majority (72.8%) of the respondent detected honey contamination by observation. Regarding contaminants, the respondent revealed brood as the prominent (31.0%) source followed by pollen (28.6%) and debris of died ants (26.2%). In association of contamination to human health, majority (52.4%) of the respondent beekeepers disclosed that it has no any impact and the remaining stated a particular person who consumed it can experience stomach pain. Moreover, 81.0% of respondents revealed contamination results in reducing honey price.

Table 21: Beekeepers perception to honey contamination and its impact to human health and price in the study sites (n=162)

Item	Response		sites				Total	χ^2	df	*P<0.05*
			Bidha	Hidi	Obda	Yubdo				
Purity of harvested honey	Uncontaminated	Freq.	26	28	29	37	120	4.640	3	0.200
		%	83.9	82.4	69.0	67.3	74.1			
	Contaminated	Freq.	5	6	13	18	42			
		%	16.1	17.6	31.0	32.7	25.9			
Purity identification means	Observation	Freq.	5	1	5	15	26	12.329	6	0.055
		%	16.1	2.9	11.9	27.3	16.0			
	Observation and testing	Freq.	24	29	33	32	118			
		%	77.4	85.3	78.6	58.2	72.8			
	Testing	Freq.	2	4	4	8	18			
		%	6.5	11.8	9.5	14.5	11.1			
Source of contamination	Ants	Freq.	0	2	4	5	11	9.116	12	0.693
		%	0	33.3	30.8	27.8	26.2			
	Brood	Freq.	4	2	3	4	13			
		%	80.0	33.3	23.1	22.2	31.0			
	Container	Freq.	0	0	1	2	3			
		%	0	0	7.7	11.1	7.1			
	Pollen	Freq.	1	1	4	6	12			
		%	20.0	16.7	30.8	33.3	28.6			
	Smoking	Freq.	0	1	1	1	3			
		%	0	16.7	7.7	5.6	7.1			
Effect of contaminated honey to human health	No effect	Freq.	2	4	7	9	22	1.690	6	0.946
		%	40.0	66.7	53.8	50.0	52.4			
	Stomach pain	Freq.	2	2	5	7	16			
		%	40.0	33.3	38.5	38.9	38.1			
	Vomiting and stomach pain	Freq.	1	0	1	2	4			
		%	20.0	0	7.7	11.1	9.5			
Effect of contaminated honey on price	Lower price	Freq.	4	4	11	15	34	0.976	3	0.807
		%	80.0	66.7	84.6	83.3	81.0			
	No effect	Freq.	1	2	2	3	8			
		%	20.0	33.3	15.4	16.7	19.0			

Analysis of honey quality

Reducing Sugars

The analyses of reducing sugars were found to vary from 69.02±0.35 to 70.54±0.56 across the area from which samples were taken. The samples taken from Elweye market showed significantly ($p<0.05$) lower reducing sugar value (69.02±0.35) than samples taken from other locations (Table 20). However, there is no significant variation ($p>0.05$) observed between honey samples collected from Yabello apiary sites and from two market points. The result obtained for the reducing sugar content of the samples coincides with the research outputs varied from 67.83 to 80.25% and 71.25 to 84.25%, before hydrolysis and total reducing sugars after hydrolysis respectively for Algerian honey (Ouchemoukh, 2004). Total reducing sugar contents in all honey samples are with in quality requirement limits (≥65%) which is set by the EU (2002).

Sucrose Content

The result of honey samples collected for sucrose content ranged from 1.02±0.10% to 2.76±0.25% Table 19. Accordingly, sucrose content for honey samples collected from Yabello (1.55%) and Elweye (1.02%) pastoral association apiary sites was found to have 46.18% and 63.04% respectively, lower than the honey samples collected from their respective market points. Subsequently, the result of sucrose content showed significant ($p<0.05$) variation between samples collected from farm get and market points. However, there is no significant variation ($p>0.05$) observed within farm gets and market points. The mean sucrose content of honey samples collected from the study areas was lower (1.02 to 2.76%) than the national average of 3.6% that was reported by Nuru (1999). Moreover, the present result is by far lower than the findings of Bogdanov and Martin, (2002), who reported 7.55% mean for the honey from Gomma district. The result revealed that honey produced by traditional beekeepers of the study sites is natural and free of any adulteration.

Moisture Content

Average moisture content examined honey samples collected from the study sites ranged from 18.87 ± 0.11 to 20.32 ± 0.57% (Table 19). The moisture content of honey samples from Elweye (18.88 ± 0.21%) and Yabello (18.87 ± 0.11%) pastoral association apiary sites didn't vary ($p>0.05$). Similarly, moisture content of honey from the two local markets Elweye (20.32 ± 0.57%) and Yabello (18.93 ± 0.53%) were not significantly differ ($p>0.05$). However, moisture contents of honey samples collected from the two farms get apiary sites is found lower and significantly different ($p<0.05$) than moisture content of honey samples collected from the two market points. The mean moisture content of honey (18.87 to 20.32 %) was recognized for the present study which is in line with previous findings of Nuru (1999) and Tessega (2009) (20.6% and 18.80 %) for Burie district of Ethiopia. The moisture content of the current study is within the requisite of international standard.

pH

Determination of pH values of honey samples collected from the study sites ranged from 4.06 ± 0.07 to 4.60±0.06 (Table 19). pH of honey samples randomly collected from Elweye (4.11 ± 0.06) and Yabello (4.06 ± 0.07) pastoral association apiary sites was found to be relatively acidic with no significant variation ($p>0.05$). Similarly, pH of honey samples collected from the two local markets Elweye (4.46 ± 0.04) and Yabello (4.60 ± 0.06) showed no significant different ($p>0.05$). Honey collected from tow market sites had higher pH values than the honey samples collected from the two farm get apiary sites. The pH values obtained for current honey samples coincides with values reported by (Nigussie *et al.*, 2012) and PH of honey ranging from 3.82 to 4.45. However, the current pH values are higher than the one reported (3.45) for **honey** samples obtained from traditional hive **for Quality Assessment in Guji Zone southern Ethiopia (**Birhanu, 2016).

Free Acidity

The analysis for free acidity of honey samples collected from the study sites ranged from 19.62 ± 1.18meq kg^{-1} to 35.13 ± 2.11 meq kg^{-1} Table 19. Free Acidity of honey samples from Elweye and Yabello pastoral association apiary sites was found to be 19.62 ± 1.18 meq kg^{-1} and 19.95 ± 1.84meq kg^{-1}, respectively, with no significant variation ($p>0.05$). Similarly, Free Acidity of honey samples collected from the two local markets Elweye (35.13±2.11meq kg^{-1}) and Yabello (33.86±1.76 meq kg^{-1}) was did show no significant difference($p>0.05$). The mean free acidity of honey samples analyzed for current study 19.62meq kg^{-1} to 35.13 meq kg^{-1} is within the acceptable limits of honey standards settled by EU in 2002.

Electrical Conductivity

Determination of Electrical Conductivity values of honey samples collected from the study sites ranged from 0.17 ± 0.04to 1.04 ± 0.11(Table 19). The Electrical Conductivity of honey samples collected from the two local markets Elweye (0.91 ± 0.09) and Yabello (1.04 ± 0.11) was showed no significant difference ($p>0.05$) and higher Electrical Conductivity values than the honey samples collected from the two farm gates apiary sites. The variation observed for the electrical conductivity of the honey samples between different sites of the study area is due to the concentration of mineral salts, organic acids and proteins and variability in floral origin which is considered as one of the best parameters for differentiating between honeys with different floral origins (Terrab et al., 2004).

Ash Content (%)

An average ash content of honey samples collected from four various study locations were presented in (Table19) .The mean ash content of honey samples varied from 0.28 ± 0.05% to 1.99 ± 0.78%. The ash content of honey samples collected from the two local markets Elweye (0.64 ± 0.12%) and Yabello (0.68 ± 0.06%) showed no significant difference($p>0.05$). The ash contents 0.28 observed for honey samples collected from Yabello farm get apiary which is coincides with Nuru (1999) reported for Ethiopian honey.

Hydroxymethylfurfuraldehyde (HMF) Content

Determination of Hydroxymethylfurfuraldehyde (HMF) values of honey samples collected from the study sites ranged from 12.25±3.72to 30.82±7.19 mgkg^{-1}Table 19. HMF values from Elweye (13.27 ± 3.38 mgkg^{-1}) was found slightly higher than values determined for Yabello (12.25 ± 3.72^{b}mg perkg) pastoral association apiary sites with no significant variation (p>0.05). On the other hand, HMF values of honey samples collected from the local markets Elweye (21.29 ± 6.30 mgkg^{-1}) was found lower than Yabello (30.82 ± 7.19 mgkg^{-1}) that showed no significant difference (p>0.05). In the contrary, HMF values of honey samples collected from the two market points were found to be significantly higher (*p<0.05*) than HMF values of honey samples collected from the two farms get apiary sites.

The HMF values recognized for samples collected under the current study is higher than naturally occurring levels of HMF which is reported as10 mg kg^{-1}. A maximum HMF level allowed in table honey in the international market is ≤ 40 mg kg^{-1}and amounts exceeding the mentioned level are considered a main indicator of honey deterioration Bogdanov and Martin (2002). The average content of HMF values that recognized for the current samples are found within the values stated for international market standard (≤ 40 mg kg^{-1}).

Table 22: Mean values of physicochemical analysis results of honey samples collected from two study sites and market points (n=96).

Parameters	ELFAR	ELMRK	YAFAR	YAMRK
RS	70.54±0.56^{a}	69.02±0.35^{b}	70.24±0.53^{a}	70.21±0.41^{a}
SC	1.02±0.10^{b}	2.76±0.25^{a}	1.55±0.24^{b}	2.38±0.20^{a}
MC	18.88±0.21^{b}	20.32±0.57^{a}	18.87±0.11^{b}	18.93±0.53^{b}
pH	4.11±0.06^{b}	4.46±0.04^{a}	4.06±0.07^{b}	4.60±0.06^{a}
FA	19.62±1.18^{b}	35.13±2.11^{a}	19.95±1.84^{b}	33.86±1.76^{a}
EC	0.18±0.04^{b}	0.91±0.09^{a}	0.17±0.04^{b}	1.04±0.11^{a}
Ash	1.99±0.78^{a}	0.64±0.12^{b}	0.28±0.05^{b}	0.68±0.06^{b}
HMF	13.27±3.38^{b}	21.29±6.30^{a}	12.25±3.72^{b}	30.82±7.19^{a}

[*]ELFAR=Elweye farm, ELMRK=Elweye market, YAFAR=Yabello farm, Yabello market,[*] RS=Reducing sugar, SC=Sucrose content, MC=moisture content, FA=Free acidity, EC=Electrical conductivity, Ash=percent ash content and HMF=Hydroximethylfurfural

[*]Means with different letters in the same row are significantly different at p<0.05

Determination on the effect of addition of table sugars as adulterant on pure honey samples collected from both farm gate and market points are presented in Table 20. Accordingly, the values 2.30, 4.44 and 12.92 for for SC, pH and HMF respectively for pure honey sample significantly increased (*p<0.05*) towards 5.90, 5.64 and 65.61 for SC, pH and HMF respectively after 1:1 table sugar addition. On the other hand, the RS (69.76 ± 1.61) value obtained for pure honey samples obtained was significantly (*p<0.05*) reduced to 42.56 ± 0.98 after table sugar addition. However, except determination of some increase and reduction of values for the parameters like, MC, FA, EC and Ash, there is no significant difference (p>0.05) observed after table sugar is added on pure honey samples.

Table 23: Mean values of physicochemical composition of pure and adulterated honey samples (n=16).

Parameters	RS	SC	MC	pH	FA	EC	Ash	HMF
Pure Honey	69.76±1.61[a]	2.30±0.46[b]	19.74±0.56[b]	4.44±0.10[b]	25.96±5.05[a]	0.52±0.23[a]	0.41±0.09[a]	12.92±4.48[b]
Min	65	1	17	4	10	0	0	3
Max	75	4	22	5	47	2	1	41
Honey + Sugar	42.56±0.98[b]	5.90±1.18[a]	21.31±0.60[b]	5.64±0.13[a]	18.17±3.54[a]	0.47±0.19[a]	0.35±0.08[a]	65.61±22.76[a]
Min	39	3	19	5	7	0	0	13
Max	46	11	24	6	33	1	1	210

**ELFAR=Elweye farm, ELMRK=Elweye market, YAFAR=Yabello farm, Yabello market,*

Conclusions and Recommendations

Beekeeping has been practiced as a sideline activity in the area by Agro-pastoral communities for income generation. The current honey production system in the area is based on traditional beekeeping system dominated by forest beekeeping which resulted for the low quantity and quality of honey. Thus traditional beekeeping system should be improved through introduction of modern beekeeping technologies for improving the production and quality of honey. Months of December, January and February are considered a dry period of the year and where large proportions of honeybee colonies usually migrate and provision of supplementary feeding is required to prevent colony migration in the study area.

The honey samples collected from farm gates apiary is characterized by low electrical conductivity, pH, acidity, HMF and high reducing sugars indicating that the honey produced in the area is predominantly floral (blossom) honey. Therefor the honey quality analysis results of the study districts, meet the international quality standards of EU and Codex.

Identification and classification of locally produced honey based on pollen analysis should be done for the characterization of honey as monofloral or multifloral honeys for branding of honey for market promotion.

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Fishery Research Results

Prevalence of parasites of commercially important fish species in Lake Harkiso

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Abstract

Cross sectional study was conducted to assess the baseline information on common fish parasites that can also be extrapolated to predict the prevalence in Lake Harkiso. A total of 73 specimens of O. niloticus (19) and C. gariepinus (54) were examined from Lake Harkiso. Of the sampled specimen, 21 (5 Oreochromis niloticus and 16 Clarius gariepinus) were positive to one or more species of parasites with overall prevalence rate of 28.76%. Clinostomum sp and Contracaecum parasites were the most prevalent parasites in the lake. Contracaecum is found in 4(80%) of the parasite positive O. niloticus and 13(81.25%) of the positive C. gariepinus. Leeches are the only external parasite identified in the lake with 3 (60%) and 15(93.75%) of the positive Oreochromis niloticus and Clarius gariepinus samples respectively. Since the detected parasites have significant importance for public health, people should be informed about the risks of eating raw or only partially cooked fish, and should not consume it. A successful parasite control program should also be implemented into the lake's management.

Key words: *Clarius gariepinus, Fish parasites, Lake Harkiso, Oreochromis niloticus, Prevalence*

Introduction

One of the problems of the fishery sector in the capture fisheries and the wild population is parasite and disease condition of fish parasite that reduces fish production by affecting the normal physiology of fish (Skelton, 2001). If left uncontrolled, it can results in mass mortalities or in some cases, can be serve as source of infection for human and other vertebrates that consumed fish (Yanong, 2008). To improve the productivity of the fishery sector, important constraints remain to be addressed Avenant-Oldewage (2001).

Parasite can also spoil the appearance of fish and usually affect the marketability of commercially produced fish, thus raising public health concerns especially in areas where raw fish is eaten (Zhokhov *et al.*, 2007). In natural systems, they may threaten the abundance and diversity of indigenous fish species. In Ethiopia, community knowledge on fish disease and their impact on the fish production in the inland waters of the country is far from adequate. This is due to the fact that there had not been systematic studies undertaken in the past years as well as community awareness is not created. The habit of raw fish eating is common among fishermen and people in Ethiopia, especially people near to water bodies. But potential fish parasites that can easily be disseminated to those water bodies are not sufficiently known (Eshetu Yimer and Mulualem Eneyew, 2003).

Objective:

- To assess the prevalence of the economically important fish parasite in the lake.

Materials and Methods

Materials used

Fishing gears (Gillnet of different mesh size including monofilaments), long line, boats with engine, different chemicals for fish as well as parasite preservation.

Site selection:

Lake Harkiso is a pouch of floodplain found in the North-East border of Lake Langano. It has open water area of 600 ha, with about 1 km S-N length and 600 m E-W width. Before starting regular sampling program a reconnaissance survey was conducted to fix the sampling sites.

Field sampling and measurements:

Samples of fishes were collected monthly from November 2021- May 2022 using different mesh size of gill nets and hooks from the selected sites of water bodies. The codes of each fish, species, sex, total length, total weight, name of the site, date of sampling, organ of fish to be sampled, types of parasites observed, number of parasite per organ and different health related notes were recorded for each fish on separate field protocol. Beside internal and external examination, eye balls were taken out using scissors and forceps, then crushed; examined.

Brain of African catfish was dissected longitudinally and the cranial cavity was washed away into Petri dish using water dropper and checked for parasites. Swim bladder of African catfish was also dissected to examine nematodes. Each parasite that gets each fish was kept in a plastic bag containing 4% formaldehyde solution.

Samples were then transported to laboratory for further laboratory studies. Prevalence was calculated for all the recovered parasites following the indications of Bush *et al*. (2001). Numerical results were analyzed by Microsoft Excel 2013. Then statistically data was analyzed using descriptive statistics and mean comparison procedure of the SPSS Software Version 21.

Results and discussion

A total of 73 specimens of *O. niloticus* (19) and *C. gariepinus* (54) were examined from Lake Harkiso. Out of these, 21 (5 *O. niloticus*, and 16 *C. gariepinus*) were infected with one or more species of different parasite with overall prevalence rate 28.76%. *Clinostomum sp* and *Contracaecum* parasite were the most prevalent parasite in the lake of which *Contracaecum* is found in 4(80%) of the infested *O. niloticus and* in 13 (81.25%) of the infested *C. gariepinus* (Table 1). Leeches are the only and dominant external parasite identified in the lake from both fish species with the occurrence of 3(60%) and 15(93.75%) of the parasite infested *O. niloticus* and *C. gariepinus* respectively.

Table 1: Prevalence of each parasite recorded among the examined fishes.

Parasites observed	Total number observed	*O. niloticus*	*C. gariepinus*	%age (of total 21 Infected)
Contracaecum sp	17	4	13	80.9
Clinostomum	11	7	4	52.3
Leech sp.	18	3	15	85.7

Sexes were evaluated to determine their effect on the results of the parasite infection, and the results are reported in Table 2. According to the findings 9 (42.8%) of the infected fish were females, and the remaining 12 (57.14%) were males.

Table 2: Prevalence of parasites on sex base of the host (n= 73).

Sex	Examined		Infected		Percentage (infected)		Total infection (%)
	Tilapia	Catfish	Tilapia	Catfish	Tilapia	Catfish	
Male	13	26	3	9	23.07	34.62	30.77
Female	8	26	2	7	25.00	26.92	26.47
Total	**21**	**52**	**5**	**16**	**23.81**	**30.77**	**28.76**

Over all prevalence of parasite infection in relation to different length were also assessed. The result shows that high prevalence (71.43%) was recorded at 31-60cm total length of fish and the lower parasites prevalence (28.57%) was observed at 10-30cm total length of fishes (Table 3).

Table 3: Prevalence of parasites in examined fish in relation to their total length.

Length (cm)	Examined		Infected		Percentage (infected)		Total infection (%)
	Tilapia	Catfish	Tilapia	Catfish	Tilapia	Catfish	
10–30	17	12	2	4	9.52	19.05	28.57
31–60	4	40	3	12	14.28	57.14	71.43
Total	21	52	5	16	23.8	76.19	100

The findings of this study demonstrated that parasite prevalence (28.76%) in *O. niloticus* and *C. gariepinus* in Lake Harkiso was lower than that reported in previous studies, which found prevalence rates of 75.67% (Temesgen, 2003) at Lake Hawassa, 73.24% (Teferra, 1990) at Lake Tana, 48.12% (Shibru and Tadesse, 1997) and 58% (Amare Tadesse, 1986) at Lake Hawassa. The variation in prevalence rates within a single lake may be due to the dynamic nature of parasitism, while the variation between these lakes is likely caused by geographic differences that provide suitable ecological niches for the parasites and/or have an impact on the susceptibility of the hosts and the availability of intermediate hosts.

The prevalence of infection varied only little between infections in males and females. This observation was in line with the conclusion made by Ochieng *et al.* (2012) that male fishes were typically more vulnerable than female fishes to infections with nematodes, cestodes, acanthocephalan, crustacean, and copepod parasites. Although the cause of this phenomena is yet unknown, it may be related to fish's spawning behavior. Male fish guard a region around the nest when breeding, keeping out other fish (females not spawning included). The males had higher prevalence because they spent more time than the females during this time in the shallow waters where the snails bearing *Clinostomum cercaria* are most frequently seen.

The prevalence of infection was positively correlated with host length. This means that as fish grows, chances of infection increase for these parasites, because a long period of exposition to infective stages, and the amount of food it consumes, which including the larval stages of this parasite increased (Mashego, 1989 and Rohde, 1993). Larger fish have lived longer (as fish grow during all their life) and, therefore, have a higher probability of encountering parasites during their life span than smaller and shorter lived fish species. Moreover, feeding habits and wide diet put fish into contact with potential intermediate hosts of nematodes, cestodes, digenea, and acanthocephalan. Aho and Bush (1993) stated that the parasite species might accumulate among food chains; this could be particularly the case for endo-parasites. Szalai and Dick (1990) mentioned that the Contracaecum spp. larvae were absent in age 0 and age 1 bass (Micropterus salmoides) but prevalence and mean intensity increased with age, for bass age 2 or older. Poulin (2006) stated that the increase in prevalence and intensity with the host length could be related, not only to accumulation of parasites in the host during its life, but also to change of diet. According to Luque and Alves (2001), correlation between the host total length and parasite prevalence and intensity is a pattern widely recorded in marine fish and documented with numerous cases in freshwater and marine fishes. Bruton (1979) also indicates *C. gariepinus* with the ranges 30-70cm feed on large juvenile fish and crustacean parasites which also similar with this study. Paperna (1996) added that infection level increase significantly with size that also correlated with this finding.

Conclusion and recommendation

The result of the present study showed that the prevalence of parasite in *O. niloticus* and *C. gariepinus* in Lake Harkiso was far lower than the previous reports from different lakes. The variation of prevalence rate might be the dynamic nature of parasitism and the variation between lakes or probably due to geographical difference offering suitable ecological niches for the parasites and/or affecting the susceptibility of the hosts and the availability of intermediate hosts.

Sex preference was observed in the prevalence of the parasite. Generally, male fish had a higher prevalence than the female fish. This is associated with the male’s behavior during breeding. The prevalence of infection was also positively correlated with host length. This means that as fish grows chances of infection increase for these parasites in this finding. *Clinostomatid digeneans* and *Contracaecum* nematodes could represent potential human health risks of eating uncooked or slightly cooked/smoked fish.

Hence, based on the findings of the current study, the following points are recommended:

- Identification of the genera *Clinostomum* and *Contracaecum* to the species level is important to check for the occurrence of zoonotic parasites.
- Consumers should not eat uncooked or slightly cooked fish and health education should be given for them on the risk of eating raw and partly cooked fish,
- Lake management should be implemented to avoid the existing contamination of the lake and to break the life cycle of parasites of fish and an effective parasite control program should be incorporated in the management of the lake.
- The study of these parasites and others should be coupled with molecular biology to enable a more accurate description of their diversity and occurrences.

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Assessment of the diversity and some population aspects of fish in the potential rivers of Oromia Region

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Abstract

The total number of valid fish species known from Ethiopian inland water bodies is about 183. The objective of this assessment was to assess fish species diversity in the potential rivers of Oromia Region. The fishes were collected from Awash, Guder, Jama, Gaba, Gibe, Sor, Ketar, Bulbula, Didesa and Gidabo for five consecutive years from July 2017 to June 2022 using gillnets of various mesh sizes (6, 8, 10 and 12 cm stretched mesh). Totally, 917 fish samples belonging to 13 different fish species were collected. The highest fish species diversity is found in Awash River followed by Gidabo River. The lowest fish species diversity is found in Katar River followed by Gaba River. Labeobarbus intermidus was dominant fish species with percent Index of relative importance (IRI) of 71.77% followed by Oreochromis niloticus with index of relative importance 23.33% and Coptodon zillii and Morymyrus with are the least dominant species with IRI of 0.02% each. From length-weight relationship, it can be stated that the two dominant fish species L. intermedius showed positively allometric growth, whereas O. niloticus showed negatively allometric growth. In order to have a better knowledge of biology and behavior of most of the species are still lacking. Therefore, further studies are required on the biology and behavior of fishes in the study using electro fishing techniques.

Keywords: *Fish diversity, Length frequency, length-weight relationship, Index of relative importance*

Introduction

Most developing countries in Asia and Africa have recognized riverine fisheries as an effective way of increasing the supply of fish as food in rural areas, at an affordable price. Riverine fisheries also provide additional income to rural farmers, thereby contributing to poverty alleviation. Riverine fisheries have added advantages in that, unlike the more conventional aquaculture practices, they are less resource intensive and need less technical skills at farmers' level.

Ethiopia has a number of lakes and rivers with substantial quantity of fish stocks. There are 10 major lakes with a total area of 7,400 km^2 and a combined length of 7,185 km of major rivers (Brook Lemma, 2012). The highest fish species diversity in Ethiopia has been recorded from Baro basin, followed by Abay, Wabishebelle and Omo-Gibe basins including rivers found in Oromia. However, endemicity seems to be highest in Abay and Awash basins. Fish fauna of Ethiopian high lands are dominated by species of fish in the family Cyprinidae.

According to Golubtsov and Mina (2003) the total numbers of valid fish species known from Ethiopian inland water bodies is about 168 to 183 including 37-57 countrywide endemics. There are also about 10 exotic fish species introduced from abroad into Ethiopian freshwaters (Shibru, 1973). At the moment, we have no clear and complete list and description of the diversity of the fish fauna of Ethiopia. Many of the drainage basins, especially the rivers, are not exhaustively explored in the country in general (Abebe

Getahun, 2002) and in Oromia in particular. The objective of this assessment were to identify the economically important fish in rivers of Oromia,

Materials and methods

Fish sampling and identification

Fish samples were collected for five consecutive years from July 2017 to June 2022. The fishes were collected from Awash, Guder, Jama, Gaba, Gibe, Sor, Ketar, Bulbula, Didesa and Gidabo. Fish specimens were collected from the rivers using gillnets of various mesh sizes (six, eight, ten and twelve cm stretched mesh). Gillnets were set into the rivers during late in the afternoon and collected early in the morning the next day. Identification of the fish specimens was made to species level using relevant taxonomic literature (Habteselassie, 2012). Both total length (TL) and total weight (TW) measurements were taken, to the nearest 0.1 cm and 0.1 g, respectively. Ultimately, voucher specimens from each species were preserved in 10% formalin solution and transported to Batu Fish and Other Aquatic Life Research Center where they were deposited. Length (L, cm) and weight (W, g) relationships were developed by regression and the b values were tested to verify if they were significantly different to the isometric (b =3).

Diversity of fish species in the rivers was calculated by Shannon diversity index, denoted as *H*, this index is calculated as:

$$H = -\Sigma p\mathrm{i} * ln(p\mathrm{i})$$

Where, H' = implies fish diversity index, N = total number of fishes, Pi = ni/N = number of fishes in (ni)

Estimation of the relative abundance of fishes in the study river was made by comparing the relative catch in number and weight in the total sampling. An index of relative importance (IRI), which is a measure of the relative abundance or commonness of the species based on number and weight of individuals in catches, as well as their frequency of occurrence was computed.

Results and discussion

Fish species diversities

The study was conducted for five consecutive years from 2017-2022 on ten major different rivers of Oromia. Thirteen different fish species were caught from those ten different rivers. The Shannon diversity index (sometimes called the Shannon-Wiener Index) is a way to measure the diversity of species in a community.

The highest fish species diversity was found in Awash River (H = 1.82747) followed by Gidabo River (H = 1.5765) as indicated in Table 1. The lowest fish species diversity was found in Katar River (H = 0.5439) followed by Gaba River.

Table 1: Composition and diversity of fishes from different Rivers

Species	Gidabo	Didesa	Sor	Gibe	Guder	Gaba	Jama	Ketar	Bulbula	Awash	Total
Synodontis schall	8	-	-	-	-	-	-	-	-	-	8
Lebeoclindaricus	1	1	-	-	2	-	-	-	1	-	5
Schilibe species	11	-	-	-	-	-	-	-	-	-	11
Labeobarbus intermidus	19	99	17	20	36	44	17	95	60	16	423
Morymyrus	1	-	-	-	-	-	-		-	-	1
Oreochromis niloticus	2	29	1	11	3	19	1	29	85	80	260
Clarias gariepinus	9	-	-	4	10	-	-	-	10	63	96
Varycohrinus	-	-	4	-	-	-	13	-	-	-	17
Coptodon zillii	-	-		-	-	-	1	-	-	3	4
Cyprinus carpio	-	-		-	-	-	-	-	-	39	39
Carassius carassius	-	-		-	-	-	-	-	-	20	20
Garra makiiensis	-	-		-	-	-	-	-	-	15	15
Garra dembecha	-	-		-	-	-	-	-	-	8	8
Enteromius paludinosus	-	-		-	-	-		-	-	10	10
Total no. of fish caught	51	128	22	35	51	63	32	124	155	254	917
Shannon diversity index (H)	1.58	0.76	0.65	0.93	0.86	0.61	0.92	0.54	0.91	1.83	

Index of relative importance

he Index of Relative Importance (Pinkas *et al.*, 1971) is calculated as the the product of the sum of the weight of fish and the percent number and the percent frequency occurrence. The relative abundance of fishes from different ten rivers was expressed using the index of relative importance (IRI) (Kolding, 1998) such that:

$$\text{IRI}\,(\%) = \frac{(\%W + \%N)\,\%F_i}{\sum_{j=1}^{s=1} (\%W + \%N)\%F_j} \,x\,100$$

According to Kolding (1989), IRI (index relative importance) among fish species is important to compute the relative abundance of fishes by using the number, weight, and frequency of occurrence of fish species in rivers from the total catch. This IRI was analysed for Nine rivers, excluding Awash, of the total 10 described in table 1 above. Fish Length-Weight record of Awash River and some samples of Bulbula River were not included in the data.

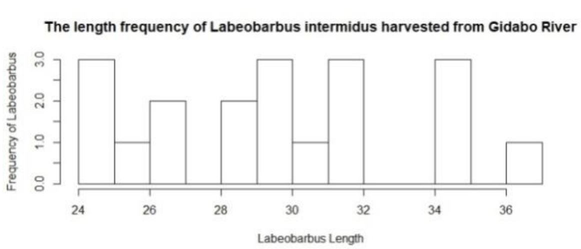
The IRI of the nine fish species caught from the 9 rivers during the study were calculated based on the number of fish caught during the study and estimating their weight from the mean values of those recorded. The index of relative importance of the fish in the rivers is given in table 2 below.

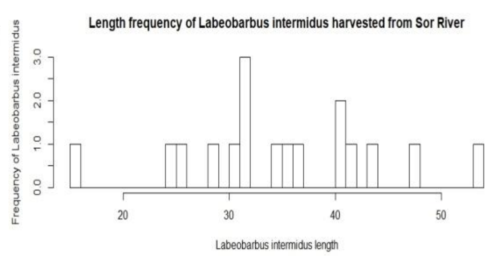
Table 2: The index of relative importance of nine different fish species harvested from nine water bodies

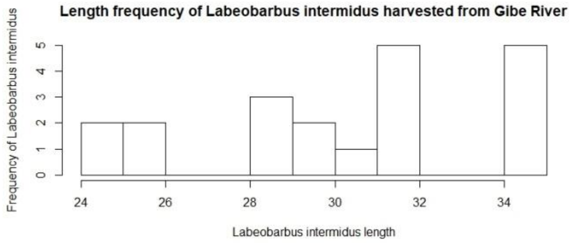
SN	Species	No.	% N	Weight (gram)	% Weight	Frequency (9)	% Frequency	IRI = (%N + %W) × %F	% IRI
1	*Synodontis schall*	8	1.21	2974.5	1.150	1	11.11	26.18	0.15
2	*Lebeobarbus clindaricus*	5	0.75	802.2	0.310	4	44.44	47.30	0.27
3	*Schilibe species*	11	1.66	1222.8	0.473	1	11.11	23.69	0.13
4	*Labeobarbus intermidus*	407	61.39	169910.6	65.696	9	100	12708.36	71.77
5	*Morymyrus*	1	0.15	430.0	0.166	1	11.11	3.52	0.02
6	*Oreochromis niloticus*	180	27.15	49973.34	19.322	9	88.88	4130.39	23.33
7	*Clarias gariepinus*	33	4.98	23571.5	9.114	4	44.44	626.22	3.54
8	*Varycohrinus*	17	2.56	9448.6	3.653	2	22.22	138.15	0.78
9	*Coptodon zillii*	1	0.15	298	0.115	1	11.11	2.96	0.02
	Total	663	100.0	258631.5	100.00			17706.77	100.0

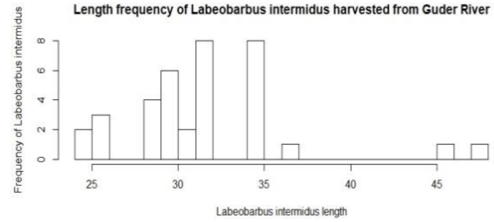
Labeobarbus intermidus was dominant fish species with percent Index of relative importance (IRI) of 71.77% followed by *Oreochromis niloticus with index of relative importance 23.33%* and *Coptodon zillii is the least dominant species with IRI of 0.02%* and followed with *Morymyrus with IRI 0.02%*. The dominance of *Labeobarbus intermedius* in rivers was also reported in previous studies; *Labeobarbus intermedius, Labeobarbus nedgia*, and *Labeo cylindricus* were the most abundant fish species, respectively, with 60.72%, 16.83%, and 14.66% index of relative importance in Sor and Geba rivers (Simagegnew *et al.*, 2017).

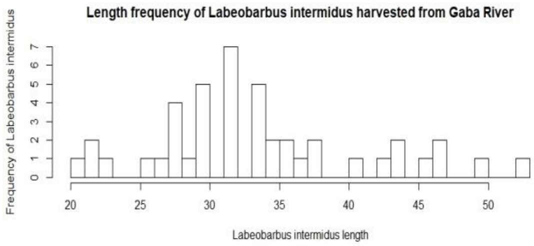
Length frequency of Labeobarbus intermidus from different rivers

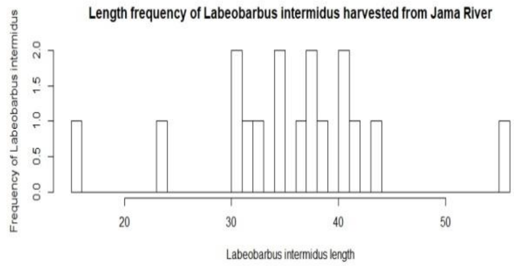


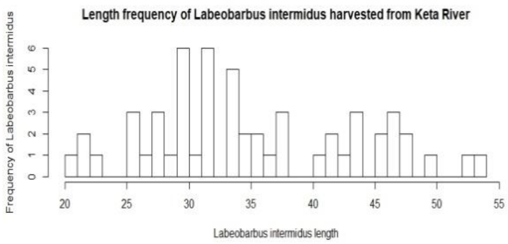


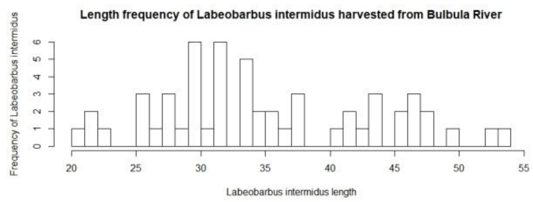




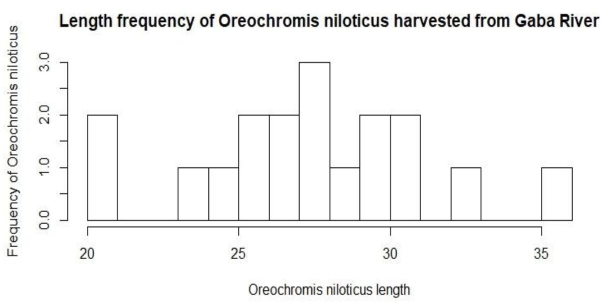


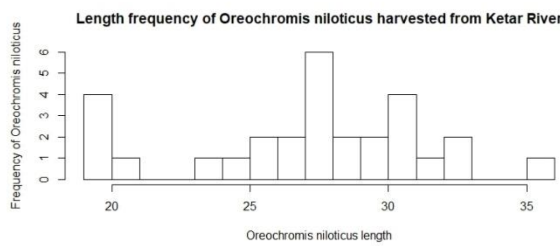




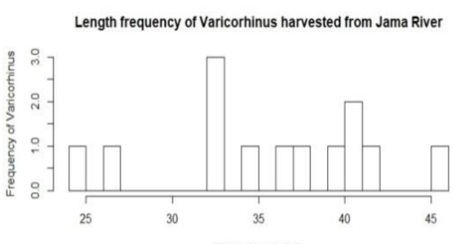


Length frequency of Oreochromis niloticus from different Rivers



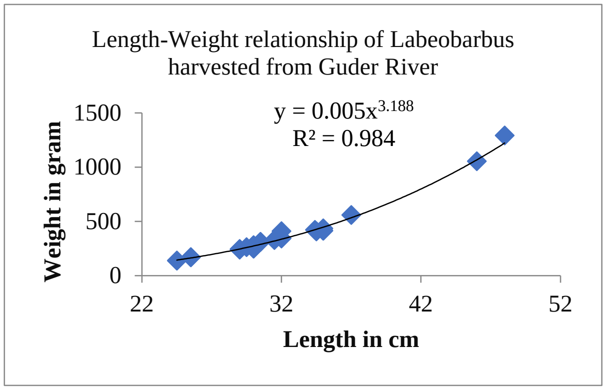


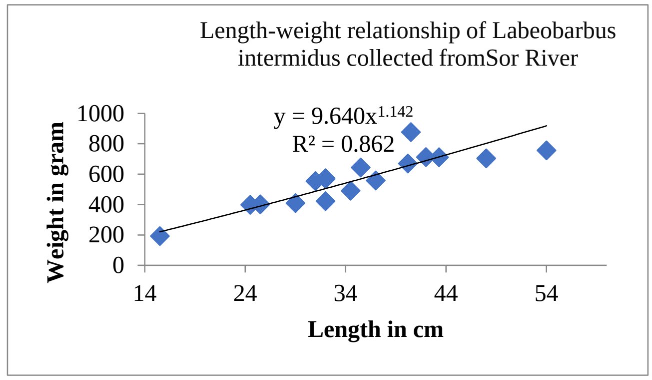
Length frequency of Varicorrhinus harvested from Jama River

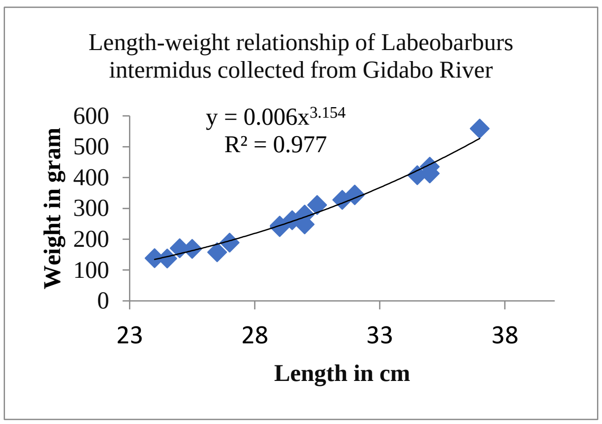


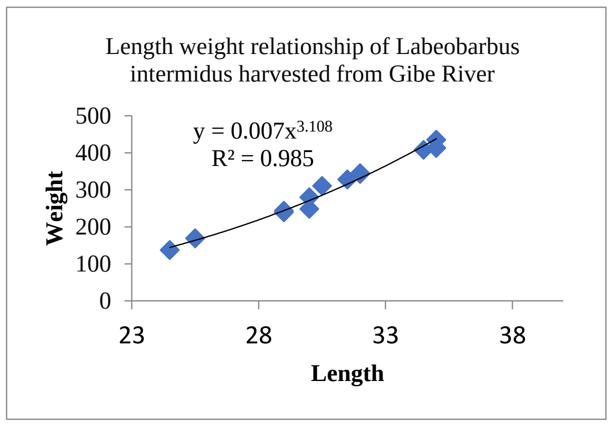
Length-weight relationship of Labeorbarbus

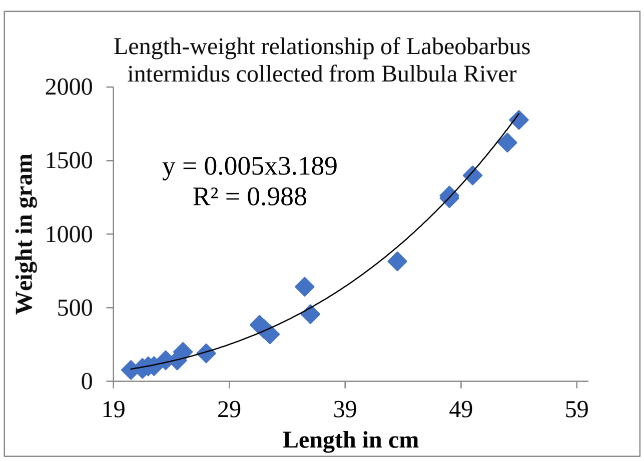
The relationships between fork length and total weight of Labeobarbus species were calculated using power function of (TW = aFL^b) where; TW – total weight (g), FL- fork length (cm), a and b are intercept and slope of regression line, respectively. If "b" = 3 then growth is isometric, if not the growth is allometric (>3 = positive allometric growth, <3= negative allometric growth). The line fitted to the data was described by the regression equation for each species. Length-weight relationship of *Labeobarbus intermidus* b value were 3.1 in Gidabo, 3.18 in Guder, 3.1 in Gibe, 3.1 in Bulbula and 3.0 in Ketar. Gizachew *et al* (2015) reported that the b- values of the reverine fishes were about 3.0; (*L. intermedius* (2.9), *L. brevicephalus* (3.0) and *L. nedgia* (2.9). The measure of fish condition can be linked to various factors such as environment, quality and quantity of food, rate of feeding, reproductive potential, water level fluctuation and disease.

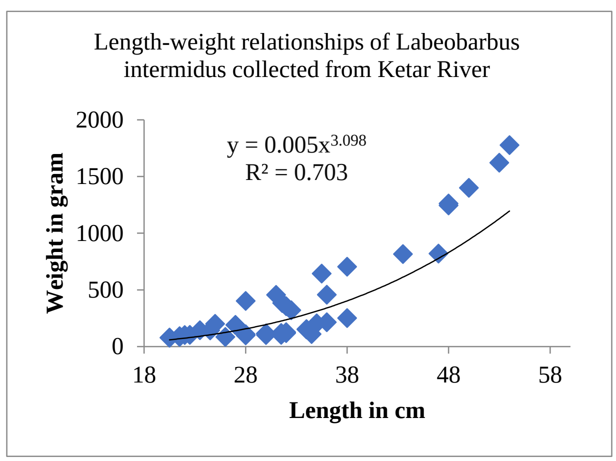








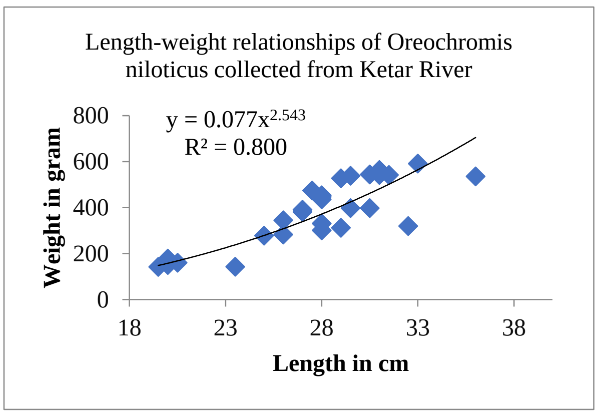


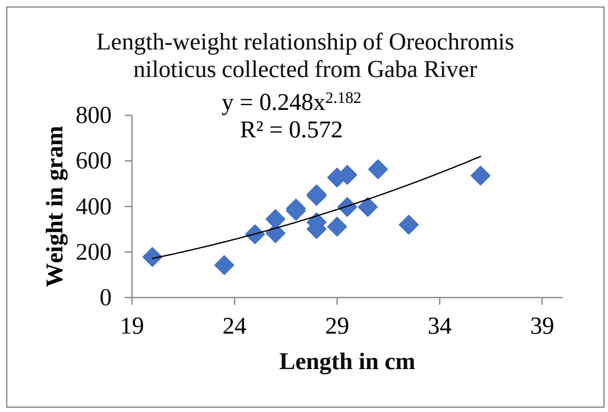


Length-weight relationship of the three most abundant species, L. intermedius, L. nedgia, and L. cylindricus, showed negative allometric growth in Sor and Geba Rivers (Simagegnew et al., 2017). In the present study, with the exception of *Labeobarbus intermidus* collected from Sor river, L.intermidus collected from Bulbula, Ketar, Gidabo, Guder, Gibe, and Ketar all shown positive allometric growth. The variations in the results between the present study and those studies could be ascribed to the differences in the number of samples, the differences in food availability, stage of fish gonad development, and spawning period.

Length-weight relationship of Oreochromis niloticus

The relationships between total length and total weight for *O. niloticus* were curvilinear. The regression coefficients for *O. niloticus* in Ketar and Gaba Rivers are less than 3 which means the fish showed negative allometric growth (Olurin, Aderibigbe, 2006). The value of “b’’ in this study (is less than reports for *O. niloticus* i.e., 2.93 in Lake Ardibo (Endalh *et al*., 2019) 2.91 in Lake Awassa (Demeke Admassu, 1990), 2.74 in Lake Tana (Zenebe Tadesse, 1997) and 3.12 in Lake Tana (Dereje Tewabe, 2014).





The fish species diversity (12) of Jemma River were *Bagrus docmak* , *Synodontis schall* , *Oreochromis niloticus*, *Ramias loti* , *Labeo forskhalii* , *Labeobarbus nedgia* , *Labeobarbus intermedius*, *Heterobranchus longifilis* , *Varicorhinus beso*, *Tilapia rendelii*, *Mormyrus kannume* and *Bagrus bajad Foroks* (Tewabe et al., 2016)

Conclusion and recommendations

Labeobarbus intermedius, and *Oreochromis niloticus* were the most dominant fish species in all rivers. From length-weight relationship, it can be stated that, of the two dominant fish species, *L. intermedius* showed positively allometric growth, whereas *O.niloticus* showed negatively allometric growth. Further investigation into food, feeding, and reproductive biology of fish species, especially in the tributaries of these rivers using electro fishing for better sample representation, and their socioeconomic aspects is recommended.

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Assessment of Commercially Important Fish Species Parasites in Potential Rivers of Oromia Region

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Abstract

The purpose of this study was to gather baseline knowledge regarding common fish parasites affecting fish species from major rivers of Oromia by collecting different fish species using gillnets of different mesh sizes and hooks. A total of 507 fish samples were collected from nine major rivers, namely Gidabo (51), Didesa (62), Sor (22), Gibe (35), Guder (47), Gaba (63), Jama (32), Ketar (124) and Bulbula (71). From the total collected and examined fishes (507) during the study period, 123 (24.26%) were found to have one or multiple parasites. The prevalence of the parasites was found in different rates and the major parasites identified during the investigation include adult Contracaecum, Clinostomum, Eustrongylides Diplostomum spp, Lernea elegans, Dolophus and Argulus. Contracaecum and Clinostomum parasites recorded in the present study could be medically important from public health point of view since the parasites can be transmitted to humans via eating raw infected fish. Therefore, further studies should be made for identification of zoonotically important common fish parasites to their species level.

Key words: *Fish, Parasites, Prevalence, River*

Introduction

The fish sector makes a vital contribution to the food and nutritional security of 200 million Africans and it provides income for over 10 million people engaged in fish production, processing and trade (ADF, 2004). More than 200 species of fish are known to occur in lakes, rivers and reservoirs in Ethiopia. Ethiopian has about 7,185 km of rivers within the country. The annual fish production potential of Ethiopia based on empirical methods on individual lake surface area and mean depth of major water bodies was estimated to be more than 90,000 tones EARO (2002).

The parasite and disease condition of fish is one of the issues facing the fishing industry in the capture fisheries and the wild population. By interfering with the normal physiology of fish, parasites reduce fish production and, if left unchecked can cause mass fish deaths or, in some cases, serve as a source of infection for humans and other vertebrates that consume fish. There are still significant issues that must be resolved in order to increase the productivity of the fishing industry Ayotunde and Okey (2007).

Additionally, parasites can detract from the aesthetic of fish and typically reduce its marketability, which raises public health concerns, particularly in regions where raw fish is consumed (Mukama, 2008). They might endanger the variety and quantity of local fish species in natural settings (Yanong, 2008). Community awareness of fish diseases and their effects on fish output in Ethiopia's interior rivers is woefully inadequate. This is because no systematic research has been done in previous years, and no community awareness has been developed (Temesgen, 2003). The practice of eating raw fish is widespread among Ethiopians in general and in the study area in particular, particularly among fishermen and those who live close to waterways. But potential fish parasites that can easily be disseminated to those water bodies are not sufficiently known.

Objective

- To assess the prevalence of the economically important fish parasite in the rivers of Oromia region, Ethiopia.

Materials and methods

Materials used: Fishing gears (Gillnet of different mesh size including monofilaments), long line, boats.

Site selection

Gidabo, Gibe, Sor, Dedessa, Guder, Gaba, Jama, Keter and Bulbula are major rivers selected in the region. Before starting regular sampling program a reconnaissance survey was conducted to fix the sites. Hence, sampling sites were selected based on geographical proximity and/or habitat similarity their distance from human settlements.

Field sampling and measurements

Fish parameters: The fishes were collected quarterly from the selected sampling sites using variety of fishing gears, which includes gill nets of various mesh sizes (6, 8, 10 and 12 cm stretched mesh size), monofilament nets with various stretched mesh sizes (5 mm to 55 mm stretched mesh size). Immediately after capture, total length (TL) and total weight (TW) of each specimen were measured to the nearest 0.1 cm and 0.1g, respectively. Each specimen were then dissected and its sex determined by inspecting the gonads.

Fish Examination, parasite collection and identification: samples of fishes were collected and examined both for external and internal parasite.

A. External examination:

The codes of each fish, species, sex, total length (TL), total weight (TW), name of the site, date of sampling, organ of fish sampled, types of parasites observed, number of parasite per organ and different health related notes were recorded for each fish on separate field protocol. Any abnormalities on the fish were recorded. A hand lens was used for quick identification of ecto-parasites on the skin and fins of the fish sample. Skin was also checked if there were capsules with metacercariae of trematodes in black dots and yellowish cysts which were sliced off the skin for further investigation.

To examine ecto-parasites on gills, the opercula were removed using scissors; the gills were removed and then placed in Petri dish containing normal lake water. Gill rakers were detached apart by forceps and examined under stereomicroscope for some worms of the class Monogenea.

To examine protozoans, scrapings from the fish skin were taken with a cover slip (22 x 22mm) near the operculum to the caudal peduncle on the lateral side of the body length, dorsal part of the head, and ventral region of the fish from head to just after the anal point and from fins especially under pectoral and ventral fins. The scraper was held at approximately 45° to the body and drawn backwards towards the tail (not against scales of fish) in a smooth movement, lifting off a small amount of mucus from the sample site. The mucus sample was then smeared onto a clean microscope slide along with a drop of lake water. The sample was then covered with a cover-slip and examined under compound microscope on 100x and 400x magnification. The samples were taken in separate cover slip for skin and fins and the numbers of similar genus parasites on each fish was later merged to estimate prevalence.

B. Internal examination:

Internally fish were examined with the following procedure. First the coelom was opened by making a ventral surface cut from the anus forward to an imaginary line at the posterior portion of the operculum. Then cut out the entire side of the coelom by cutting a rectangle of skin from behind the operculum, anterior to the anus, and ventral to the backbone. Secondly, by following the digestive system from the esophagus to the anus and listing the number of parasite found on different organ. Thirdly, small and large intestines were cut out and wash bottles were used to flush out the inside so that parasite like; tapeworms will come out the back end.

The eye balls were taken out using scissors and forceps, then crushed; examined under the stereo microscope and dissecting microscope. Brains of African catfish were dissected longitudinally and the cranial cavity was washed away into Petri dish using water dropper and checked for parasites. Swim bladder of African catfish was also dissected to examine nematodes.

Each parasite that gets each fish was kept in a plastic bag containing 4% formaldehyde solution. With regard to the technique and method used in fixing, preserving and identification of each parasite specimens, the appearance and procedures of Paperna (1996), Yamaguti (1971) and Bykhovskaya-Pavlovskaya (1964) was used as a guideline.

Result and discussions

Occurrence of parasites in sampled fishes

Over all prevalence in relation to sampling site: Fish samples were collected from Gidabo (51), Didesa (62), Sor (22), Gibe (35), Guder (47), Gaba (63), Jama (32), Ketar (124) and Bulbula (71). From the total collected and examined fishes (507) during the study period, 123 (24.26%) were infected with single or multiple parasites. From sampled rivers, Ketar and Bulbula shows the highest parasite prevalence with 26.83% and 17.07% respectively. The prevalence of the parasite was found in different rate and *Contracaecum, Clinostomum, Eustrongylides Diplostomum spp*, lernea elegans, Dolophus and Argulus were among identified parasites with different prevalence rate (Table 1).

Table 1. Parasite prevalence across the rivers, and occurrence in fish species examined (n= 507)

	Fish Species										
Rivers	Examined	Infected	S. schall	L. clindaricus	Schilibe species	L. intermidus	Morymyrus	O. niloticus	C. gariepinus	Varycohrinus	Prevalence (%age)
Gidabo	51	9	0	1	2	2	1	2	1	0	17.65
Didesa	62	11	0	0	0	7	0	4	0	0	17.74
Sor	22	7	0	0	0	5	0	1	0	1	31.82
Gibe	35	7	0	0	0	3	0	3	1	0	20.00
Guder	47	19	0	2	0	11	0	1	5	0	40.43
Gaba	63	11	0	0	0	9	0	2	0	0	17.46
Jama	32	5	0	0	0	3	0	1	0	1	15.63
Ketar	124	33	0	0	0	0	0	21	0	12	26.61
Bulbula	71	21	0	0	0	7	0	9	5	0	29.58
Total	**507**	**123**	**0**	**3**	**2**	**47**	**1**	**44**	**12**	**14**	**24.26**

Figure 1 shows that the prevalence of parasites in sampled fishes in each river. Relatively high prevalence (40.42%) was recorded at Guder River followed by Sor (31.8) and the lower parasites prevalence (15.62%) was observed at Jama River.

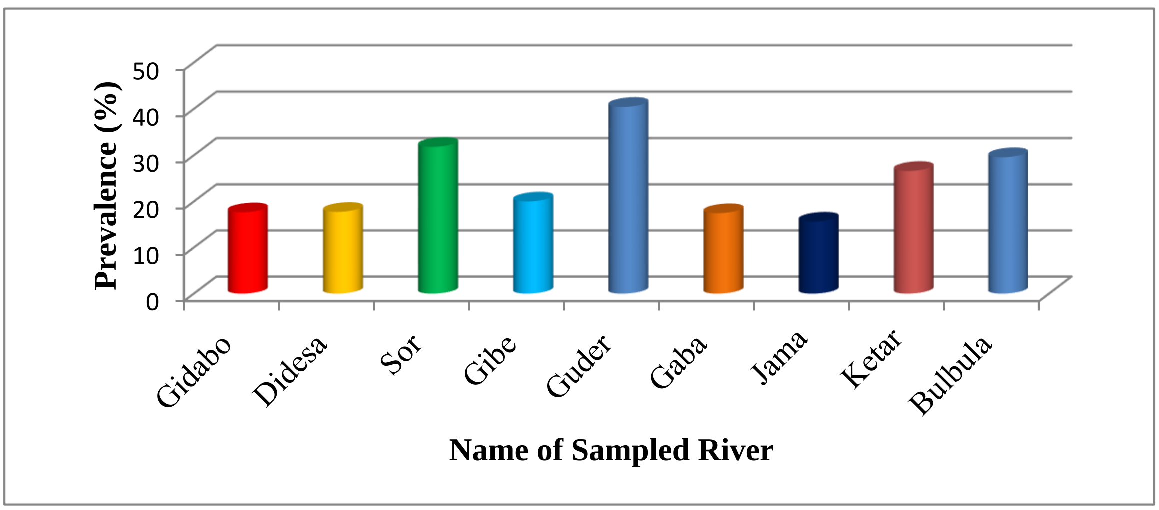

Figure 1: Parasite prevalence from each river

Parasite prevalence in relation to sex

Sexes were assessed to observe their influence on the parasite infection results. When analyzing the infection rate of all examined parasites larvae by host sex, of the two hundred ninety seven (297) males examined, 74(24.92%) were infected. On the other hand, out of two hundred ten females examined, 49(23.33%) were infected by different parasite species (Table 2).

Table 2: Sex based prevalence of parasites (n: 507)

Sex	number of examined	number of infected	%age (infected)
Male	297 (58.58%)	74	24.92
Female	210 (41.42%)	49	23.33

Parasite prevalence across the fish species were also assessed for its association with sex of the fish species. According to the findings, from total examined *Labeoburbus intermidius*, 31 (16.58%) samples of the 187 examined male and 16 (12.50%) samples of the 128 examined female were found to be positive (Table 3) showing that the males are slightly affected by the parasites than the females. The number of sample for some fish species are very few that the percentage of small observation can be misleading.

Table 3: Host sex based prevalence of parasites for each fish species (n: 507)

Sex	Status	Fish Species									Total
		S. schall	L. clindaricus	Schilibe spp	L. intermidus	Morymyrus	O. niloticus	C. gariepinus	Varycohrinus	Coptodon zillii	
Male	Examined	6	5	8	187	1	66	17	6	1	297
	Infected	0	3	1	31	1	27	8	3	0	74
	%Infected	0.00	60.00	12.50	16.58	100.00	40.91	47.06	50.00	0.00	24.92
Female	Examined	2	0	3	128	0	54	12	11	0	210
	Infected	0	0	1	16	0	17	4	11	0	49
	%Infected	0.00	0.00	33.33	12.50	0.00	31.48	33.33	100.00	0.00	23.33
Total	Examined	8	5	11	315	1	120	29	17	1	507
	Infected	0	3	2	47	1	44	12	14	0	123
	%Infected	0.00	60.00	18.18	14.92	100.00	36.67	41.38	82.35	0.00	24.26

The results of the present study showed that the parasite prevalence observed in the riverine fish was 24.26%; the value of which is far lower than the previous reports of 58% (Amare, 1986), 48.12% (Shibru and Tadesse, 1997) and 75.67% (Temesgen,2003) at Lake Hawassa, and 73.24% (Teferra, 1990) at Lake Tana. The variation of prevalence rate might be the dynamic nature of parasitism and the variation between these water bodies are probably due to geographical difference offering suitable ecological niches for the parasites and/or affecting the susceptibility of the hosts and the availability of intermediate hosts. Since, the definitive hosts of *Clinostomum* species are birds like herons, darters, cormorants and pelicans. Trematodes are established in the mouth and pharynx of these piscivorous birds (Paperna, 1991). It is likely that a large population of piscivorous birds around the lake harbor the adult parasites. *Contracaecum* species that infect freshwater fish are usually found as adults in fish-eating birds, such as cormorants and pelicans. Larval stages are seen in cyprinids (carp and related species), ictalurids (channel catfish), centrarchids (sunfish and bass), tilapia and other cichlids, and percids (perch).

Among helminthes identified during the study period from sampled fish species, *Clinostomatid* digeneans and nematodes of the genus *Contracaecum* could represent human health risk factors by eating raw or smoked meat of parasitized fish. *Clinostomum complanatum* is known to cause laryngopharyngitis infections in humans as was reported in Japan (Kakizoe et al., 2004) and Near East (Paperna, 1980) resulting apparently from ingesting inadequately cooked fish. The works of Dias *et al.* (2003) and Paperna (1996) also reveal that egret and cormorant birds are definitive hosts for *C. complanatum* though it failed to become established in pelicans (Finkelman, 1988).

Contracaecum spp. recovered range of prevalence in most rivers substantially accords with the findings of Eshetu Yimer (2003) in Lake Tana (59.8%) were the most common larval nematodes. Also from Lake Chamo, Eshetu Yimer *et al*. (1999) observed a prevalence of 2.09% for this genus. Identification of these larval nematodes to species level is difficult unless it is supported by DNA sequencing technique linked with their adult identification from the definitive bird hosts. Encapsulated larval nematodes are known to cause fibrous capsule (Paperna, 1980) and the non-encapsulated larvae cause extensive tissue damage by migration. Apart from this, the larval stages of *Contracaecum multipapillatum* were reported as potentially zoonotic parasite in Mexico (Vidal-Martinez *et al.*, 1994). Another parasite species, the nematode *Eustrongylides ignotus* was also reported to be infectious to humans (Barros *et al*., 2004) and the presence of the genus Eustrongylide*s* in mesentery of African catfish from Lake Chamo was indicated by the work of Eshetu Yimer *et al*. (1999).

Conclusion and recommendations

Apart from economic and public health importance, parasites impair fisheries activity. At the lakes and Rivers the harvested fish, fishing equipment and fishermen are loaded on the too narrow boat. Therefore, parasites that detach from the fish host can bite the bare foot of fishermen causing pain, bleeding and breakage of skin that might allow the entrance of other organisms which may cause anxiety and fear among young fishermen employed in the job. The present study showed that the proportion of parasites differ in prevalence. It may be difficult to draw a definite conclusion that particular parasites is definitely absent from a particular water body. Thus further investigation in all water bodies are needed to determine the level of parasite infestation and their seasonal dynamics. *Clinostomum,* Eustrongylide*s* and *Contracaecum* species could therefore represent potential health risks of eating uncooked or slightly cooked fish. Based on the above findings and conclusion the following recommendations are forwarded:-

- Medical survey on occurrence of laryngopharyngitis should be done on people eating uncooked/ smoked fish,
- Identification of the genera Clinostomum and Contracaecum to species level is important to check for the occurrence of zoonotic parasites
- Consumers should not eat uncooked or slightly cooked fish and health education should be given for them on the risk of eating raw and partly cooked fish.

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Assessment of Status of Cultured Fish Parasites in Selected Districts of Oromia Region

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Abstract:

This Study was conducted to assess the status of cultured fish diseases in selected district of Oromia region by collecting different fish species. Thus, a total of 351 specimens of O. niloticus (207), C. gariepinus (75) and Lebeoburbus intermidus (69) were examined. Different genera of fish parasites both from internal organs and external body surfaces of examined fishes were identified. The result revealed that 97(27.6%) from examined total fishes were infected with one or more genera of fish parasite. Among the three fish species examined of which ninety seven (97) were positive from the total (n: 351), sixty two (62) of them were infected by Contracaecum larvae (17.66% of the total and 63.9% of the infected) while thirty one (8.8% of the total and 31.9% of the infected), and sixteen (4.55% of the total and 16.5% of the infected) were Clinostomum sp and Eustrongylides respectively. These indicate higher prevalence of Contracaecum parasite during study period. On the other side, from assessed external parasites of fish, Dactylogyrus sp. had the highest prevalence 11.3% followed by Argulus sp. (8.2%) and Lernaea sp. (5.1%). It is crucial to take the right precautions to avoid parasite infestation and Potential transmission to consumers.

Key words: *Fish, Pathogen, Parasites, Prevalence*

Introduction

As per FAO data, aquaculture has been the fastest growing food producing sector in the world, with an average annual growth rate of 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (Leung and Bates, 2013). However, progress of aquaculture has caused some unwarranted activities both for the species and environment. At the same time, over exploitation of fisheries and anthropogenic stress on aquatic ecosystems has placed pressure on wild fish populations. The consequence has been the emergence and spread of an increasing array of new diseases change. As has been noticed in other food producing sector, aquaculture has been adversely affected due to frequent occurrence of disease outbreaks mostly due to intensive culture practices for higher economic gain (Walker and Winton, 2010).

Although aquaculture is the fastest-growing food production sector in the world, its production and trade is challenged by diseases of aquatic organisms (Stentiford *et al*., 2012). The aquatic environment creates its own specific dares for disease control, with the spread of pathogens through water creating different patterns of disease to those observed in terrestrial animal production (Barton, 2011). Disease epidemiology also varies globally, with outbreaks of aquatic animal disease at lower latitudes progressing more rapidly and resulting in higher mortality which require effective control systems to be developed (Leung and Bates, 2013).

Disease is one of the major constrains to aquaculture and may eventually become a limiting factor to the economic success of the fish farmers and it will result in production losses and public health problems. It also cause deterioration in the food value of fish and may even result in their mortality. Aquatic animal health therefore has major social and economic impacts on the people, businesses, communities and economies that rely on aquatic animal production (Komar and Wondover, 2007). Therefore, the knowledge of the diseases of major concern in a fish farming system is necessary in order to assess the risk factors influencing their introduction/spreading and defines the measures useful to their prophylaxis and control (Paperna *et al*., 1984; Paperna, 1996).

Losses due to mortality and retardation of growth of fish in ponds were estimated in different countries of the world. In China, it was estimated that around 10% culture area is suffering from disease, with annual losses of fish production around 15% (Wei, 2002). In Bangladesh the loss was estimated at about US$ 3.38 million in 1988 (Barua, 1994). The finding in those two countries indicated the importance of assessing the impact of disease in aquaculture in order to develop farmers-oriented primary fish health management.

Objective: To investigate the status of parasitic fish diseases in different pond of Oromia Region.

Materials and methods

Study area

The study was conducted in four selected districts of two zones of Oromia region viz., Adama and Ada'a districts of East Shewa zone and Serbo and Omo Nada districts of Jimma zone due to abundance of fish resources and functionality of fish pond in the selected areas.

Jimma Zone is divided in to 13 weredas (hosting a total population of over 2.2 million) with an agro-ecological setting of highlands (15%), midlands (67%) and lowlands (18%). The zone is one of the major coffee growing areas of Oromia region, well-endowed with natural resources contributing significantly to the national economy of the country. Major crops grown, other than coffee, are maize, teff, sorghum, barley, pulses (beans and peas), root crops (enset-false banana and potato) and fruits. Teff and honey production are another sources of cash after coffee. Enset is a strategic crop substantially contributing to the food security of the zone and is especially important in Setema and Sigimo weredas (highlands). Jimma Zone reliably receives good rains, ranging from 1,200 - 2,800 mm per annum. In normal years, the rainy season extends from February to October (Dechassa Lemessa, 2000).

East Shewa is located at center of Oromia, connecting the western regions to the eastern ones. This zone is bordered on the south by the West Arsi Zone, on the southwest by the Southern Nations, Nationalities and Peoples Region, on the west by Southwest Shewa Zone and Oromia Special Zone Surrounding Finfinne, on the northwest by North Shewa, on the north by the Amhara Region, on the northeast by the Afar Region, and on the southeast by Arsi. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this Zone has a total population of 1,356,342 population, of whom 696,350 are men and 659,992 women; with an area of 8,370.90 square kilometers, East Shewa has a population density of 162.03. While 340,225 or 25.08% are urban inhabitants, a further 664 or 0.05% are pastoralists. A total of 309,726 households were counted in this Zone, which results in an average of 4.38 persons to a household, and 296,342 housing units (Wikimedia, 2022).

Fish collection

Samples of fishes were collected quarterly by fishing nets from the selected ponds. After capture, total length (TL) and total weight (TW) of each specimen was measured to the nearest 0.1 cm and 0.1g, respectively.

Pathological examination

External examination: The codes of each fish, species, sex, TL, TW, name of the site, date of sampling, organ of fish sampled, types of parasites observed, number of parasite per organ and different health related notes were recorded for each fish. Any abnormalities on the fish were recorded. A hand lens was used for quick identification of ecto-parasites on the skin and fins of the fish sample. Skin was also checked if there are capsules with metacercariae of trematodes in black dots and yellowish cysts which were sliced off the skin for further investigation.

Parasites on gills were examined by removing the opercula using scissors; the gills were then placed in Petri dish containing normal lake water. Gill rakers were detached apart by forceps and examined under microscope for some worms. Protozoans were examined by scraping fish skin and taken with a cover slip (22 x 22mm) near the operculum to the caudal peduncle on the lateral side of the body length, dorsal part of the head, and ventral region of the fish from head to just after the anal point and from fins especially under pectoral and ventral fins. The mucus samples were smeared onto a clean microscope slide along with a drop of lake water and covered with a cover-slip and examined under compound microscope on 100x and 400x magnification.

Internal parasite examination*:* To examine internal parasite the coelom was opened by making a ventral surface cut from the anus forward to an imaginary line at the posterior portion of the operculum. Then, by following the digestive system from the esophagus to the anus and listing the number of parasite found on different organ. Finally, small and large intestines were cut out and wash bottle were used to flush out the inside so that parasite comes out the back end.

The eye balls were taken out using scissors and forceps, then crushed; examined. Brain of African catfish was dissected longitudinally and the cranial cavity were washed away into Petri dish using water dropper and checked for parasites. Each parasite that gets each fish was kept in a plastic bag containing 4% formaldehyde solution.

Parasites fixation, preservation and identification

With regard to the technique and method used in fixing, preserving and identification of each parasite specimens, the appearance and procedures of Paperna (1980), Yamaguti (1971) and Bykhovskaya-Pavlovskaya (1964) were used as a guideline. Larva nematodes were fixed in 4% formalin and later stored in the saline solution. Adult nematodes were fixed in hot formalin to insure their relaxation and preserved in 4% formalin mixed in 1% glycerin to avoid accidental drying. Trematodes were fixed in AFA (Alcohol Formalin Acetic acid).

Data management and analysis

The whole data were processed by the MS Excel program and collected raw data was entered into Microsoft excel data sheets and analyzed using SPSS-21 statistical software. Descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of infested fish. Statistical significance was set at $p<0.05$.

Result and discussions

Overall Prevalence

A total of 351 specimens of *O. niloticus* (207), *C. gariepinus* (75) and *Lebeoburbus intermidus* (69) were examined. Different genera of fish parasites both from internal organs and external body surfaces of examined fishes were identified. The result revealed that 97(27.6%) from examined total fishes were infected with one or more genera of fish parasite. The common endo-parasites were recorded as, *Contracaecum, Clinostomum* and Eustrongylides (**Table 1**). *Dactylogyrus sp., Argulus sp., Lernaea sp., and Ichthyophthirus sp.,* are among assessed and identified ecto-parasites.

Prevalence of each parasite species identified during study period

The prevalence of each parasite shows different rates in fishes (*O. niloticus*, *Lebeoburbus intermidus* and *C. gariepinus*) that were recorded during the study period. Among the three fish species examined of which ninety seven (97) were positive from the total (n: 351), sixty two (62) of them were infected by *Contracaecum* larvae (17.66% of the total and 63.9% of the infected) while thirty one (8.8% of the total and 31.9% of the infected) were infected by *Clinostomum sp* and sixteen (4.55% of the total and 16.5% of the infected) were infected by *Eustrongylides.*. These indicate higher prevalence of *Contracaecum* parasite in cultured fish during study period. On the other side, from assessed external parasites of fish, *Dactylogyrus sp.* had the highest prevalence of 11.3% followed by *Argulus sp. (*8.2%) and *Lernaea sp.* (5.1%) respectively.

From the examined and identified as infected fish species the prevalence of *Contracaecum* parasite was recorded as 39(62.9%) in *O. niloticus and* 21(33.8%) *C. gariepinus.* The result showed significant infection differences were occurred between the two fish species. On the other hand the prevalence of *Clinostomum sp* was higher in *C. gariepinus* and lower in *O. niloticus* **(Table 1)**.

Table 1: prevalence of each parasite recorded among the examined fishes.

Parasites observed	Number observed	*O.niloticus*	*C. gariepinus*	*L.intermidus*	%age (Infected)
Contracaecum spp	62	39	21	2	63.9
Clinostomum	31	5	17	9	31.9
Eustrongylides	16	7	3	6	16.5
Dactylogyrus sp.	11	3	6	2	11.3
Argulus sp.	8	2	4	2	8.2
Lernaea sp.	5	0	5	0	5.1
Ichthyophthirus	3	0	3	3	3.09

The present finding goes in line with the finding of study conducted in Sebeta ponds (Marshet *et al*., 2018), which revealed from sampled *O. niloticus* about 91(71.9%) was infested with one or multiple parasites. In this finding about seven genera of fish parasites were identified but eleven genera of both external and internal parasites were identified in Sebata study site. In contradict with current finding which identified *Dactylogyrus sp*.(11.3) as the most prevalent external parasite, Marshet *et al.* (2018) identified *Trichodina spp.* from skin and gills and *Cichlidogyrus spp*. from gills as the most prevalent external parasites in Sebeta ponds with prevalence of 37.50 and 33.59% respectively.

Overall prevalence in male and female fishes

Sexes of fish were assessed to observe their influence on the parasite infection results and the findings are presented in (Table 2). According to the result, out of infected fishes, 61 (62.8%) were males and the remaining 36 (31.2%) were females (Table 2).

Table 2: Prevalence of parasites on sex base of the host (n:351).

Sex	Number of examined	Number of infected	percentage (infected)
Male	191	61	31.9
Female	160	36	22.5

Sexes were also assessed to observe their influence on the parasite infection results on basis of fish species examined. According to the findings from total examined male catfish 19 (46.34%) were found to be positive (**Table 3).**

Table 3: Host sex based prevalence of parasites for each fish species (n: 351)

Sex	Status	*O. niloticus*	*C. gariepinus*	*L.intermidus*	Total
Male	Examined	117	41	33	191
	Infected	33	19	9	61
	%Infected	28.21	46.34	27.27	31.94
Female	Examined	90	34	36	160
	Infected	20	9	7	36
	%Infected	22.22	26.47	19.44	22.50
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	%Infected	25.60	37.33	23.19	27.64

There was slight difference between the infection of males and females in prevalence of infection. This observation agreed with the finding of (Ochieng *et al.*, 2012) that male fishes were generally more susceptible than female to infections with nematodes, cestodes, acanthocephalan, crustacean and copepod parasites. This phenomenon is however still unclear but can be associated with the breeding habit of the fishes. During breeding, male fish takes care of a territory around the nest, keeping off other fish (females not spawning included). During this period the males spend more time than the females in the shallow waters where the snails harboring the cercaria of *Clinostomum* are mostly found, and this is probably why the males had higher prevalence.

Overall prevalence in relation to fish length classes

The result shows that the prevalence of parasites in sampled fishes in relation to their total length (cm). Relatively high prevalence (35.7%) was recorded at 30-60 cm total length of fish and the lower parasites prevalence (16.4%) was observed at 10-30 cm total length of fishes. And there was statistically significant variation ($p < 0.05$) observed between the two total body length categories.

Table 3: Prevalence of parasites in examined fish in relation to their total length.

Total length (cm)	Number of examined	Number of infected	%age (Infected)
10 – 30	197	39	19.8
31 – 60	154	58	37.6

x^2: 9.98 P = 0.019

Lengths were also assessed to observe their influence on the parasite infection results on basis of fish species examined. According to the findings from total examined lower sized fish 39 (19.8%) were found to be positive which is lower when compared with higher size fish (Table 4**).**

Table 4: Host length based prevalence of parasites for each fish species (n: 351)

Total length (cm)	Status	*O.niloticus*	*C. gariepinus*	*L.intermidus*	Total
10-30cm	Examined	129	21	47	197
	Infected	19	11	9	39
	%Infected	14.73	52.38	19.15	19.80
31-60	Examined	78	54	22	160
	Infected	34	17	7	58
	%Infected	43.59	31.48	31.82	36.25
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	% Infected	25.60	37.33	23.19	27.64

The prevalence of infection was positively correlated with host length ($p < 0.05$). This means that as fish grows, chances of infection increase for these parasites, because a long period of exposition to infective stages, and the amount of food it consumes, which including the larval stages of this parasite increased (Mashego, 1989). Larger fish have lived longer (as fish grow during all their life) and, therefore, have a higher probability of encountering parasites during their life span than smaller and shorter lived fish species. Moreover, feeding habits and wide diet put fish into contact with potential intermediate hosts of nematodes, cestodes, digenea, and acanthocephalan. Aho and Bush (1993) stated that the parasite species might accumulate among food chains, this could be particularly the case for endo-parasites. Szalai and Dick (1990) mentioned that the Contracaecum spp. larvae were absent in age 0 and age 1 bass (*Micropterus salmoides*) but prevalence and mean intensity increased with age, for bass age 2 or older. Poulin (2006) stated that the increase in prevalence and intensity with the host length, could be related, not only to accumulation of parasites in the host during its life, but also to change of diet. According to Luque and Alves (2001), correlation between the host total length and parasite prevalence and intensity is a pattern widely recorded in marine fish and documented with numerous cases in freshwater and marine fishes. Brutol (1974) also indicates *C. gariepinus* with the ranges 30-70cm feed on large juvenile fish and crustacean parasites which also similar with this study. Paperna 1974 added that infection level increase significantly with size that also correlated with this finding.

This finding agrees with the finding of Petchimuthu *et al.*, (2018) which revealed that larger fishes are more susceptible to infections in comparison with small fish i.e. larger fishes were heavily parasitized than smaller ones. The highest prevalence value (23.71%) was found in length groups above 10 cm and lowest prevalence value (14.28%) was found in 3-5 cm length groups. The highest abundance value (3.32) was found in 5-10 cm length groups but lowest (0.35) was found in 3-5 cm length groups.

Overall prevalence in relation to fish body weight

It is clear from the result in Table 5 that the prevalence of parasites in fish examined in relation to their body weight is higher in weight class 401-600 g (81.4%) while lower in class of 16-200gr (5.2%). Statistically significant variation was seen when the three weight categories were tested by Chi Square test.

Table 5: Prevalence of parasites in examined fish in relation to their total body weight (gr).

Total weight (gr)	Number of examined	Number of infected	%age (Infected)
16-200	83	5	6.02
201-400	127	13	10.24
401-600	141	79	56.02
x^2: 69	P = 0.0001		

Overall prevalence in relation to sampling sites

The prevalence of parasites in fish examined in relation to sampled sites were checked and the result showed no significant variation observed between the site (P>0.05) (Table 6). Study by Marishet *et al.*, (2018) revealed as there is a significant difference (p<0.05) in prevalence of parasite in the different study sites. This might be attributed to differences in the diversity and availability of invertebrate intermediate hosts and fish eating birds to complete its developmental cycle.

Table 6: Prevalence of parasites in examined fish in relation to their sampling district.

Sampling District	Number of examined	Number of infected	%age (Infected)
Wonji	184	47	25.54
Godino	51	18	35.29
Serbo	49	11	22.45
Omo Neda	67	21	31.34

The prevalence of parasites in fish examined in relation to sampled zones was also checked and the result showed no significant variation was observed between the two zones (Table 7).

Table 7: Prevalence of parasites by fish species and their sampling districts.

Zones	Status	*O.niloticus*	*C. gariepinus*	*L.intermidus*	Total
East Shewa	Examined	134	32	69	235
	Infected	30	19	16	65
	%Infected	22.39	59.38	23.19	27.66
Jimma	Examined	73	43	0	116
	Infected	23	9	0	32
	%Infected	31.51	20.93	0.00	27.59
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	%Infected	25.60	37.33	23.19	27.64

Conclusion and recommendations

Infestation with parasites is the biggest danger to fish culture systems. Every year, numerous fish species are infected by various forms of parasites, which have a considerable negative impact on fish productivity. The analyzed fish species in the study sites contained parasites from a combined total of 7 genera, both external and internal. All of the fish that were sampled had either one or more parasite infections. Fish samples from each study site had external parasites including *Dactylogyrus sp., Argulus sp.,* and *Lernaea sp*. on them. These parasites can be spread from site to site along with the fish. Control techniques must be developed, to limit the free transfer of fish fingerlings from place to place including from the natural water bodies to stock into other water bodies or intensive and small-scale fish farms.

On the other hand, further research should be done to identify parasites at the species level using molecular methods and parasite genomics of culture fish species. In studies of fish parasites, biotic parameters like stocking density and abiotic factors like water chemistry and water quality that can affect the abundance of parasites should also be taken into account. In general, appropriate measures should be implemented to avoid parasite infection and to save key fish species from extinction. In order to prevent the aquaculture business from suffering this significant economic loss on a farmer level each year, control of these parasite illnesses should be given top priority.

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On-Station Evaluation of Juvenile African Catfish (*Clarias gariepinus*) Growth Performance under different Stocking Densities in Concrete Channel Tanks at Batu, Oromia.

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Abstract

African catfish (Clarias gariepinus) is the most suitable candidate species for Aquaculture, largely farmed next to Tilapia. However, its production in intensive pond-culture was not yet practiced in Ethiopia. The main objective of this experiment was to evaluate its growth performance and survival rate at different stocking densities. Fingerlings of African catfish of similar size were collected from Koka Reservoir, introduced to concrete ponds at Batu and acclimatized for 15 days. The fish were then stocked into 4.8 m^3 experimental ponds at lower stocking density (T3=10 fish/M^3), at medium (T2=20fish/M^3) and higher stocking density (T1=30 fish/m^3) in duplicate. All the fish were fed with 35% crude protein pelletized feed and water quality parameters were monitored. Data were collected for five months, and analysis made using one-way-ANOVA at $p< 0.05$ significance level for mean separation. Survival rates of fish were 100%, 98.95% and 96.45%, in lower, medium and highest stocking densities, respectively. Daily growth rates (g/fish/day) were 0.76, 0.91 and 0.81 in T1, T2 and T3, respectively and all values are in good growth performance range. Average mean length (cm) and weight (g) of the fish in T1 were statistically significantly different from T3 for the first four months. Individual fish weight gains in all the treatments were promising. Total biomass across all the treatment showed differences, with large biomass (7.41 kg/m^3) obtained in T1 followed with T2 (6.06 kg/m^3) and T3 (2.71 kg/m^3). Therefore, considering higher biomass as a target in the economics of fish culture, the highest stocking density of 30 fishs/m^3 was recommended

Key words: *Clarias gariepinus* , Growth performance, Stocking density

Introduction

Aquaculture is one of the several alternatives to alleviate animal protein deficiency which is becoming one of the critical issues in developing countries. Globally, aquaculture is contributing to food and nutritional availability, household food security, income generation, job opportunity, poverty reduction and improving living standards in many developing countries (Prajith *et al*., 2011). Fish are rich source of high quality protein, amino acids, vitamins, fats, fatty acid, omega series and minerals (Elsadig *et al*.., 2017). However, the gap between demand and supply of food fish has been widening due to a decline of capture fishery production and a rapid growing of global population. Aquaculture will be the only way to boost food fish production and very recently aquaculture products are exceeding capture fishery.

There are different Aquaculture candidate fish species in the world. Of these, African catfish (*Clarias gariepinus*) is the most suitable candidate species, largely farmed next to Tilapia (*Orechromis niloticus*) (World Fish Center, 2008). This is because of its ideal merits (Eding and Kamstra, 2001), which includes fast growth rate at high stocking densities, high feed conversion efficiency, good meat quality, better yield, better adaptation under captivity, high resistance to environmental stressors, and year round production. Normally catfish are opportunistic feeders. Naturally they are adaptable to a wide range of feed, a bottom feeder under natural system; however can come to the surface and feed in groups at the water surface under captivity. Catfish can survive depleted dissolved oxygen level for a long time using its arborescent organs to breathe atmospheric air (Viveen *et al.*, 1986). In addition, the fish can grow to an average market size of 700 to 800 g in 8 months at stocking densities of 5 to 5.7 fish/m^2 when fed with 38% CP (Hecht *et al*., 1988).

Stocking density is one of the main factors determining the growth and the final biomass of fish harvested. It has also been found to be one of the principal factors in regulating aquatic behavior of *Clarias gariepinus* and also an important parameter in fish culture operation, since it has direct effect on the growth, survival and production (Hecht, 2013). A review of world fisheries also indicated that the contribution of aquaculture can only be realized if a number of issues including stocking density of aquaculture species are addressed (FAO, 1995). Environmental variables, farming conditions and food availability are other factors that can affect fish growth under culture conditions.

Some Fish farmers are also aware that catfish can tolerate large range of dissolved oxygen and water deficits and started exercising increased high density farms. However, increased stocking density results in high mortality unless waste produced is strictly controlled. That means, aquaculture production and productivity can rise by utilizing improved and modern aquaculture techniques and facilities such as rising of fish stocking density to the optimum, improving pond management, modern waste control and providing fish with quality feed.

These days, some African countries like Nigeria, Uganda, South Africa, Kenya and Ghana have industrially formulated fish feeds. Consequently, fish farming is growing at different scale; some countries like Nigeria use catfish under intensive monoculture farming (Abou-Zied, 2016). In Ethiopia, pelletized fish feed producing company has started very recently. However, fish feed production is demand based and hence impossible to get industrial feed except under special order. This indicates the stage of aquaculture is yet underdeveloped, despite high number of unemployment, large gap in food and nutrition and plenty of suitable aquaculture potential areas in the country. The objective of this experiment is therefore, to evaluate growth performance, survival rate and final production of *Clarias gariepinus* at different stocking densities in concrete ponds in the Central Rift Valley of Ethiopia.

Materials and Methods

Description of the Study Area

This study was conducted in the central Rift Valley of Ethiopia, Batu (Ziway) Fish and Other Aquatic Life Research Center which is found at 1640 m.a.s.l. The experiment was conducted under small rectangular concrete ponds of 4.8m^3 water volume each.

Experimental design

Six concrete ponds each having a volume of about 4.8m^3 were cleaned, disinfected and filled with ground water 15 days after disinfection. Pond water depth was maintained at o.80 cm and left for 15 days to stabilize. Juveniles of African catfish (*Clarias gariepinus)* were collected from wild (Koka reservoir), acclimatized for 15 days, measured (at size of 15 $\pm$ 0.3 cm mean length and 18 g average weight), counted and stocked to the experimental tanks randomly. The experiment has three treatments with two replications, arranged in completely randomized design. A total of 568 juveniles of African catfish were subjected to three treatments.

T1: 282 Fish, 30fish/m^3
T2: 188 fish, 20 fish/M^3
T3: 96 fish, 10 fish/M^3

The fish were fed with 35% CP *Alema* commercial pelletized feed at a feeding rate of 3% body weight daily delivered in split twice at 9:00 am and 4:00 pm for the whole experimental period, 150 days. Feed adjustment was done for each treatment after collecting the fish sample data, and calculation made depending on average biomass change every month. The pond water was refreshed twice weekly to maintain the 80 cm water depth after gentle stirring from the bottom to avoid the turbid bottom following the procedure of Viveen *et al.*(1986).

Measurements

Fish total length (TL_{cm}) & total weight (TW_g) were recorded monthly whereas water physico-chemical parameters including temperature, pH, dissolved oxygen (DO) were taken twice weekly. Plankton sampling was done monthly just to control algal bloom if any (Edmondson and Weinberg, 1971).

Survival rate was calculated as:

$$\boldsymbol{Survival\ rate}\ (\%) = \frac{\boldsymbol{Number\ of\ fish\ harvested}}{\boldsymbol{Number\ of\ fish\ stocked}} \boldsymbol{X\ 10}0$$

Daily growth rate was calculated as:

$$\textbf{Daily Growth Rate}(\mathbf{g\ /day}) = \frac{\boldsymbol{Mean\ Final\ Weight(g) - Mean\ Initial\ Weight(g)}}{\boldsymbol{Experimental\ Days}}$$

Mean differences in fish length and weight among the stoking densities were analyzed using one-way analysis of variance (ANOVA) at 5% probability using multiple range test.

Result and discussion

Table 1: Some water quality parameters of the experimental fish tanks

Parameter	Standard	T1	T2	T3	
K µg/ml		15.85	15.45	15.3	
Na µg/ml		199.55	195.35	194.35	
Phosphate		1.87	2.15	2.58	***Water***
DO		>3.3	>4.00	>4	***quality***
NO_2-N		4.32	5.97	4.37	***paramete***
NH_3		-	-	-	***rs***
Ca µg/ml		117	114.85	114.1	
TDS g/l	<25	2.32	1.22	1.17	Water
Salinity (PPT)		1.21	1.19	1.85	physico-
Resistivity Ω		427	419	422	chemical
Secchi-disc		36.4	26.4	20.8	paramete
Avg. Temperature		25	25	25	rs

measured *in-situ* twice weekly during the experimental period, and lab analysis of water quality test done at the end of the experiment are presented in Table 1. Water temperature ranged between 20.34℃ and 27.45℃ with mean temperature of 25℃. The highest water temperature was recorded in April and the lowest in July.

Growth response

Relatively lower survival rate of 96.45% was recorded in the higher stocking density (T1) as compared to the 98.95% and 100% recorded in T2 and T3, respectively. Higher stocking density results in crowded conditions which make the fish suffer from the high density stress due to aggressive feeding interaction, waste production and reduced feed intake, resulting in growth retardation and even death (Bjoemsson, 1994). In this particular experiment, the highest stocking density of 30 fish/m^3 was in fact not much when compared to a study done by Hecht (2013) with stocking density of 250 fish/m^3. Survival rate of 96.45% recorded in the current study was high as compared to the previous report of 75% by Hecht perhaps due to relatively lower stocking density, better pond management and frequent water exchange. Generally, mortality has inverse relationship with stocking density.

Growth performances of the treatments were seen in terms of increment in length, weight and daily growth rate recorded during 150 days of the experimental period. Daily growth rates (g/fish/day) of 0.76, 0.91 and 0.81 obtained in T1, T2, and T3, respectively in the current experiment were higher than the result reported by Akinwole and Faturoti (2005) which was 0.26 $\pm$ 0.02 in hatchery. However, the current values are lower than a daily growth rate of 4.03-6.25 g/fish/day reported by Akinwole and Faturoti (2005) in grow out ponds even in higher stocking densities. This difference in daily growth rates depends on the size of the growing fish with the bigger fish attaining higher weight every day than a fingerling. The combined mean length as indicated in Table 2 below showed significant difference across all the treatments in the first and fourth months (March and June), but almost no significant differences in the rest of the months.

In most of the experimental period, T2 and T3 were not significantly different in mean length and mean weight, though higher performance was recorded in T2. Correspondingly, combined mean for fish weight showed similar trend with mean length. Despite appreciable weight gain recorded across all the treatments, T2 andT3 have shown better mean growth performances, and the difference between their mean growth performances were not significant except for the month of June. The closer results could be due to relatively smaller stocking density, which indicates that smaller differences in stocking densities between the treatment groups may not immediately result into higher significant difference in growth. Different literatures indicated that catfish biomass production varies as the technique goes from simple to more intensive. The biomass rarely exceeds 1.5 tonnes/ha/year or 30 kg/200m^2 pond under extensive poly culture farm with tilapia. However, catfish can grow from 1g to 800g in the temperature range of 26-28℃, better water re-circulation and when fed with 30-35% protein pelletized feed for 8 months (Hecht, 2013). Ayinla (2007) also reported different growth performance ability of catfish under static pond conditions ranging from 15 - 20 tons/ha/cycle and can be improved to 25-40 tons/ha/cycle under flow-through conditions.

Table 2: Summary of fish growth performance (Mean ± SD **)** across the experimental months.

Parameter	Treatment	Experimental months					
		0	1	2	3	4	5
Fish total length (cm)	T1	15	15.87±0.28^{b}	17.73±0.30^{a}	21.21±0.33^{a}	24.31±0.38^{c}	26.84±0.37
	T2	15	16.61±0.32^{ab}	18.48±0.36^{a}	22,33±0.40^{a}	27.20±0.36^{a}	27.38±0.86
	T3	15	17.28±0.33^{a}	18.74±0.38^{a}	22.28±0.43^{a}	25.61±0.40^{b}	27.61±0.45
	p< 0.05		*	ns	ns	*	ns
weight (g)	T1	18	27.03±01.5^{b}	40.19±1.86^{a}	63.09±2.8^{b}	95.59±4.18^{c}	131.27±5.74^{b}
	T2	18	31.60±1.8^{b}	46.17±2.87^{a}	77.22±3.8^{a}	137,93±6.08^{a}	155.49±8.21^{a}
	T3	18	36.86±2.05^{a}	46.16±2.81^{a}	75.11±4.2^{a}	111.05±5.34^{b}	139.11±7.25^{ab}
	p< 0.05		*	ns	*	*	*

To assess the overall experimental results in fish growth performances, data on fish weight gain and length were collected and analyzed. This was assumed to show the trend of fish growth through different months, as different months are characterized by different environmental variables that may directly affect fish growth, feed availability, waste production and the overall physiology of the fish. As shown in Fig 1, and 2, the mean monthly growth in terms of length seems to be the mirror image of the growth in terms of weight gain on the graphs. Fish growth showed progressive performance across all the treatments of the experiment, with T2 showing higher growth, though the difference is still non-significant when compared with T3 in most of the months. Mean fish length was significantly different between T1 and T3 during the first month, but no significant difference was observed in the rest of the months. This will be a good indicator for the possibility of catfish farming at higher stocking density. Moreover, increase in culture period for more months may be required to get more biomass and the best performing stocking density.

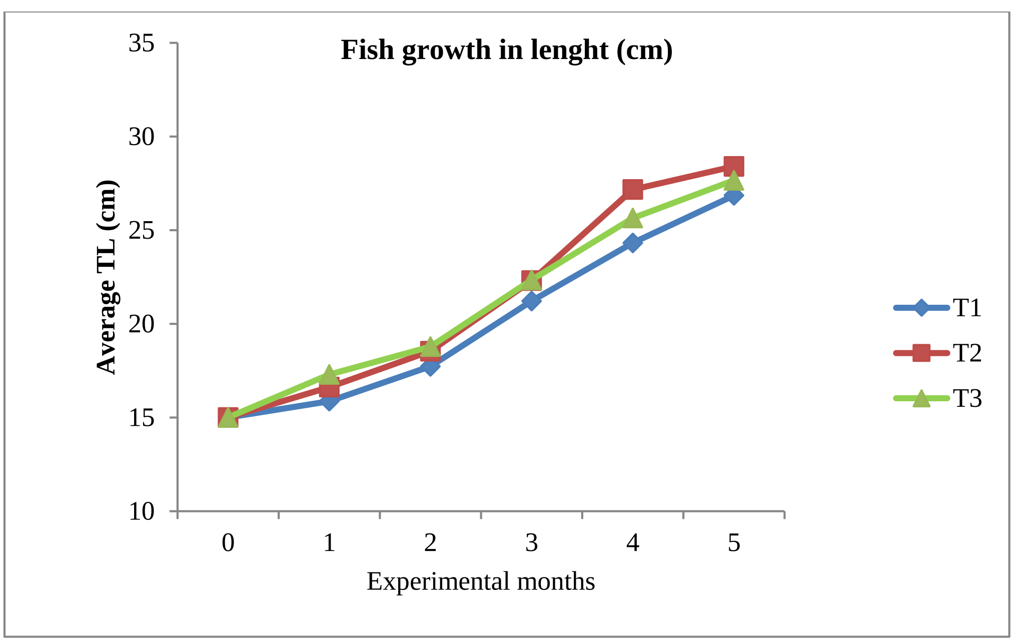

Figure 1. Monthly growth of the fish in length.

Fish growth in weight (g)

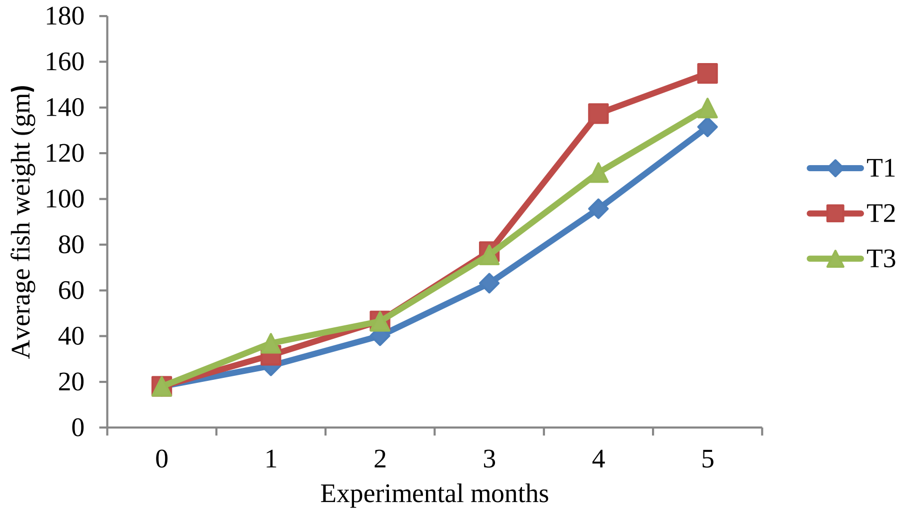

Figure 2. Monthly growth of fish in weight.

Individual fish growth performance is the key parameter to be used to recommend the best fish growth performance biologically. However, individual performance is not the only parameter to recommend aquaculture profitability. Total biomass, total feed and seed cost should be considered in profitability analysis. Although the mean growth parameters values of the three treatments look similar, biomass of the three treatments has observable production difference (Figure 3).

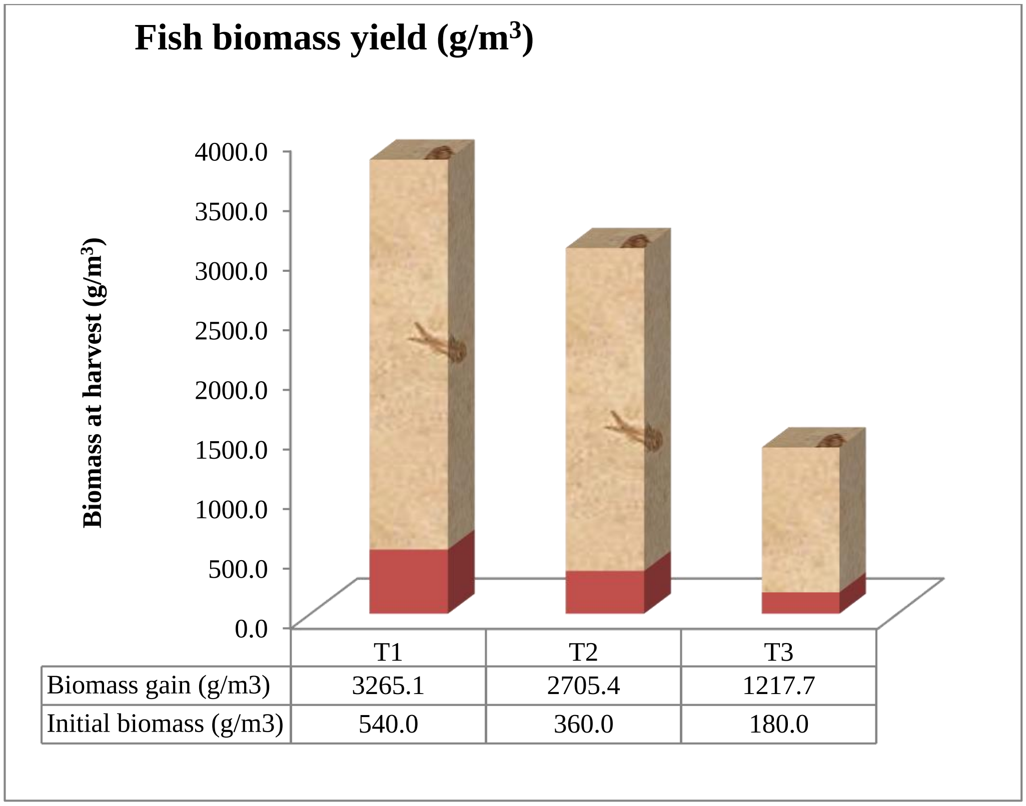

Figure 3. Fish biomass recorded per treatment at the end of the experiment.

The fish in all the treatments were fed pelletized industrial feed of 35% crude protein at a daily feeding rate of 3% body weight. The biomass gain of the fish in the treatments was presented in Figure 3. The fish biomass gains per 1M^3 volume were 3,265.1 g, 2,705.4 g and 1,217.7 g in T1, T2 and T3, respectively, during the 150 experimental days. The highest total biomass per unit volume (3,805.1 g/M^3) and the highest biomass gain (3,265.1 g/M^3/150days) was recorded in T1.

Based on the feed price, the amount delivered to the experimental fish and the weight gain recorded, the production cost was estimated for each of the treatment in the experiment. It was calculated that the cost of feed to produce a kilogram of fish in T1, T2 and T3 were 122.33 Birr, 129.99 Birr and 132.06 Birr respectively. In this particular experiment it was found that the fish production cost per kg of yield is low in the higher stocking density (T1) and higher in the lower stocking density (T3).

Fish feed is the most determinant factor in profitability of aquaculture business and is apparently expensive as compared to the life fish price. From the real market, the current market price of catfish (whole) at landing site is 50.00-70.00 birr/kg which is by far smaller than the production cost calculated in this experiment. Fish is usually collected from wild in the country, with minimal cost that the price did not consider the production cost in Aquaculture. The market price of fish meat looks cheaper than the price of other animals in the local market. Currently, a kilogram of catfish fillet is 150.00 birr whereas a kilogram of cattle meat is 600.00 birr, which means the price of cattle meat is 4 times higher than the price of fish fillet. Despite the price escalation for both fish and meat in Ethiopia, the rate at which the price of meat increases is lower for fish. This can be attributed to the feeding culture of Ethiopians and low preference of Catfish on market when compared with other fish species like tilapia, trout and Nile perch.

On the other hand, the feed cost for the catfish was too high for the reason that high protein pelletized fish feed is available only in a single food processor at local market and it is too expensive (5,000.00 – 7,500.00 birr/kg currently). Therefore, catfish production under intensive farming system with less expensive local feed my be feasible.

Table 3: Fish growth performances under three treatments during 150 experimental days.

Growth parameters	**treatments**		
	T1	**T2**	**T3**
Culture duration (days)	150	150	150
Number of stocked fish	282	190	96
Number of harvested fish	272	188	96
Survival rate (%)	96.45	98.95	100
Initial weight(g)	96.45	18	18
Average final weight (g)	131.50	154.90	139.77
Average final length (cm)	21.2	22.61	22.35
Daily growth rate			
(g/fish/day)	0.76	0.91	0.81
Final biomass (g/m^3)	3,805.1	3,065.4	1,397.7
Net biomass gain (g/m^3)	3,265.1	2,705.4	1,217.7

Conclusion and recommendations

Stocking density affects survival rate, growth rate and final biomass of African catfish in channel tank. Fish survival rate decreased with increasing stocking density, though all the recorded survival rates were high at the evaluated densities. Mean fish length and weight were lower at the highest stocking density of 30 fish/m^3, while differences between the lower stocking densities of 10 fish/m^3 and 20 fish/m^3 were not statistically significant. However, the fish total biomass is higher at the highest stocking density, showing the possibility of even higher stocking densities for the catfish culture. Fish production cost per unit product of the catfish was lower for high stocking density and increased as stocking density decreases. The calculated production cost in all the treatments were higher than the product value; inferring that the production according to the current system is not economically profitable. Pelletized high quality fish feed is also new arrival and too expensive which is the major cause for high production cost. Locally available formulated feeds with more stoking density will perhaps be economically feasible under commercial system and therefore, further studies are required to be conducted using high stocking densities with locally formulated high protein feeds.

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Feed resources and Rangeland Research Results

Registration of '*Morka*' Late Maturing Cowpea *(Vigna unguiculata (L.)* Variety

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Abstract

Morka is a name given for cowpea (Vigna unguiculata (L.) variety with pedigree designation of ILRI-IT83D-442. The variety was developed and released by Bako agricultural research center for production in the low and mid altitude areas of western Oromia and similar agro ecologies in the country. Morka was tested at Bako, Billo and Gute sub sites of Bako Agricultural Research Center during 2019 and 2020 main cropping season along with 10 other genotypes. Morka was selected for its best and stable yield performance and verified at on-station and on farmers' field during 2021. This variety has higher herbage DM and seed yield advantages of 72 and 41.58%, respectively over the standard check 'Adulala'. It was tolerant to ascochayta blight and cercospora leaf spot which were economically important cowpea diseases in the study areas. Therefore, the variety is recommended for the low and midland of Western Oromia and similar agro-ecologies.

Keywords: *Morka,* Cowpea, Yield stability, Resistance, Crude protein, late maturity

Introduction

Cowpea [*Vigna unguiculata* (L.) is one of the widely cultivated multipurpose legume crops, growing in tropical and subtropical regions of Africa, Asia, and Central and South America (Alemu *et al*., 2016). Nutritionally, cowpea plays a crucial role in the lives of millions of people in developing countries, providing a major source of dietary protein complementing low-protein cereal and tuber crops (Timko and Singh, 2008). Its seed and leaves are rich sources of high-quality protein, which provides an excellent supplement to the lower quality feeds (Ndiaye M., 2007). Likewise, cowpea has become a commercial food crop for resource limited small holder farmers and its fodders are very important for animal feeding. Cowpea also offers the alternative of seed production. The seed contains 1.8% fat and 60.3% carbohydrates and a rich source of calcium and iron (Mafakheri K. *et al.,* 2017). In Ethiopia, cowpea is cultivated primarily for its edible seeds, although limited use of its leaf as green vegetable is reported (Alemu *et al*., 2016). However, its productivity among smallholder farmers in the country is limited by several constraints, such as pest and disease incidences, weeds peculiarly striga gesneroides, poor quality seeds, low soil nutrient availability, drought, erratic rainfall, and heat (Beshir *et al.,* 2019).

Cowpea is classified in different maturity groups such as early, medium and late maturing varieties. Despite its importance in providing quality feed, there are very few improved cowpea varieties were released and registered in our country. Therefore, there was a need to evaluate the agronomic and yield potential of late maturating genotypes across various agro-ecological zones to identify the best-bet varieties for efficient utilization. Accordingly, one late maturing cowpea variety, named '*Morka*' has been officially released owing to its better yield potential and quality to address the feed demand of mixed crop-livestock farming systems. On the average, this late cowpea variety needs 80 days to reach 50% of flowering and 120 days to reach seed maturity stage. Therefore, this paper presents the forage yield performance, herbage qualities, agro-ecological adaptation, reaction to major diseases and pests and other morpho-agronomic and management recommendations for '*Morka',* the recently released cowpea variety.

Varietal Origin and Evaluation

Morka is the name given for a released late maturing cowpea variety with the pedigree of 'ILRI IT83D-442'. *Morka* and the other cowpea genotypes were collected from ILRI and evaluated for their growth and agronomic traits in 2017. Therefore, forty nine (49) genotypes from late maturing cowpea type were promoted to the preliminary variety trial during the 2018. Again, the top ten selected genotypes were evaluated at regional variety trial (RVT) stage against standard check (Adulala) over locations and years. For variety verification trial, *Morka* and other two candidate varieties were selected for their best and stable yield performance and verified at on-station and on-farmers' field. The National variety release committee (NVRC) evaluated the varieties under field conditions and *Morka* variety was officially released in 2022.

Varietal Characters and Adaptation

Morka variety is late maturing and of erect growth habit. It takes an average of 80 days to reach 50% flowering and 120 days for its seed to reach maturity. The released variety had an average plant height of 112.4 cm at optimum biomass harvest and 14.3 number of pods per plant and 12 number of seeds per pods (Table 1). It has light brown seed color and is a large seeded variety. On the other hand, it had better crude protein and *IVOMD* and lower fiber fractions than all genotypes that were evaluated in the same environment.

Herbage Dry Matter and seed yields and stability

Combined analysis indicated that forage DM yield varied significantly among the tested cowpea genotypes/varieties (Table 1). *Morka* variety produced the highest herbage DM yield (5.04 t/ha) followed by ILRI-25128 (4.39 t/ha) while the standard check (Adulala) gave the lowest herbage DM yield (2.93 t/ha). *Morka* variety has 72% dry matter yield advantage over standard check (Table 3). Besides, seed yields differed significantly ($p<0.01$) ranging from 10.1 to 15.51 qt/ha. *Morka* variety gave the optimum mean seed yield (14.3 qt/ha) next to genotypes ILRI-25128 (15.51 qt/ha) and ILRI-12691 (14.45qt/ha) while *Adulala* variety gave the lowest grain yield (10.1 qt/ha). The GGE biplot analysis revealed that the released variety, *Morka* (ILRI-IT83D-442) fall in the concentric circle near to average environment axis, suggesting its potential for wider adaptability with better herbage yield performance (Fig 1). Mostly, genotypes that fall in the central (concentric) circle are considered as ideal environments and stable genotypes (Yan *et al*., 2001).

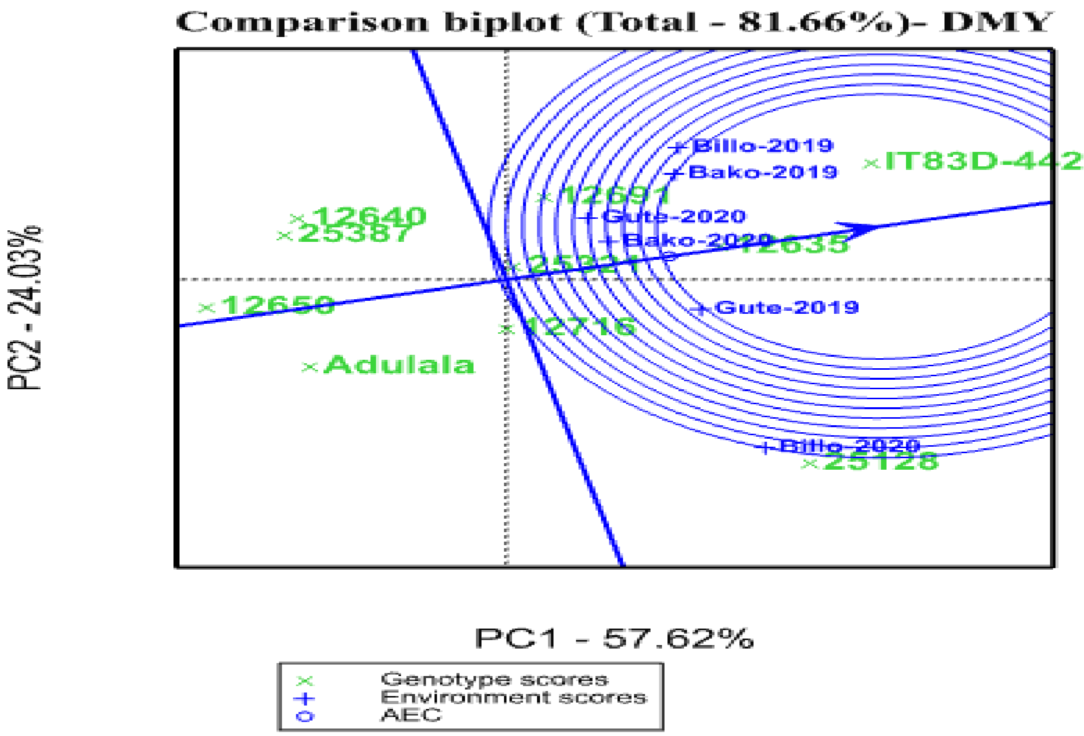

Fig1. GGE bi-plot based on genotype-focused scaling for comparison of genotypes for their yield potential and stability

Quality Parameters

Data on the nutritional content of the varieties/genotypes indicated that *Morka* variety had higher crude protein (CP) and in vitro organic matter digestibility (IVOMD) contents of 23.87% and 61.51%, respectively. The released variety had CP and IVOMD yield advantages of 20.68% and 4.01%, respectively, over the standard check (Table 3). *Morka* variety also contained comparatively lower ash (6.787%) than the other tested genotype/varieties. As regards to fiber contents, the lowest ADF (29.59%) and NDF (49.69%) were registered by *Morka* variety (Table 2).

Reaction to Diseases and Pests

The major cowpea diseases in the study areas were ascochayta blight and cercospora leaf spot. Based on 1-9 scoring scale, *Morka'* scored a mean of 2.11 for ascochayta blight and 1.3 for cercospora leaf spot (Table 1). Therefore, the released variety was regarded as a tolerant variety to these major diseases at all locations.

Table 1: Mean yields, agronomic traits, crude protein, IVOMD and disease reaction of *Morka* variety and other genotypes with their standard check

Genotypes /Varity	Yields and agronomic parameters						Disease score (1-9)	
	HDMY	SY	Leafiness	PPP	SPP	Pht	AB	CLS
ILRI-12650	2.74^{e}	10.34^{c}	8.0^{cde}	12.5	11.3	106.4^{c}	3.67^{a}	1.67
ILRI-12635	4.3^{b}	11.09^{c}	8.4^{c}	14.7	11.6	105.4^{c}	2.67^{abc}	1.83
ILRI-12716	3.65^{c}	10.75^{c}	8.1^{cd}	12.9	12.1	107.1^{c}	3.33^{a}	1.33
ILRI-12691	3.96^{ab}	14.45^{ab}	8.8^{b}	14.3	12.0	114.4^{ab}	2.0^{ab}	1.0
ILRI-25387	3.01^{e}	10.99^{c}	8.1^{cd}	13.2	11.5	107.0^{c}	2.83^{abc}	1.17
*Morka (*ILRI-IT83D-442)	5.04^{a}	14.3^{ab}	9.6^{a}	13.8	11.4	119.3^{a}	2.11^{bc}	1.3
ILRI-25128	4.39^{b}	15.51^{a}	8.2^{cd}	14.1	11.8	110.1^{bc}	1.83^{c}	1.5
ILRI-25321	3.65^{cd}	13.75^{b}	8.0^{cde}	12.9	11.7	109.6^{bc}	3.67^{a}	1.67
ILRI-12640	3.01^{de}	11.03^{c}	7.6^{e}	13.9	12.4	109.5^{bc}	2.67^{abc}	1.83
Adulala	2.93^{e}	10.1^{c}	7.9d^{e}	12.8	11.2	108.8^{bc}	3.0^{ab}	1.67
CV %	0.63	1.73	7.5	19.7	13.8	8.7	22.43	26.68
LSD (0.05)	26.19	21.41	0.4	1.8	1.1	6.3	1.07	0.69
p-value	<0.001	<.0001	<0.001	0.195	0.465	<0.001	0.015	0.2205

Pht=plant height, PPP=pod per plant; SPP= seed per plant; GY= seed yield; HDMY= herbage dry matter yield; LSD= least significant deference, CV=significant variation in %.; AB= Ascochayta blight; CLS= Cercospora leaf spot

Table 2. Mean Chemical compositions of *Morka* variety and other cowpea varieties

Genotypes /Variety	DM	Ash	CP	NDF	ADF	ADL	IVOMD
Morka	90.66	6.787	23.87	49.69	29.59	5.33	61.01
ILRI-12691	90.49	6.923	23.6	50.16	28.89	4.963	61.31
ILRI-25128	90.64	6.89	22.98	55.4	31.83	5.453	61.09
Adulala	90.76	7.18	19.78	54.24	33.99	5.633	59.14

DM = Dry matter, CP = Crude protein, NDF= Natural detergent fiber, Acid detergent fiber, ADL = Acid detergent lignin, IVOMD = in vitro organic matter digestibility.

Table 3: Average forage dry matter yield (t/ha) and seed yield (qt/ha), crude protein and *in vitro organic matter digestible* yields advantage of cowpea varieties over the standard check

Genotypes /Variety	DM yield	% increase	Seed yield	% increase	CP yield	% increase	IVOMD yield	% increase
Morka	5.04	72.01	14.30	41.58	23.87	20.68	61.51	4.01
ILRI-12691	3.96	35.15	14.45	43.07	23.60	19.31	61.31	3.67
ILRI-25128	4.39	49.83	15.51	53.56	22.98	16.18	61.09	3.30
Adulala	2.93	-	10.10	-	19.78	-	59.14	-

DM = Dry matter; CP = Crude protein; and IVOMD = In vitro organic matter digestibility.

Table 4. Agronomic and morphological characteristics of newly released '*Morka'* variety of cowpea

Characteristics	**Description**
Species	*Vigna unguiculata* (L.)
Variety name	*Morka*
Adaptation	Low to midland areas
○ Altitude (m.a.s.l):	1400-2000
○ Rainfall (mm):	600-880
○ Soil type:	Almost all soil type except water logging areas
Seed rate (kg/ha):	36
Spacing (cm)	20 b/n plants and 40 between rows
Planting date:	Late June to early-July
Fertilizer rate: (kg/ha):	P_2O_5: 46; N: 18, at planting
Height at 50 flowering (cm):	119.3
Number of pods per plant	13.8
Number of seeds per pods	11.4
Leafiness (on 1-10 scale)	9.6
Seed color	Red brown
Propagation:	By seed
Growth habit:	Erect
Life span	Annual
Maturity group	Late
Days to 50% flowering	80
Days to seed maturity:	120
Crop pest reaction:	
○ Ascochayta blight	2.11
○ Cercospora leaf spot	1.3
Biomass yield in DM bases (t/ha	5.04
Seed yield (qt/ha:	14.3
Leaf to steam ratio:	1.2
Fodder quality (%):	
○ DM:	90.66
○ CP:	23.87
○ IVOMD:	61.01
○ Ash:	6.787
○ NDF:	49.69
○ ADF:	29.59
○ ADL:	5.33
Special merits:	Wide brunch leaf
Year of release:	2022 G.C.
Breeder/maintainer:	IQQO/Bako ARC

Conclusion

Morka is superior variety compared to the standard check in herbage and seed yields in the multi-location trials across the testing environments with good yield and stability. It has good agronomic traits and better nutritional quality due to higher content of CP and IVOMD and lower ash and fiber parameters (NDF, ADF & ADL). *Morka* variety is tolerant to the common cowpea diseases (Ascochayta blight and Cercospora leaf spot) in the study areas. Therefore, smallholder farmers and other stockholders inhabiting around mid and lowland areas of western Oromia and other areas with similar agro-ecologies can grow *Morka* variety with its full agronomic and other management recommendations. The released variety is maintained at Bako agricultural research center.

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Registration of '*Qophee'* Early Maturing Cowpea *(Vigna unguiculata (L.)* Variety

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Abstract

'Qophee' is a name given for early maturing cowpea (Vigna unguiculata (L.) variety with pedigree designation of 'ILRI-11114'. The variety was developed and released by Bako agricultural research center for production in the low and mid altitude areas of western Oromia and similar agro ecologies in the country. Originally it was obtained from International Livestock Research Institute (ILRI). The trial was conducted at Bako, Billo and Gute sub sites of Bako Agricultural Research Center during 2019 and 2020 main cropping season. The variety was tested along with 10 other genotypes. Qophee was selected for its best and stable yield performance and verified at on-station and on farmers' field during 2021. This variety has higher herbage DM and seed yield advantages of 36.79 and 45.61%, respectively over the standard check 'Adulala'. It was tolerant to ascochayta blight and cercospora leaf spot which were economically important cowpea diseases in the study areas. Therefore, 'Qophee' is recommended as an early maturing variety for the low and midland of Western Oromia and other similar agro-ecologies in the country.

Keywords: Crude protein, Disease Resistance, Early maturity, Qophee, Vigna unguiculata, Yield stability

Introduction

Cowpea (*Vigna unguiculata* (L.) is an important tropical and subtropical leguminous crop, grown for seed, vegetable, green manure, fodder, medicinal purposes and as cover crop to minimize water losses and maintain soil fertility in rain-fed conditions (Akbar *et al*., 2010). It is an indigenous crop to sub-Saharan Africa and the entire plant serves for either human consumption or animal feed (Boukar *et al.*, 2011). Its seed and leaves are rich sources of high-quality protein (23-35 and 27-34%, respectively, which provides an excellent supplement to the lower quality cereal or root and tuber protein consumed (Ndiaye M., 2007). Moreover to its role as food, cowpea has become a commercial crop for resource limited small holder farmers and its fodders are very important for animal feeding. The seed also contains 1.8% fat and 60.3% carbohydrates and a rich source of calcium and iron (Mafakheri K. *et al.*, 2017). The dry haulms of cowpea are used as fodder for livestock particularly during the dry season when animal feed is scarce. However, cowpea production is constrained by several factors, some are biotic such as insects, diseases and pest weeds peculiarly Striga gesneroides while others are abiotic as terminal drought, erratic rainfall, soil salinity and heat (Sobda Gonné, 2013). These climate variability, terminal drought which usually occurs during flowering and pod filling stage of the crop could cause as high as 80% yield loss (Agbicodo *et al.*, 2009).

Early maturing varieties are considered climate smart cultivars since they have the ability to escape terminal drought as well as pests and diseases damage that normally occur later in the cropping season. The concept of early maturity in cowpea is a combination of early flower initiation and short seed filling period (Kauret *et al*., 2009). Early maturity constitutes an important adaptation in agro-ecological zones with short growing seasons particularly in the arid and semiarid tropics (Abadassi J., 2015, Adeyanju *et* *al.*, 2007). They provide first food sooner than any other crop, thus shortening the hunger period (Ayo-Vaughan *et al*., 2011). Early maturing varieties provide additional seed for the main season cropping and are also suitable for rotation with cereals (Pswarayi *et al.*, 2007). In a crop rotation program, it can significantly improve soil nitrogen levels by nitrogen fixation or by incorporation in soil as a green manure crop. However, there are very few improved cowpea varieties were released and registered in our country. Hence, there was a need to evaluate the adaptability and yield potential of early maturating varieties across various agro-ecological zones to identify the best-bet varieties for efficient utilization. Accordingly, one early maturing cowpea variety, named '*Qophee'* has been officially released owing to its better yield potential and quality to address the feed demand in the study areas. Therefore, this paper presents the forage yield performance, herbage qualities, agro-ecological adaptation, reaction to major diseases and pests, and other agro-morphological traits and management recommendations for the recently released cowpea variety '*Qophee'*.

Varietal Origin and Evaluation

Qophee (ILRI-11114) is an early maturing cowpea variety developed and released by Bako Agricultural Research Center for low and midland areas of western Oromia and other areas with similar Agro ecologies in the county. Originally, *Qophee* and other 87 early maturing cowpea genotypes were obtained from ILRI and evaluated for their growth characteristics, agronomic traits, persistence, and resistance to pests and diseases at observation nursery in 2017. Then, 36 promising genotypes were promoted to the preliminary variety yield trial during the 2018 cropping season. Here, the genotypes were evaluated for their herbage yield, seed yield and reaction to diseases. Based on the results, 10 superior genotypes were selected for further multi-location trial at regional variety trial (RVT). These genotypes were evaluated against standard check Adulala across three locations (Bako, Billo and Gute) for two years (2019-2020). Three promising genotypes and standard check were planted in 2021 on 10 x 10 m^2 plots at nine locations for variety verification trial. The National Variety Release Committee evaluated the varieties under field conditions. Finally *Qophee* confirmed to be a superior early maturing cowpea variety for low and midland areas of western Oromia and other areas with similar agro ecologies in the country in 2022.

Varietal Characters and Adaptation

The released varieties, Qophee, is characterized by growth habit of semi erect with bush and branched leaves. Seed color is red brown and on the average it needs 60 days to reach 50% flowering and 90 days to reach for seed maturity stage. *Qophee* variety had a plant height of 111.44 cm at optimum biomass harvest and had 18.61 and 11.96 number of pods per plant and number of seeds per pods, respectively. (Table 1).

Herbage Dry Matter and Seed Yields and Stability

The released variety *Qophee* produced high mean herbage dry matter yield of 4.35 t/ha with 36.79% yield advantages over the standard check which produced 3.18 t/ha. The mean seed yield was higher (15.66 qt/ha) for genotype 82D-504-4 followed by *Qophee* (15.26 qt/ha) and ILRI-11990 (14.49 qt/ha) having the seed yield advantages of 49.43, 45.61 and 38.26%, respectively over the check which produced 10.48 qt/ha. (Table 3). Based on the criteria of the Eberhart and Russell (1966) regression model, *Qophee* variety can be considered as more stable than other tested genotypes. The GGE biplot analysis revealed that the released variety, *Qophee*, fall into the concentric circle near to the average environment axis, indicating that it is ideal genotype in terms of higher yielding ability and stability. Genotypes that fall in the central circle are considered as ideal for the environments and is a stable genotypes (Yan *et al*., 2001)

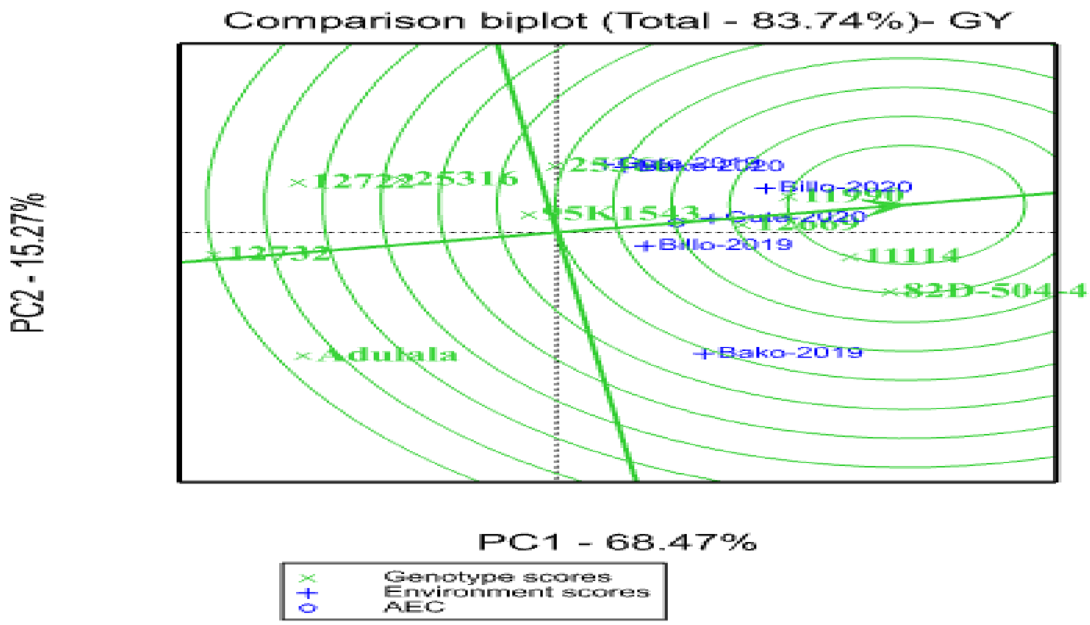

Fig1. GGE bi-plot based on genotype-focused scaling for comparison of genotypes for their yield potential and stability

Quality Parameters

Data on the nutritional content of the varieties also indicated that the crude protein content was higher for *Qophee* variety with mean values of 26.44% followed by ILRI-11990 (25.07%). The IVOMD was higher (61.73 %) for *Qophee* variety followed by ILRI-11990 and ILRI-82D-504-4 with mean values of 61.61% and 60.21%, respectively. As regards to fiber content, the lowest values of 28.44 and 47.16 %, were registered by *Qophee* variety for ADF and NDF, respectively, (followed by ILRI-11990 (28.45 and 47.8%), in that order (Table 2). Generally, *Qophee* variety gave the highest CP yield and IVOMD yield advantages, 27.48 and 3.2, respectively, over the standard check (Table 3).

Reaction to Diseases and Pests

The major cowpea diseases in the study areas were Ascochayta blight and Cercospora leaf spot. On 1-9 rating scale, *Qophee* scored a mean of 2.27 and 1.27 for Ascochayta blight and Cercospora leaf spot, respectively, indicating that this new cowpea variety is tolerant to these diseases. The disease score results for the varieties and the checks are summarized in Table 1.

Table 1: Mean yields, agronomic traits and disease reaction of *Qophee* variety and other genotypes

Genotypes/Varity	**Yields and agronomic Parameters**						**Disease score (1-9)**	
	HDMY	**SY**	**Leafiness**	**PPP**	**SPP**	**Pht**	**AB**	**CLS**
Qophee (ILRI-11114)	4.35^{a}	15.26^{a}	9.06^{a}	18.61^{a}	11.96	111.44	2.27^{c}	1.27
ILRI-11990	3.58^{bc}	14.49^{ab}	8.44^{b}	17.06^{b}	11.90	106.94	2.28^{c}	1.33
ILRI-12669	3.52^{bcd}	14.03^{ab}	8.28^{b}	15.91^{bcd}	11.80	102.11	2.83^{abc}	1.33
ILRI-12722	3.33^{bcd}	11.35^{cde}	8.11^{bcd}	15.71^{bcd}	11.80	107.17	2.67^{abc}	1.28
ILRI-12732	2.97^{de}	10.0^{e}	7.78^{de}	16.06^{bcd}	11.38	106.39	2.5^{bc}	1.17
ILRI-25316	2.72^{e}	12.02^{cd}	8.00^{cde}	16.47^{bc}	11.28	106.44	3.17^{ab}	1.38
ILRI-25366	3.85^{ab}	12.79^{bc}	8.33^{bc}	15.88^{bcd}	12.16	111.39	2.0^{c}	1.32
ILRI-82D-504-4	3.68^{bc}	15.66^{a}	8.83^{a}	16.77^{b}	12.48	112.28	2.17^{c}	1.37
ILRI-95K1543	3.44^{bcd}	12.88^{ab}	7.89^{de}	15.22^{cd}	12.23	111.02	2.33^{bc}	1.53
Adulala	3.18^{cde}	10.48^{de}	7.67^{e}	14.58^{d}	12.58	103.50	3.5^{a}	1.28
LSD (0.05)	0.56	1.81	0.349	1.5	1.0	9.276	0.94	0.69
CV %	24.71	21.25	6.4	14.4	12.3	13.1	21.59	26.68
p-value	<0.001	<0.001	<0.001	< 0.001	0.147	0.343	0.0411	0.4742

HDMY= herbage dry matter yield; GY= seed yield; Pht=plant height, PPP=pod per plant; SPP= seed per plant; LSD= least significant deference, CV=significant variation in %. CP= Crud protein; IVOMD= in vitro organic matter digestibility; AB= Ascochayta blight; CLS= Cercospora leaf spot

Table 2. Mean Chemical compositions of *Qophee* variety and other cowpea genotypes

Genotype/Variety	**DM**	**Ash**	**CP**	**NDF**	**ADF**	**ADL**	**IVOMD**
*Qophee (*ILRI-*11114)*	90.71	5.97	26.44	47.16	28.44	4.61	61.73
ILRI-11990	90.48	6.253	25.07	47.8	28.45	5.05	61.61
ILRI-82D-504-4	90.85	5.967	21.24	51.92	30.98	5.19	60.21
Adulala	90.74	7.39	20.74	51.57	30.75	5.49	59.82

DM = Dry matter, CP = Crude protein, NDF= Natural detergent fiber, Acid detergent fiber, ADL = Acid detergent lignin, IVOMD = in vitro organic matter digestibility

Table 3: Average forage dry matter yield (t/ha) and seed yield (qt/ha), crude protein and *in vitro* organic matter digestible yields advantages of cowpea varieties over the standard check

Genotype/Variety	DM yield	% increase	Seed yield	% increase	CP yield	% increase	IVOMD yield	% increase
Qophee	4.35	36.79	15.26	45.61	26.44	27.48	61.73	3.19
ILRI-11990	3.58	12.58	14.49	38.26	25.07	20.88	61.61	2.99
ILRI-82D-504-4	3.68	15.72	15.66	49.43	21.24	2.41	60.21	0.65
Adulala	3.18	-	10.48	-	20.74	-	59.82	-

DM = Dry matter; CP = Crude protein; and IVOMD = In vitro organic matter digestibility.

Table 4. Agronomic and morphological characteristics of *Qophee* variety as compare to the standard check (Adulala)

Characteristics	Description
Species	*Vigna unguiculata* (L.)
Variety name	Qophee
Adaptation	Low to midland areas
o Altitude (m.a.s.l):	1400-2000
o Rainfall (mm):	600-880
o Soil type:	Almost all soil type except water logging areas
Seed rate (kg/ha):	36
Spacing (cm)	20 b/n plants and 40 between rows
Planting date:	Late June to early-July
Fertilizer rate: (kg/ha):	P_2O_5: 46; N: 18, at planting
Height at 50 flowering (cm):	111.44
Number of pods per plant	18.61
Number of seeds per pods	11.96
Leafiness (on 1-10 scale)	9.06
Seed color	Red brown
Propagation:	By seed
Growth habit:	Semi erect
Life span	Annual
Maturity group	Early
Days to 50% flowering	60
Days to seed maturity:	90
Crop pest reaction:	
o Ascochayta blight	2.27
o Cercospora leaf spot	1.27
Biomass yield in DM bases (t/ha	4.35
Seed yield (qt/ha:	15.26
Leaf to steam ratio:	1.4
Fodder quality (%):	
o DM:	90.71
o CP:	26.44
o IVOMD:	61.73
o Ash:	5.97
o NDF:	47.16
o ADF:	28.44
o ADL:	4.61
Special merits:	Wide brunch leaf
Year of release:	2022 G.C.
Breeder/maintainer:	IQQO/Bako ARC

Conclusion

Based on the results obtained, better herbage DM yield was recorded for the newly released '*Qophee'* variety. The study also indicated that, *Qophee* variety had a better nutritional quality due to the highest contents of CP and *IVOMD* and lowest fiber contents. The stability analysis indicated that *Qophee* variety is more stable when compared to other varieties evaluated in the study. So, *Qophee* variety is ideal forage variety in terms of high yielding ability and stability as compared to other tested genotypes. Therefore, *Qophee* variety was released and registered in 2022 for production in the mid and low altitude areas and similar agro ecologies in the country. The released variety is maintained at Bako agricultural research center.

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Effects of Combined Application of Biochar and Inorganic Fertilizers on Yield and Nutritive Value of Chomo Grass (*Brachiaria humidicola*) in Western Oromia, Ethiopia

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Abstract

The study was carried out to evaluate the growth performance, yield and chemical composition of chomo grass (Brachiaria humidicola) by application of different levels of coffee husk biochar and inorganic fertilizers (NPS and urea). The experimental design was a Randomized Complete Block Design (RCBD) with three replications. The treatments were: T1= Zero fertilizer, T2= 100% Biochar (5 t/ha), T3= 75% Biochar (3.75 t/ha) and 25% inorganic fertilizer (37.5 kg/ha), T4=50% Biochar (2.5 t/ha), 50% inorganic fertilizer (75 kg/ha), T5=25% Biochar (1.25 t/ha) and 75% inorganic fertilizer (112.5 kg/ha), T6=100% inorganic fertilizer (150 kg/ha)for chomo grass. The result revealed that the combined application of biochar and inorganic fertilizer significantly affected (P<0.05) phonological variables, growth parameters except plant height, dry matter and seed yield, and chemical composition of the grass. Delayed days to 50% flowering (126.3 days) and days to maturity (163 days) were obtained due to the application of 100% of biochar and 75% biochar with 25% inorganic fertilizer. The highest number of leaves per plant and leaf to stem ratio was recorded for T6 and T2 while the lowest was from T1 and T3. The study results indicated that dry matter, seed yield, crude protein and fiber contents of chomo grass can be improved by the combined application of biochar and inorganic fertilizer. The partial budget analysis also affirmed the combined application of 50% biochar with 50% recommended inorganic fertilizer (T4) gave a higher net benefit. Therefore, it is advised to use a combination of 50% biochar with 50% inorganic fertilizer (T4) to enhance the dry matter yield and nutritive value of chomo grass in the study area.

Key words: Biochar, chemical composition, Chomo grass, dry matter yield, fertilizer, seed yield

Introduction

The forage productivity is low in quality and quantity due to the decline of soil fertility, rainfall variability, poor agronomic practice, and poor accessibility of quality seed. Productivity can mainly be improved through the use of improved forages, the application of fertilizers, and other good agronomic practices. Many grass species like Chomo grass have been introduced in Ethiopia. Chomo grass is one possible perennial improved grass that can be grown on farms and used by small-holder farmers. Chomo grass grows better in areas where a wide range of soil types from very acid-infertile (P^H 3.5) and annual rainfall is between 600 -2800 mm and altitude ranges between 1000 m and 2400 m (Abera and Alan, 2013). The productivity of Chomo grass in the ranges of 7-34 t/ha/year depending on soil fertility and its nutritive value ranges between 5-17% CP when compared with natural pasture. Currently, farmers used Chomo grass for degraded land rehabilitation, sources of income in addition to forage use (Geleto and Tulu, 2020).

Fertilizers are normally used to increase forage yield and quality, but since plant tissue reflects the mineral constituents of the soil in which the plants are grown, quality is also greatly influenced (Miles and Manson, 2000). Inorganic fertilizer application is essential for plant growth and productivity of forage grass. However, the increased cost of inorganic fertilizer and application of recommended doses is difficult to be afforded by farmers. Hence, an easily accessible and low cost organic source of plant nutrients for supplementing and enhancing chemical fertilizer should be substituted. In this context, integrated nutrient management would be an available strategy for the effective use of inorganic fertilizer with the addition of organic soil amendment. Biochar is a carbon-rich solid material that is produced during pyrolysis. Pyrolysis is a thermos chemical process where biomass is heated in the absence of oxygen (Yaman, 2004). The application of biochar to soil causes numerous soil changes, ranging from chemical, physical, and biological effects (Alemayehu *et al.,* 2020). Biochar is a recently well-known organic component of an integrated nutrient supply system, which improves soil health, increases productivity, and releases some amount of macro and micronutrients (Xu *et al.,* 2015). The ability to retain nitrogen and prevent its leaching can increase nutrient use efficiency, thereby maintaining crop yield under small nitrogen applications (Zhang *et al.,* 2015). However, the physical, chemical, and nutritional properties and thus the quality of biochar depends on the chemical composition of the feedstock used, the pyrolysis system, and production conditions, including temperature and residence time (Lie *et al.,* 2017). Soil study in the western area indicated that decline of total nitrogen which is below the critical level due to nitrogen leaching problems as the area received high rainfall and farmers have a limited cultural practice to integrate chemical fertilizer with organic amendments for enhanced forage production around study area condition is lacking. So far biochar has been applied to amend soil for crop production and no information is available as to its application for grass production mainly around the study area. Hence, the objective of this study was to evaluate the effect of application of different levels of biochar and inorganic fertilizer on the growth performance, productivity and chemical composition of chomo grass.

Materials and Methods

Description of the study site

The experiment was conducted at on station site of Haro sabu Agricultural Research Center in Kellem Wollega and Nedjo in West Wollega during the year 2019 – 2021 main cropping seasons. Haro sabu is located at $8^{o}9$'N latitude, $35^{o}23$'E longitude and has an altitude of 1515 m a.s.l. It has a warm humid climate with an average annual minimum and maximum temperatures of $14^{o}C$ and $30^{o}C$, respectively. The area receives an average annual rainfall of 1000 mm. Nedjo is located at $9^{o}30$'N latitude and $35^{o}30$'E longitude with an altitude of 1821 m a.s.l. The means of annual minimum and maximum temperatures were $12^{o}C$ and $26^{o}C$, respectively. The area receives an average annual rainfall of 1300 mm. Both the test locations have uni-modal rainfall distributions and represent the midland area.

Experimental biochar preparation

The coffee husk used as a feedstock was taken from a nearby coffee processing enterprise located in the study area. After separating impurities it was dried in the sun until the moisture content of about 15%, and then taken to the pyrolysing place. The prepared coffee husks were processed by the process of pyrolysis with a temperature of $350^{0}c$ (approximately) for 2 hours in a dug hole. After it was pyrolysed, it was watered to cool down; the biochar was taken and spread out in the sun (air-dried). Finally, the biochar was collected and made ready till it was used.

Planting material, experimental design and treatments

The planting material was Chomo grass (*Brachiaria humidicola*). A Randomized Complete Block Design (RCBD) was employed with three replications consisting of six fertilizer levels. The total area of land 25 m x 15 m was selected and cleared by removing all unwanted materials before ploughing. Then, the selected land was ploughed to make a fine field and fallowed by harrowing using hoe and rack to break down the clods. The experimental field was divided into 3 blocks with 18 plots. Each plot size was 4 m x 3 m and consisted of six rows with 1m between rows. The seed rate of 6 kg/ha was used. The spacing between plots and blocks was 1 m and 1.5 m, respectively. Treatments were assigned to each plot within a block by SAS generated randomization code. The assigned plots were top-dressed using biochar at the rate of 5 t/ha for 15 days prior to planting chomo grass. The application was done in the assigned plots by incorporating coffee husk biochar into the top 15 cm of the soil with the aid of a hoe. The rate of application was made following the recommendation suggested by Dennis and Kou (2013).

Table .1: Treatment arrangements for the six fertilizer levels

Treatments	**Level of fertilizer**
T1	Zero fertilizer application (control)
T2	Biochar alone (100%) = 5 t/ha
T3	75% Biochar (3.75 t/ha) and 25% inorganic fertilizer (25 kg/ha NPS and 12.5 kg/ha urea)
T4	50% Biochar (2.5 t/ha) and 50% inorganic fertilizer (50 kg/ha NPS and 25 kg/ha urea)
T5	25% Biochar (1.25 t/ha) and 75% inorganic fertilizer (75 kg/ha NPS and 37.5 kg/ha urea)
T6	Inorganic fertilizer alone (100%)= 100 kg/ha NPS and 50 kg/ha urea)

The assigned plots were fertilized with NPS at a rate of 100 kg/ha at the time of planting and Urea was applied at the rate of 50 kg/ha after establishment. Weeds were controlled through a manual and slashing inters row spaces to reduce weed competition within the replications and pest inspection was carried out every day during the whole trial period.

Data collected

Phonological and agronomic parameters

Days to 50 % flowering for chomo grass was determined by recording the number of days after planting when half of the plants were flowering. Also, days to maturity were determined by recording the number of days from planting to the time when the plant seed get matured by continuous visual observation (Akililu and Alemayehu, 2007). Growth parameters like plant height and leaves number per plant were recorded at the forage harvesting stage from five sample plants selected randomly from each plot area, and tagged using thread on the plant. Plant height (cm) was determined using a steel ruler and measuring the vertical from the ground to the last leaf (flag leaf) of the main shoot. The number of leaves per plant was determined by counting the total number of leaves from the main five randomly selected plants in each plot and the average of five plants was taken for each plot.

Biomass and seed yield

The biomass yield of forages per plot was evaluated at 50 % flowering based on continuous visual observation. Samples were collected from two inner rows of each plot and replication and harvested at stubble 5 cm height of cutting. The harvested green forage was weighed plot wise using a sensitive field balance. The fresh sub sample was measured from the inner rows of each plot, weighed and chopped into small pieces (2-3 cm), labeled and kept in separate perforated bags. 300 gm fresh weight of subsamples was taken from each plot and dried in an oven at 65 °C for 72 hours to constant weight. The dry weight of sub sample was reweighed to have an estimate of dry matter production as per the formula suggested by Tarawal (1995). Ripen seeds of Chomo grass, along with the inflorescence was mowed at the right stage of seed development and sweating immediately after harvest and left under a shed to assist the final maturation. Thereafter, the seeds were gently trashed to separate the seed from the sheaves, cleaned and weighed using a sensitive balance to determine seed yield.

Laboratory analysis

From each plot, subsamples of grass were taken and dried in a forced draft oven at 60°C for72 hours and then, ground using a Wiley mill to pass through a 1mm sieve screen for chemical analysis. The AOAC (1990) procedure was used for the determination of DM, Ash and CP. The DM content was determined by oven drying at 105°C for 24 hours. The ash component was determined by igniting the dried sample in a muffle furnace at $600^{O°}$C overnight. The nitrogen is determined using the micro-Kjeldahl technique. The CP was calculated as 6.25 x N. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the Van Seost method (1991).

Partial budget analysis

A partial budget analysis of dry matter yield for the selection of the economically feasible and profitable levels of biochar applied to the soil in combination with the inorganic fertilizer rate was done according to the CIMMYT procedure (CIMMYT, 1988). To estimate economic parameters DM yield was valued at an average open market price of 2.52 Ethiopian birr kg^{-1} and the cost of NPS and urea fertilizers were 16.25 and 15.60 ETB/kg, respectively. The costs of organic fertilizer preparation and application were estimated. The potential responses of the grass toward the added fertilizers ultimately determine the economic feasibility of fertilizer application (CIMMYT, 1988).

Data Analysis

The data were analyzed by using GLM (ANOVA) with SAS software (SAS, 2009 version 9.3). Significantly different means were separated and compared using Least Significant Difference (LSD) test at 5% significance level. The model used for data analysis was:

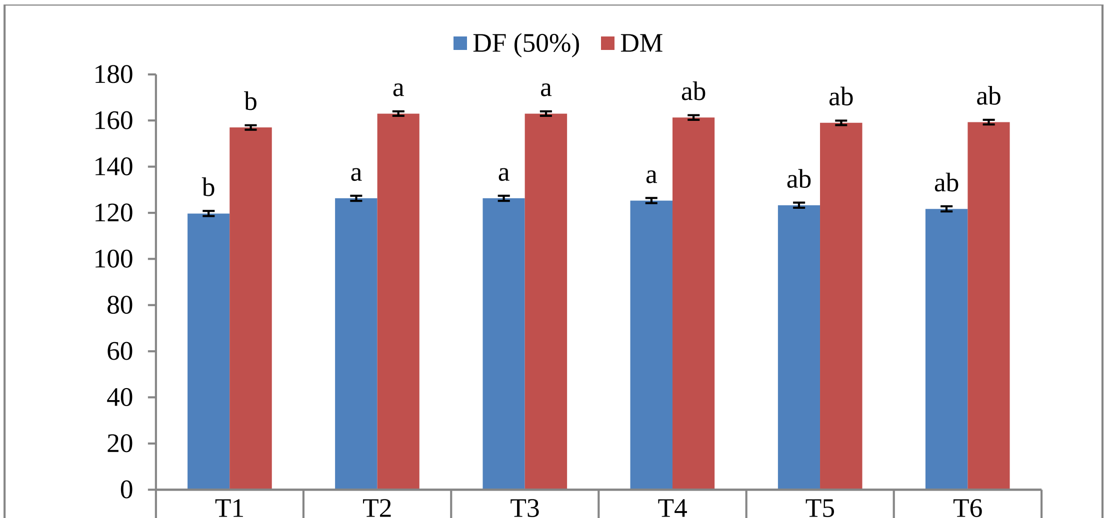
$$\mathbf{Y_{ij} = \mu + B_i + T_i + e_{ij}}$$

Where; Y_{ij} = Response variable; μ = Overall mean*;* T_i = Treatment effect; B_i = Block effect*;* e_{ij} = Random error.

Results and Discussion

Phonological parameters of chomo grass

Analysis of variance showed a combination of biochar and inorganic fertilizers significantly (P<0.05) affected days to 50% flowering and days to maturity of chomo grass (Figure1). Delayed days to flowering (126.3) and maturity (163) were observed for biochar alone (T2) and 75% biochar with 25% inorganic fertilizer application (T3) while the earliest (119.7 and 157) was observed in the control treatment (T1). This could be due to the application of biochar with inorganic fertilizer promoting vegetative growth, as it retains and contains a high amount of nutrients especially nitrogen for fast vegetative growth and longer photosynthetic apparatus by delayed flowering (Zheng *et al.,* 2013). Similarly, Zelalem *et al.* (2009) reported that increasing nitrogen and phosphorus fertilization levels significantly delayed the day required for 50% flowering. Delayed days to maturity results indicated that the days to physiological maturity were prolonged in response to the increased level of biochar and inorganic fertilizer.



	T1	T2	T3	T4	T5	T6
DF (50%)	119.7	126.3	126.3	125.3	123.3	121.7
DM	157	163	163	161.3	159	159.3

T1= Zero fertilizer, T2= 100% Biochar (5 t/ha), T3= 75% Biochar (3.75 t/ha) and 25% inorganic fertilizer (37.5 kg/ha), T4=50% Biochar (2.5 t/ha), 50% inorganic fertilizer (75 kg/ha), T5=25% Biochar (1.25 t/ha) and 75% inorganic fertilizer (112.5 kg/ha), T6=100% inorganic fertilizer (150 kg/ha).

Fig.1. Days to 50% flowering and maturity of chomo grass at different level of biochar andinorganic fertilizer application

Agronomic parameters of Chomo grass

Plant height

The result showed that plant height was not significantly (P>0.05) affected by biochar and inorganic fertilizer applications (Table 2). The mean plant height of chomo grass in different levels of fertilizers application was 89.58 cm at Haro sabu and 81.14 cm at Nedjo sites. The overall mean plant height (85.36 cm) of chomo grass with the higher plant height (89.06 cm) was recorded at 75% biochar with 25%

inorganic fertilizer application (T3) while the lower (81.76 cm) was recorded at 25% biochar with 75% inorganic fertilizer (T5) numerically. This difference was due to the application of biochar and inorganic fertilizer that incites the plant cell division and increased the plant height. This result contrary to the findings of Tadesse *et al*. (2022) reported that the application of biochar with inorganic fertilizer at different levels significantly affected the plant height of *Chloris gayana* and *Panicum coloratum*in South-western Ethiopia.

Number of leaves per plant

The number of leaves per plant was significantly varied at Haro sabu between treatments due to the combined application of biochar and inorganic fertilizer though not significant at the Nedjo site. At Haro sabu site, the highest number of leaves per plant was recorded for T6 (4.4) followed by T3 (4.2), and the lowest was recorded for the control treatment (3.4). While at the Nedjo site, the higher leaves number per plant (4.67) was recorded for T5, T2 and T6, and the lower leaf number was recorded for T1 (3.93). The number of leaves per plant of chomo grass across sites showed significant differences among treatments (Table 2). The highest number of leaves per plant (4.4) was recorded at 25% biochar with 75% inorganic fertilizer application (T5) which was at par with other treatments except for the control treatment (T1) while the lowest (3.67) was recorded in neither biochar nor inorganic fertilizer application (T1). Combined application of inorganic fertilizer with biochar has improved the number of leaves per plant as compared to the control treatment. This result is in agreement with Saarnio *et al.* (2013) who reported that biochar application integrated with fertilizer helps to increase plant leaf number. Xu *et al*. (2015) also reported that there was a significant improvement of leaf photosynthesis and capacity on biochar amended soils, which they attributed to increased leaf number and soil available nitrogen. Koul (1997) reported that the level of fertilizer increased the number of leaves per plant increased due to the ability of fertilizer that promoted leaf yield via cell division and elongation. The number of leaves per plant is an excellent indicator of herbage yield and nutritional quality and a higher amount of leaves has a higher content of protein and digestible dry matter (Miller, 1994).

Table 2: Mean plant height, number of leaf per plant and leaf to stem ratio at harvest of chomo grass tested at Haro sabu and Nedjo site

Treatments	PH (cm)			NLPP			LSR		
	Haro sabu	Nedjo	Mean	Haro sabu	Nedjo	Mean	Haro sabu	Nedjo	Mean
T1	88.53	84.86	86.70	3.40^{b}	3.93	3.67^{b}	0.43^{b}	0.49^{ab}	0.46^{ab}
T2	88.53	81.67	85.10	4.13^{ab}	4.67	4.30^{a}	0.50^{ab}	0.51^{a}	0.51^{a}
T3	94.60	83.53	89.06	4.20^{a}	4.20	4.20^{a}	0.42^{b}	0.38^{b}	0.41^{b}
T4	88.4	80.60	84.50	3.80^{ab}	4.33	4.07^{ab}	0.49^{ab}	0.47^{ab}	0.48^{a}
T5	86.33	77.2	81.76	4.13^{ab}	4.67	4.40^{a}	0.47^{ab}	0.40^{ab}	0.43^{ab}
T6	91.13	79	85.06	4.40^{a}	4.67	4.43^{a}	0.54^{a}	0.47^{ab}	0.51^{a}
Mean	89.58	81.14	85.36	4.01	4.34	4.17	0.47	0.45	0.46
CV (%)	8.84	7.48	8.05	10.52	10.41	9.74	11.49	15.14	13.55
$LSD_{(5\%)}$	ns	ns	ns	0.76	ns	0.48	0.10	0.12	0.075

$^{a-b}$ means with different letters in a column are significantly different (P<0.05).

PH=plant height; NLPP=number of leaves per plant; LSR=leaf to stem ratio; CV= coefficient of variance; LSD= least significance difference; cm= centimeter; ns= non-significant.

Leaf to stem ratio

Combined application of different levels of biochar with inorganic fertilizer showed significant differences ($P<0.05$) in the Leaf to Stem ratio of chomo grass at the forage harvesting stage (Table 2). The highest LSR (0.54 and 0.51) was recorded in sole inorganic fertilizer application (T6) and biochar alone (T2) at Haro sabu and Nedjo site, respectively. While the lowest (0.42 and 0.38) was recorded in 75% biochar with 25% inorganic fertilizer application (T3) at both sites. From the overall mean of LSR of chomo grass, the highest LSR (0.51) was recorded at sole inorganic fertilizer application (T6) and biochar alone (T2) while the lowest (0.41) was recorded in75% biochar with 25% inorganic fertilizer application (T3). Higher leaves to stem ratio is generally an indication of the better nutritional value of grass. This is might be due to the slow release of nutrients from biochar that helps to enhance the increment of leaf than stem. The current result agreed with Xu *et al.* (2015) who reported that there was a significant improvement in leaf photosynthesis and capacity on biochar-amended soils, which they attributed to increased leaf number.

Dry matter and seed yields of Chomo grass

The analysis of variance showed that dry matter yield of chomo grass was significantly ($P<0.05$) affected by application of biochar and inorganic fertilizer as well as at each testing site (Table 3). The highest DM yield (5.34 t/ha) was obtained from T3 and followed by T6 (5.26 t/ha), T4 (5.21t/ha) at Haro sabu, while T5 (4.02 t/ha) gave highest dry matter yield and followed by T4 (3.64 t/ha) at Nedjo site. The lowest yield (3.82 and 2.79 t/ha) was recorded in control treatment (T1) at Haro sabu and Nedjo site, respectively. Integrated application of biochar with inorganic fertilizer enhanced DM yield of chomo grass and this might be attributed to improved soil fertility, nutrient availability and water retention as a result of biochar characteristics added to the soil in addition to inorganic fertilizer. This result is in line with earlier reports (Saarnio *et al.,* 2013 and Patil *et al.,* 2016) who reported that the highest above ground biomass was in the treatment with fertilizer addition and highest level of biochar application to the non-fertilized and non-biochar amended soil. Tufa *et al.* (2022) noted that above ground biomass of maize increased due to combined application of biochar with NPS fertilizer at the western Ethiopia. They indicated that fresh and dry biomass yield increased with increasing fertilizer levels due to nitrogen and other macro elements which played an important role in plant growth and physiological processes and enhance vegetative growth, consequently increase plant weight by producing more dry matter.

The overall mean DM yield was 4.08 t/ha which ranged from 3.30 t/ha to 4.43 t/ha. Integrated application of 50% biochar and 50% inorganic fertilizer rates (2.5 t/ha x 50 kg/ha NPS + 25 kg urea) (T4) resulted in the highest DM yield, which was at par with the dry matter yield harvested from other treatments except the control treatment (T1). However, the grass planted neither biochar nor inorganic fertilizer (T1) gave the lowest dry matter yield of 3.30 t/ha. This result indicated that combined application of biochar with inorganic fertilizer increased DM yield as compared to neither inorganic fertilizer nor biochar (T1) and biochar alone application (T2).The result is in line with Tadesse *et al.* (2022) who reported that the highest dry biomass yield of *Chloris gayana and Panicum coloratum* recorded at combined application of biochar with inorganic fertilizer at the South western Ethiopia. Dutta *et al.,* (2003) noted that the use of organic together with chemical fertilizers, compared to the addition of organic fertilizers alone, had a higher biomass yield and hence soil health for biomass yield production. Moreover, this study is in line with that of Mokonnen *et al*. (2020) and Sarwal *et al.* (2008) who reported that straw yield was significantly increased by the application of organic fertilizers along with the application of inorganic fertilizers.

Table 3. Dry matter and seed yield of Chomo grass as affected by fertilizer levels across sites

Treatments	DMY (t/ha)			Seed yield (kg/ha)		
	Haro sabu	Nedjo	Mean	Haro sabu	Nedjo	Mean
T1	3.82^{b}	2.79^{c}	3.30^{b}	74.39^{bc}	34.73	54.56^{bc}
T2	4.22^{b}	3.56^{ab}	3.89^{ab}	62.20^{c}	36.09	49.15^{c}
T3	5.34^{a}	3.11^{bc}	4.23^{ab}	89.87^{ab}	42.62	66.25^{ab}
T4	5.21^{a}	3.64^{ab}	4.43^{a}	76.67^{bc}	37.16	56.92^{bc}
T5	4.66^{ab}	4.02^{a}	4.34^{a}	82.48^{ab}	44.81	63.65^{ab}
T6	5.28^{a}	3.30^{b}	4.29^{a}	99.5^{a}	43.64	71.57^{a}
Mean	4.75	3.41	4.08	80.85	39.84	60.34
CV (%)	17.27	14.70	22.19	19.49	27.41	25.01
LSD $_{(5\%)}$	0.98	0.59	0.74	18.87	ns	12.40

$^{a-c}$means with different letters in a column are significantly different (P<0.05).

DMY=dry matter yield; CV= coefficient of variance; LSD= least significance difference; t=tone; ha=hectare; kg=kilogram; ns= non-significant.

The effect of biochar and inorganic fertilizer application was significantly affected (P<0.05) seed yield of chomo grass at Haro sabu site though non-significant variation was observed among the treatments at Nedjo site. Chomo grass gave higher seed yield at Haro sabu as compared to Nedjo, which could be due to differences of soil and climatic factors of the sites. The establishment and growth of chomo grass was slow and seed yield was obtained by harvesting four to five weeks later the first head start shattering. The highest seed yield (99.50 and 44.81 kg/ha) was obtained from T6 and T5 followed by T3 and T6 while the lowest seed yield (62.20 and 34.73 kg/ha) in sole biochar (T2) and control treatment (T1) for Haro sabu and Nedjo site, respectively. The overall mean seed yield significantly varied (P<0.05) among treatments. The highest seed yield (71.57 kg/ha) obtained from sole inorganic fertilizer application (T6), which was at par with T3 (66.25 t/ha) and T5 (63.65 t/ha), while the lowest seed yield (49.15 kg/ha) obtained in biochar alone (T2). This result is in agreement with Getnet *et al.*, (2003) who reported that seed yield among different nitrogen fertilization rates had a significant effect and the highest seed yield was obtained by applying high amount of nitrogen fertilizer.

Chemical composition of Chomo grass

The analysis of variance showed combined application of biochar with inorganic fertilizer at different levels were significantly (P<0.05) affected the chemical composition of chomo grass (Table 4). DM content was varied among treatments, and the highest DM content recorded at 50% biochar with 50% inorganic fertilizer application (T4) followed by inorganic fertilizer applied alone (T6) though the lowest (91.99%) was obtained in 75% biochar with 25% inorganic fertilizer application (T3). The highest ash content (8.42 %) was obtained from application of biochar alone followed by combined application of 75% biochar and 25% inorganic fertilizer (T3), while the lowest (7.73%) was obtained in application of 25% biochar and 75% inorganic fertilizer (T5). This result could be due to addition of biochar to the soil which resulted increment of minerals.

The crude protein content of chomo grass was significantly ($P<0.05$) varied among the treatments when the field was incorporated with different levels of biochar and inorganic fertilizer as compared to control. The highest CP content (10.75%) was obtained from 50% biochar and 50% inorganic fertilizer application level (T4) followed by 25% biochar and 75% inorganic fertilizer application (T5), while the lowest CP content (7.21%) was obtained at zero application of fertilizers (control treatment). Higher CP content of chomo grass was observed due to combined application of biochar and inorganic fertilizer at different levels as compared to zero application of fertilizer and sole application either organic or inorganic fertilizer. This is might be due to combined application of organic and inorganic fertilizer allows continuous growth of the vegetation, which was fresh even during forage harvest as compared to alone and none fertilizer application. This result is in line with Tadesse *et al.* (2022) reported that higher crude protein content was recorded in the combination of biochar with inorganic fertilizer application whereas lowest in no biochar and inorganic fertilizer application for *Chloris gayana* and *Panicum coloratum*. Similarly, Abdi *et al*., (2015) reported that level of fertilizer application affected the CP content of the grass and it increased with increasing level of fertilizers.

Table 4: Chemical composition (%) of chomo grass

Treatments	Parameters					
	DM	Ash	CP	NDF	ADF	ADL
T1	92.37^{c}	7.79^{cd}	7.21^{f}	63.86^{a}	41.15^{a}	7.88^{a}
T2	92.4b^{c}	8.42^{a}	7.55^{e}	61.84^{d}	39.84^{b}	6.86^{c}
T3	91.99^{d}	8.34^{a}	9.29^{c}	63.02^{b}	38.04^{e}	6.57^{d}
T4	92.83^{a}	8.13^{b}	10.75^{a}	63.16^{b}	39.2^{c}	6.91^{c}
T5	92.51^{b}	7.73^{d}	9.87^{b}	62.44^{c}	38.49^{d}	6.81^{c}
T6	92.75^{a}	7.94^{c}	8.4^{d}	63.83^{a}	36.52^{f}	7.54^{b}
Mean	92.47	8.05	9.51	63.02	38.87	7.09
CV(%)	0.06	1.12	1.28	0.22	0.26	1.41
$LSD_{(5\%)}$	0.11	0.16	0.22	0.26	0.18	0.18

$^{a-f}$ *means with different letters in a column are significantly different (P<0.05).*
DM=dry matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; CV= coefficient of variance; LSD= least significance difference.

Analysis of variance showed that crude fibers (NDF, ADF and ADL) of chomo grass were affected by application of different levels of biochar and inorganic fertilizer at forage harvesting stage (Table 4). The highest crude fibers (63.86, 41.15 and 7.88 %) were observed from control treatment for NDF, ADF and ADL, respectively. Though, the lowest crude fibers were recorded in sole biochar (61.84%), sole inorganic fertilizer (36.52%) and 75% biochar with 25% inorganic fertilizer application (6.57%) for NDF, ADF and ADL, respectively. Combined application of organic (biochar) and inorganic fertilizer at different levels were reduced the fiber contents of chomo grass as compared to grass planted without fertilizer. This is might be due to increased growth rate of new leaves and shoot which are lower in plant structural components because of biochar and inorganic fertilizer application. In addition, biochar retain water and nutrients in the soil and slowly release as plant needed which made grass green and has soft leaves. The current result is in agreement with Yuksel (2010) who reported that the crude fibers concentrations of fertilized herbage was significantly lower in treatment with additions fertilizer than in treatment without added fertilizer. A high NDF that had above 72 % will cause low intake of forage (Lima *et al.*, 2002) and as NDF percentages increase, dry matter intake generally will decrease. This result is agreement with Abdi *et al.,* (2015) who reported that ADL show significant difference in different rate of urea fertilizer used.

Economic analysis

The outcomes revealed that the combination of organic (biochar) and inorganic fertilizer applied together produced a higher dry matter yield than the control. The partial budget analysis reveals that the application of 50% biocharwith50% recommended inorganic fertilizer (T4) resulted in the highest net benefit of (8499.4 ETH ha^{-1}) with a marginal rate of return (52.25%). This was followed by 75% biochar with 25% inorganic fertilizer application (T3) with a net benefit of (8383.6 ETH ha^{-1}), while the minimum net benefit of 7476.6 ETH ha^{-1} was obtained from the application of sole recommended inorganic fertilizer (T6) (Table 5). In comparison to the control treatment, the combined application of 50% coffee husk biochar with 50% inorganic fertilizer (T4) and 75%biochar with 25% inorganic fertilizer (T3) provided 12.42% and 12.27% of net benefits, respectively. Based on this finding farmers produce forage in the study area chose to apply 50% of biochar with 50% of recommended inorganic fertilizer because it produced the highest adjustable dry matter yield.

Table 5.Partial budget analysis of different levels of fertilizers for DMY of Chomo grass

Variables	Different levels of Biochar (t/ha) and Inorganic fertilizer (kg/ha)					
	T1 (control)	T2 (100% biochar)	T3 (75% biochar + 25% IF)	T4 (50% biochar + 50% IF)	T5 (25% biochar + 75% IF)	T6 (100% IF)
Average DM yield (t/ha)	3.30	3.89	4.23	4.43	4.34	4.29
Adjusted yield-10%(t/ha)	2.97	3.5	3.81	3.99	3.91	3.86
Gross benefit (ETB/ha)	7603.2	8960	9753.6	10214.4	10009.6	9881.6
TVC (ETB/ha)		1025	1370	1715	2060	2405
NB (ETB/ha)	7603.2	7935	8383.6	8499.4	7949.6	7476.6
MRR (%)	-	32.37	56.96	52.25	16.82	-5.26
Dominance	-	D			D	D

Unit cost of NPS fertilizer: *16.25 ETB/kg; unit cost of urea fertilizer= 15.60 ETB/kg; unit price of DMY* **=** *2.52 birr/kg; ETB= Ethiopian birr; TVC= total variable cost; NB= net benefit; MRR= marginal rate of return; D= dominated; IF= inorganic fertilizer.*

Conclusion

The current study revealed that Biochar combined with inorganic fertilizer application significantly influenced ($P<0.05$) the phonology, growth, yield parameters, and chemical composition of chomo grass. Higher rates of biochar combination with inorganic fertilizer delayed days to 50% flowering and maturity as compared to other treatment combinations. Integration of biochar with inorganic fertilizer did not significantly vary on plant height though a significant difference was observed in the number of leaves per plant and leaf to stem ratio. The dry matter and seed yield of chomo grass was affected by the combined application of biochar with inorganic fertilizer and higher dry matter and seed yield was obtained in the integration of biochar with inorganic fertilizer as compared to control and biochar alone application. The crude protein content of chomo grass significantly varied among the treatments when the field was incorporated with different levels of biochar and inorganic fertilizer as compared to the control.

Higher CP content of chomo grass was observed due to the combined application of biochar and inorganic fertilizer at different levels as compared to zero application of fertilizer and sole application of either organic or inorganic fertilizer. Contrarily, the combined application of organic (biochar) and inorganic fertilizer at different levels reduced the fiber contents of chomo grass as compared to grass planted without fertilizer. The partial budget analysis showed that the combined application of 50% biochar with 50% recommended inorganic fertilizer produced the maximum net benefit (8499.4 ETB ha^{-1}) and a marginal rate of return of (52.25%), whereas the sole applied inorganic fertilizer produced the lowest net benefit (7476.6 ETB ha^{-1}). Therefore, it is advised to use a combination of 50% level of biochar with 50% of recommended inorganic fertilizer (T4) to enhance the dry matter yield and nutritive value of chomo grass.

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Determination of Cutting Frequency for Optimum Herbage Yield and Nutritive Value of Desho Grass (*Pennisetum glaucifolium*) in Western Oromia, Ethiopia

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Abstract

The experiment was conducted to determine the cutting frequency of desho grass for optimum dry matter yield and quality of forage.Treatments consisted of three cutting frequencies of desho grass (30 days, 45 days and 60 days).Treatments were arranged in RCBD with four replications.Agronomic parameters, dry matter (DM) yield and chemical composition of the grass were collected and analyzed based on the standard procedures. Results showed that cutting frequency had a significant influence on agronomic parameters and chemical composition, while no significant influences on DM yield of thegrass.The highest plant height and leaf numbers per plant were recorded at longer days of cutting frequency (60 days) than shorter days of cutting frequency (30 days), while tillers density per plant was more at 30 days than at 60 days of cutting frequency. Higher DM yield (8.64 t/ha) was obtained by cutting desho grass at 30 days intervalswhich wasnot significantly different from the rest treatments. Crude protein decreased with longer harvesting intervals and vice versa for ADF content in desho grass. Generally, cutting desho grass at 30 to 45 days resulted in good DM yield and nutritive value in the study areas.

Keywords: Chemical, composition, Cutting frequency, Desho grass, DM yield

Introduction

Feed problem in terms of quantity and quality is the major factor that hinders the development of livestock production in the mixed crop-livestock production system. In mixed crop-livestock production system, natural pasture and crop residues are the major feed resources that did not satisfy the farm animals for boosting milk and meat production in Ethiopia. Hence, to alleviate this problem establishment or development of improved forage like grass is crucial.

Desho grass is a multipurpose grass used for soil and water conservation, animal fodder and a means of income for smallholder farmers in Ethiopia (Leta *et al*., 2013; Asmare, 2016). Amongst the improved forage promoted in Ethiopia, Desho grass could play an important role in providing a significant amount of biomass yield with good agronomic and management practices (Ecocrop, 2010). Desho grass is a perennial forage grass that has the potential to produce high biomass production per unit of area. It is a highly popular, drought-tolerant species, and has a good chemical composition (Asmare, 2016). Due to its rapid growth rate, desho grass provides regular harvests, even reaching monthly cuts during the rainy season.

The ultimate objective of any process of forage production is to harvest high dry matter of good forage quality. However, because DM yield and quality are negatively correlated in most forage species, devising an appropriate cutting system is essential to optimize the yield and quality of a given forage harvest. The development of an appropriate system of harvest for perennial forage species depends on the ecological characteristics of the growing area. Environmental factors, along with various methods of harvesting, have a great influence on the nutritive value of forage (Enoh *et al.,* 2005). Stage of development is the most important consideration in developing a cutting system of any forage for a given area. Correct cutting management must be implemented to optimize the production of forage grass.On the other hands, information on appropriate cutting frequencies to optimize the yield and nutritive values of desho grass is lacking. Therefore, this study was conducted to determine the appropriate cutting frequency of desho grass for optimum DMyield and nutritive values.

Materials and Methods

Description of the study area

The experiment was conducted at on-station site of Haro sabu Agricultural Research Center and Kombo sites during the main cropping seasonof 2020 and 2021. The sites represent the midland areaoften ranging in altitude from1500 to 1750 m.a.s.l. The farming system of the study areas isthe mixed crop-livestock production system. The average temperature of the experimental area ranged from 24 to 25^{0}C. Annual rainfall ranges between 1200 to 1250 mm. The research site is located in sub-humid climatic zone (Haro sabu, 2012).

Establishment of desho grass, treatments and experimental design

Desho grass (Kulumsa-DZF-592) variety was considered for this research experiment. Three cutting frequencies (30, 45 and 60 days) were used as a treatment and planted at the beginning of the main rainy season. The treatmentswerearranged in a Randomized complete block design (RCBD) with four replications.The gross plot comprised six rows of 3 m length (6 x 0.5 m x 3 m = 9 m^{2}). The grasses were planted by vegetative part in the form of root splits with intra and inter row spacing of 0.25 m and 0.5 m, respectively (Worku *et al.,* 2017). The spacing between plots and blocks was maintained at 1 m and 1.5m, respectively. Fertilizer was applied at the rate of 100 kg ha^{-1} DAP and 50 kg ha^{-1} urea at planting time (Leta *et al.,* 2013). Management practicessuch asweeding weredone as early as possible by hoeing and hand weeding during the establishment stage uniformly to all plots and subsequently as required.

Data collection and measurements

At each cutting frequency, harvest and sampling procedures were made from the center of two rows of each plot at 8 cm above the ground level for forage yield determination. Plant heights were measured for ten plants randomly selected before harvest by using a stick meter. The tiller number and leaf number per plant were counted based on five randomly selected plants from each plot. Weight of the total fresh weight was recorded from each plot in the field just after mowing using a field balance. The estimated 300gm sub-sample was taken from each plot and dry in an oven dried for 72 hours at a temperature of 65 ^{0}c to determine the dry matter yield.

Chemical analysis of feed samples

Samples of desho grass at each treatment were taken from each plot and dried in a forced draft oven at 65 ^{0}c for 72 hours and the dried samples are ground using Wiley mill to pass through a 1 mm sieve screen forchemical analysis. DM and ash contents were determined using the procedures described by the

AOAC, 1990. Total nitrogen was determined by the kjeldhal procedure (AOAC, 1990) and Crude protein was calculated by multiplying the percent nitrogen by the factor of 6.25. Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were analyzed by Van Seost method (1991).

Statistical analysis

Differences among treatments/cutting frequencies were tested using analysis of variance (ANOVA) procedures of the SAS general linear model (GLM) to compare treatment means (SAS, 2009). Least significance differences (LSD) at 5% significance level was used for the comparison of means. The data was analyzed using the following model:

$$\mathbf{Y_{ij} = \mu + B_j + T_i + \varepsilon_{ij},}$$

Where: Y_{ij} = Response (dependent) variable of ij^{th}, μ = Overall mean, Ti = i^{th} effect of cutting frequency, Bi = j^{th}effect of block, ε_{ij} = Random error.

Results and Discussion

Combined analysis of variance

Combined analysis of variance of agronomic and dry matter yield parameters of the desho grass is presented in Table 1. The result indicated that the evaluated desho grass varied significantly (P<0.05) for plant height, number of tillers per plant and number of leaves per plant among cutting frequency, while DM yield didn'tshow any significant difference.The location also contributed significantly to the study's variation in yield and growth parameters of desho grass. Year had a non-significant effect on yield and growth attributes, except in plant height. The effect of interaction of cutting frequency, location andyear interaction also revealed significant differencesfor growth attributes, while it didn't showeda significant difference for dry matter yield.

Table1. Combined analysis of variance for growth and yield parameters of Desho grass

Source of variation	**Df**	**Mean square**			
		PH	**NTPP**	**NLPP**	**DMY**
Replication	3	13.04ns	39.33ns	0.06ns	6.98ns
Cutting frequency (CF)	2	3300.91**	499.16**	14.13**	9.14ns
Location (Loc)	1	158.8**	860.21**	1.08*	682.74**
Year (Yr)	1	54.61*	116.56ns	0.12ns	23.75ns
CF*Loc*Yr	3	130.07**	167.65*	0.55*	2.72ns
Error	46	10.64	57.30	0.14	8.37

*df= degree of freedom; PH= plant height; NTPP= number of tillers per plant; NLPP= number of leaves per plant; DMY= dry matter yield; CF= cutting frequency; Loc= location; Yr= year; ns= non-significant; *= significant at (P<0.05); **= significant at (P<0.01)*

Agronomic characteristics of desho grass

The results of the effect of cutting frequencies on agronomic parameters of desho grass are presented in Table 2. Except for thenumber of tillers per plant at Kombo site, other plant agronomic parameters were significantly affected by cutting frequency across the sites. Significant variation ($P<0.05$) between the frequency of harvest of desho grass was observed in plant height at Haro sabu and Kombo sites. Lower plant heights of 20.25 cm and 22.52 cm were observed, respectively, at Haro sabu and Kombo sites when desho grass was harvested at 30 days interval. On the other hand, higher plant heights of 43.95 cm and 54.20 cm were, respectively, observed at Haro sabu and Kombo sites at the 60 days harvesting frequency. The overall mean of the plant height showed a similar trend of increasing with decreasing cutting frequency. This could be due to the effect of additional days of harvesting interval which might have enhanced massive root development and efficient nutrient uptake by the grass. The result was in agreement with the findings of Wadi *et al.* (2004) who reported that the plant height of four *Pennisetum* species cut at a 90 day interval was higher than grasses cut at a 60 day interval. Moreover, among testing sites, plant height recorded at Kombo site was higher as compared to Haro sabu site and this could be attributed to the variations in climate conditions.

Table 2. Agronomic parameters of desho grass as affected by cutting frequency across locations

Cutting frequency (days)	**Plant height (cm)**			**Number of tiller/plant**			**Number of leaves/plant**		
	Haro sabu	**Kombo**	**Mean**	**Haro sabu**	**Kombo**	**Mean**	**Haro sabu**	**Kombo**	**Mean**
30	20.25c	22.52c	21.38c	49.95a	56.20	53.07a	4.30c	4.15c	4.22c
45	38.85b	44.87b	41.86b	47.92a	53.57	50.75a	4.97b	5.57b	5.27b
60	43.95a	54.20a	49.07a	35.70b	49.20	42.45b	5..87a	6.32a	6.10a
Mean	34.35	40.53	37.44	44.52	52.99	48.75	5.05	5.35	5.20
$LSD_{(0.05)}$	3.12	3.83	2.34	7.79	ns	5.44	0.40	0.43	0.27
CV (%)	8.52	8.87	8.71	16.42	15.46	15.72	7.47	7.57	7.44
SE	1.03	1.27	0.81	2.58	2.89	1.89	0.13	0.14	0.09

cm= centimeter, CV= coefficient of variance; LSD= least significance difference; SE= standard error;
[a-c]Means with different letters in a column/row are significantly different ($P<0.05$).

The number of tillers per plant varied significantly ($P<0.05$) among the cutting frequencies at Haro sabu, though not at Kombo site (Table 2). The highest number of tillers per plant was recorded from more repeated cutting (30 days) while less harvest frequency (60 days) gave a lower number of tillers per plant at both sites. From the overall mean result, more tillers per plant (53.07) was obtained from 30 days of frequent cutting which was not significantly different with the 45 days cutting frequency (50.75). The 60 days cutting frequency showed relatively low tillers density per plant. In the present study, tillers density per plant showed a decreasing trend with cutting frequencies of the grass decreased. This is attributed to additional tiller development being higher in more frequent cutting as compared to intermediate and less cutting frequencies. The current result was in agreement with the findings of Wadi *et al.* (2004) in which the tiller number of four *Pennisetum* species cut at a 90 days interval was reported to be lower than that of the grasses cut at a 60 days interval.

The number of leaves per plant was significantly different ($P<0.05$) among the cutting frequencies of the grass. At both sites, cutting desho grass at 60 days gave higher leaf numbers than cutting at 45 and 30 days intervals. The overall mean obtained in the two sites indicated that the number of leaves per plant of the grass was significantly affected by cutting frequencies. As harvesting frequency increased, the number of leaves per plant decreased.Similar to this result, Ansa and Garjila, (2019) reported that leaves production of elephant grass reduced when cutting intervals were reduced or when cutting frequency became higher.

Dry matter yield of Desho grass

Dry matter yield was not significantly different (P>0.05) among the cutting frequencies of desho grass (Figure 1). However, numerically as one would expect, dry matter yield per cut of the grass decreased with increasing intervals of harvests, from a mean of 8.64 t/ha/cuts at 30 days interval to 7.22 t/ha/cuts at 60 days interval. This might be due to more tiller numbers of the grass at more frequent cutting (30 days)than lessfrequent cutting (60 days). The result is in line with the results of Darmawati and Purbajanti, (2018) which revealed that Elephant grass produced more DM yield when harvested at 4 weekly intervals than at 8 weekly harvest intervals. In contrast with this study, Lounglawan *et al.* (2014) reported a significant difference in dry matter yield of King Napier grass between different cutting intervals/frequencies.

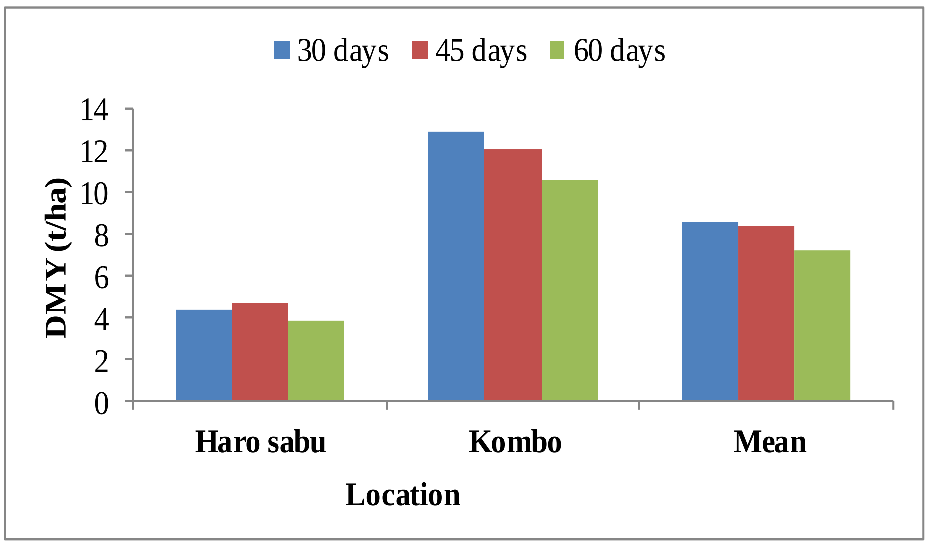

Figure 1.Dry matter yield of desho grass as affected by cutting frequency across locations

The trend of increased dry matter yield with an increment of harvest frequency in this study is in agreement with the findings of Munyasi *et al.* (2015). Furthermore, this result is in agreement with Hogling *et al.* (2005) who reported that more frequent harvest promoted re-growth and tillering in fodder grasses than less frequent harvesting.On the contrary, the present result differed from the findings of Arshad *et al.* (2010) who noted that elephant grass cut at 60 days intervals produced more DM yield than the one harvested at 30 or 45 days interval. Wadi *et al.* (2004) also found that Napier grass cut at 90 days interval gave high DM yield than 60 days cutting intervals. Hare*et al.* (2019) reported that hybrid brachiaria produced more dry matter yield as a result of longer intervals between cuttings in Thailand. Timbong-Jones *et al.*(2015) reported that DM yield decreased with increasing harvest frequency of *Cynodon nlemfuensis*.

At Haro sabu site, desho grass harvested at 45 days cutting frequency yielded insignificantly higher DM yield of 4.31 t/ha than those harvested at other cutting frequencies. This was below the dry matter yield observed at Kombo site. This could be attributed to the variations in climatic conditions between the study sites. Saddul *et al.* (2004) also reported variations in forage yields between locations as a result of differences in climatic patterns.

Chemical composition of Desho grass

As shown in Table 3, cutting frequency had a significant effect ($P<0.05$) on all the chemical compositions of the grass. the highest DM content was recorded at 60 days cutting whereas the lowest was recorded when the grass was cut at 45 days frequency. With regard to Ash content the grass cut at more cutting frequency (30 days) gave the highest ash content (7.93%) whereas the lowest was (5.85%) obtained from 60 days cutting frequency. Ash content decreased with increased cutting interval. This might be due to the natural dilution and translocation of nutrients to the roots during the growth and development of plant tissue. This trend is similar to the findings of Lounglawan et al. (2014) who tested the cutting interval of 30 to 60 days for King Napier grass.

Crude protein is greater importance and it is commonly stated that forage with higher crude protein has superior feeding value (Qureshi, 1992). The crude protein of desho grass varied significantly ($P<0.05$) with cutting frequency. The highest average CP content (10.05%) was obtained at the cutting frequency of 30 days while the lowest (8.14%) was obtained from the grass cut at 60 days frequency. This indicated that CP content reduced with longer harvest or cutting intervals. In terms of cutting interval at harvest, more frequent cutting resulted in younger and leafier plants which were reflected in the higher crude protein content of the grass, compared with the less frequent cutting. Increasing the number of cuts increased CP content because grass turns to be relatively leafy (AyalaTolares *et al.,* 2000). Similar to the current study, Timbong-Jones *et al.* (2015) reported that the CP content of *Cynodon nlemfuensis* increased with increasing harvest frequency. Other several findings (Arshad *et al.*, 2010; Hare *et al.,* 2019; Lounglawan *et al.,* 2014) indicated increased crude protein content with reduced cutting intervals of some grasses. The decrease in crude protein percentage as harvesting intervals increase is likely to occur due to maturation of the grasses and hence, ultimately utilization of nutrients by the grasses.

Table 3.Chemical composition (%) of desho grass as affected by cutting frequency

Cutting frequency (days)	**Parameters**					
	DM	**Ash**	**CP**	**NDF**	**ADF**	**ADL**
30	93.39^{ab}	7.93^{a}	10.05^{a}	60.3^{a}	46.46^{b}	16.48^{a}
45	93.18^{b}	5.96^{b}	9.25^{b}	58.83^{b}	45.62^{c}	14.0^{c}
60	93.44^{a}	5.85^{b}	8.14^{c}	55.62^{c}	47.09^{a}	15.65^{b}
Mean	93.33	6.58	10.14	58.25	46.39	15.37
CV (%)	0.14	1.20	1.67	0.29	0.45	1.10
$LSD_{(0.05)}$	0.22	0.13	0.23	0.29	0.36	0.29
SE	0.06	0.03	0.06	0.08	0.10	0.08

DM= dry matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; CV= coefficient of variance; LSD= least significance difference; SE= standard error.
$^{a-c}$Means with different letters in a column significantly different ($P<0.05$).

The NDF content was significantly different due to cutting frequency. The result showed that the NDF content ranged from 55.62% for 60 days cutting frequency to 60.3% for 30 days cutting frequency with a mean of 58.25%. Inthe present study, increasing the cutting interval reduced the NDF concentration of desho grass. Timbong-Jones *et al.* (2015) reported that the NDF content of *Cynodon nlemfuensis* increased with increasing harvest frequency. However, the current result is contrary to the findings of Hare *et al.* (2019) who found increased NDF content of hybrid brachiaria due to increased cutting intervals in Thailand. Furthermore, Lounglawan *et al.* (2014) found that the NDF content of Napier grass showed a significant increment as the cutting interval increased.

ADF and ADL content of desho grass also showed significant variation among the cutting frequencies (Table 3). There was an inconsistent relation between ADF and ADL across cutting frequencies. However, less cutting frequency (60 days) resulted in the highest ADF content (47.09%) whereas the lowest value (45.62%), was obtained from the grass cut at 45 days. This result concurs with that of Hare *et al.* (2013) in which ADF content of hybrid brachiaria increased due to increased cutting intervals in Thailand. Grass cut at 30 days interval gave significantly the highest ADL (16.48%) while the lowest (14%) was obtained from the grass cut at 45 days. In agreement with the current study, Timbong-Jones *et al.* (2015) reported an inconsistent relation between lignin content and harvest frequency for *Cynodon nlemfuensis* grass evaluated in the western part of Accra plains, Ghana.

Conclusion

The present study clearly shows that cutting frequency has a marked effect on agronomic parameters and chemical composition of desho grass but not on dry matter yield across locations over years. More repeated cutting frequency resulted in high tillering and dry matter production whereas plant height and leaf numbers were decreased as cutting frequencies increased. Ash and crude protein content decreased as the cutting interval increased from 30 to 60 days. The appropriate cutting frequency will depend on the usage to which the forage is put and what combination of yield and quality is desired. Generally desho grass harvested every 30 to 45 days gave good DM yield and nutritive values and hence, these cutting frequenciesarerecommended for the study areas.

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Season Based Application of Bush Control Techniques for Unaddressed Bush Species (*Vachellia senegal)* in Borana Zone, Southern Ethiopia

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Abstract

The study was conducted to evaluate bush controlling techniques on encroaching bush species (Vachellia Senegal) in Miyo district of Borana zone for three consecutive years. A total area of 2.5 hectares of rangeland encroached by Vachellia species was replicated into two plots for each four seasons. Each plot was subdivided into five sub-plots to receive five treatments; cutting at 0.15m above ground+2, 4 D application on stump (T1), cutting at 0.15m above ground + a mixture of 75% 2,4-D and 25% water application on stump (T2), cutting at 0.15m above ground + kerosene application on stump (T3), cutting at 0.5m above ground + debarking (T4) and no cutting (T5). Data on different parameters including basal and litter covers, soil erosion and compaction, dead and re-sprouted encroaching tree/shrub species were collected before and after treatment applications. The result indicated that for all seasons, different parameters were significantly influenced ($p < 0.05$) by the applied treatments. Among the tested bush controlling treatments, T1 was more effective in bush controlling in dry (78 %) and cool dry (71 %) seasons as compared to other treatments. Similarly in the dry season, the responses of T2, T3, and T4 in controlling V. senegal were 69 %, 63 % and 61 % respectively. The result also showed that different bush controlling techniques were found to improve the basal and litter cover, species richness, species evenness, and biomass yield of rangeland. The evaluated techniques were also indicated in decreased soil erosion and soil compaction. Increased in basal cover and changed in vegetation structure of rangeland of bush thinned plots were related with reduction of encroaching canopy thickness and regenerated perennial herbaceous species. Due to better accessibility, cost effectiveness and friendly to environment T4; cutting at 0.5m above ground and debarking was recommended for pastoralist to be applied at dry season. Besides, reseeding important herbaceous species with enough periods of resting shall be considered for improving degraded rangelands such as less recovery potential of bush cover areas.

Keywords: *Basal cover, Bush controlling, Rangeland; Grass and non-grass; species composition; Species diversity*

Introduction

Borana rangelands in the southern parts of the country were considered to be one of the best grazing lands (Coppock 1994). However, these rangelands are experiencing increasing pressure from livestock and human populations, bush encroachment and tick infestation (Ayana 2007). Mismanagement coupled with disregard of indigenous knowledge makes bush encroachment one of the major problems in many pastoral areas of Ethiopia. In Borana rangelands, where for century's episodic climatic events and the use of fire regulated vegetation dynamics, the natural balance between grasses and trees has shifted and bush cover has become a major threat to pastoral grazing management. *Vachellia drepanolobium*, *Vachellia mellifera* and other *Vachellia* species are the dominant encroachers of southern Ethiopia (Gemedo *et al.*, 2006b; Bikila *et al.*, 2014). According to Gemedo *et al.* (2006a), in the Borana rangelands, woody plant cover increased from 50% as reported in the late 1990s by Oba (1998) to 60%. Bushes are transforming open grazing lands into impenetrable thicket-forming noxious trees/shrubs and suppressing desirable grasses and non-grasses through competition, thus becoming unsuitable for browsing and grazing (Tamene 1990). Oba *et al.* (2000) reported that bush encroachment in the Borana rangelands reduces livestock productivity and survival particularly during drought years, when forage scarcity is the greatest.

Local and international non-governmental organizations and some government departments are conducting range rehabilitation, involving hand clearing of woody species along highways and near settlements, on an experimental basis which is not successful. Nowadays, the successful results we brought in controlling two encroaching bush species namely: *Vachellia mellifera* and *V. drepanolobium* made different NGOs (CARE, GPDI etc) and GOs (zonal and district level pastoralist development offices, Pastoralist Community Development Project (PCDP)) to widely implement the best techniques in the vast rangelands of Borana. Similarly, promising result was attained by pouring 2, 4-D over stumps and thoroughly debarking stumps of encroaching bush species like *Vachellia drepanolobium* and *Vachellia mellifera* soon after cutting at 0.15m above ground (Bikila *et al.* 2014).

However, information about the effects of bush encroachment control techniques in terms of the responses by the encroaching bush species like *Vachellia senegal* which are aggressive in nature to inhibit the growth of herbaceous vegetation under their canopy is lacking. Furthermore, information on controlling this bushes species through chemical and mechanical methods across the four prominent seasons in the Borana rangeland is poorly documented. Hence the objectives of study was to determine the response of herbaceous species to different bush control techniques and to identify the best seasons and techniques for controlling *Vachellia senegal* species

Materials and Methods

Site selection

The study was conducted in Miyo district, Borana zone of Southern Ethiopia. Based on the priority of the pastoral community and other stakeholders, top encroaching bush species for the district was selected. Selection of the encroaching tree/shrub species (*Vachellia senegal)* was based on their relative dominance over the district. During the project conduction, indigenous knowledge of the community on the nature of bush encroachment was considered through participatory approach. A total area of 2.5 ha rangeland unit which was encroached by the target bush species was delineated soon after assessment and identification of the intended site.

Treatments and experimental design

The treatments were consist of cutting at 0.15m above ground + 2, 4 D application on stump (T1), cutting at 0.15m above ground + a mixture of 75% 2,4-D and 25% water application on stump (T2), cutting at 0.15m above ground + kerosene application on stump (T3), cutting at 0.5m above ground + debarking (T4) and no cutting (T5).The experiment was laid out in a randomized complete block design with two replicates. The selected 2.5 hectare rangeland from the target district was laid out in twelve plots to accommodate five treatments across the four prominent seasons (4 seasons * 5 treatments * 2 replicates). Each of the treatment was laid out on a 25 m x 25 m land that was separated from each other with a border space of two meter. The plots were located adjacently and treatments were allocated randomly to the plots. The target species were marked during cutting process and the areas were fenced using locally available materials. About 25 ml of pure 2, 4D, 25 % diluted 2, 4D and kerosene were used to apply on the stump of bush species.

Sampling and measurements

Dry matter, basal cover, litter cover; herbaceous vegetation composition, density and frequency were assessed in 0.5mx0.5m quadrate. All vegetation attributes were collected prior to treatment application and at the end of the study period to see the effects of bush encroachment controlling action. The scores used for basal cover and litter cover were based on the criteria developed for semi-arid rangelands in southern Africa (Baars *et al.* 1997). Dry matter yield of herbaceous species was determined after drying in oven at 105 °C for 24 hours. Dead and re-sprouted bush species were recorded at the end of the study period.

Statistical analysis

The dry matter, basal cover, litter cover and other vegetation attributes were sorted by treatment and seasons and considered as experimental units for data analysis in a randomized complete block design. The parameters were subjected to ANOVA, using the GLM procedure of SAS Version 9 computer software package (SAS, 2002). Means were tested for significance using Least Significance Difference and differences were declared significant at $P<0.05$.

Results and discussion

Response of bush control techniques on herbaceous structure

A total of 34 herbaceous species were identified in the bush control techniques applied plots (Table 1). Based on their life forms, 47 % annual herbaceous and 53 % perennial herbaceous species were identified. The result also indicated that plots applied with bush controlling techniques increased the botanical composition by 52 % as compared to none applied (control) plots.

In the plots of bush controlling techniques, both highly and less desirable species had the highest proportion (38% and 38 %) followed by desirable species (24 %). Of the total herbaceous species identified, about 47 % were grasses species. The result showed that higher proportion of botanical composition were recorded in plots with bush control techniques applied than the control plots. In the study areas, from grass species *Aristida kenyensis* species were highly dominated grass species. From 16 grass species recorded (Table 1), eleven species had highly desirable species, four had desirable and one had less desirable species. From the highly desirable groups of grass species *Cenchrus ciliaris, Chrysopogon aucheri* and *Chloris roxburghiana* were recorded highly in the bush thinned plots than un-thinned plots. *Commelina Africana* was the highly desirable non-grass species that identified in the bush control treatment applied plots. Among the herbaceous species recorded in the study plots *Ocimum forskalei* was common and dominated the plots, followed by *Aristida kenyensis*. This significant difference in botanical composition along treatments and control plot is due to the reduction of the impact of bush density on the growth of herbaceous species. Therefore, reduction of bush density has positive effect on the recovery of rangeland herbaceous species.

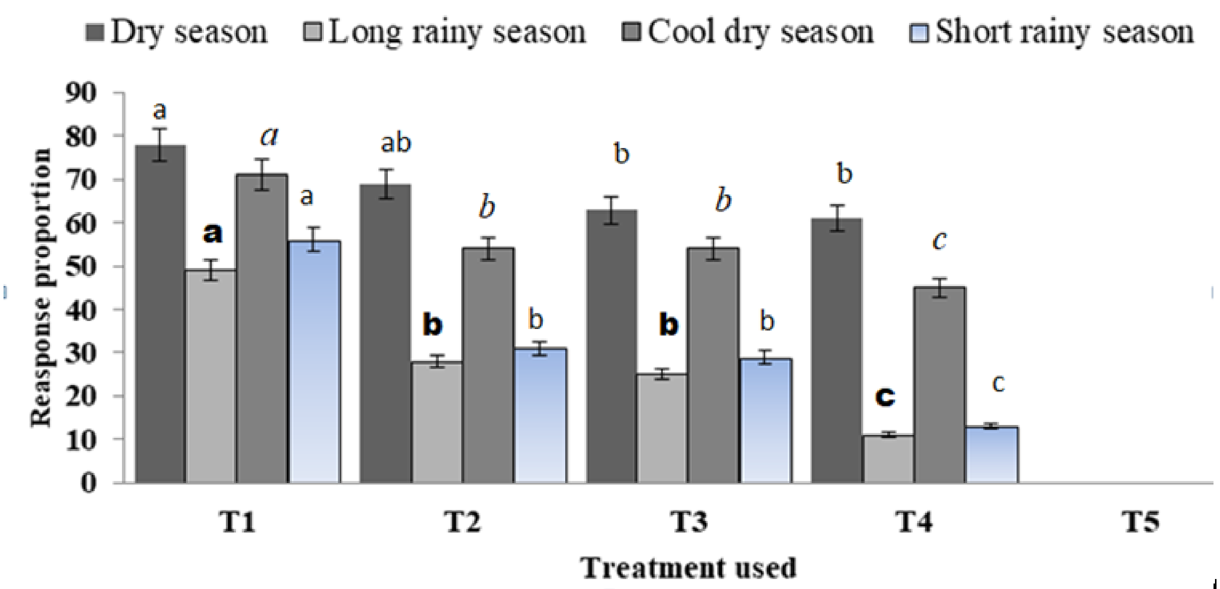
Table 1: Response of bush control techniques on botanical composition herbaceous species

Scientific Name	Growth form	Life form	Desirability	2,4,D	2,4,DW	Kerosene	Debarking	Control
Chrysopogon aucheri	Grass	P	HD	1.38	0.67	0.77	1.75	0.00
Actiniopteris radiate	Forbs	A	LD	0.55	0.00	0.00	0.00	0.00
Digitaria milanjiana	Grass	P	HD	7.71	0.33	9.23	0.44	0.52
Barleria spinisepala	Forbs	P	D	3.31	3.01	3.85	7.89	8.85
Commelina Africana	Forbs	A	HD	0.55	1.00	0.38	0.88	0.52
Ocimum forskalei	Forbs	P	LD	41.32	47.83	36.54	15.35	15.63
Plectranthus barbatus Andr.	Forbs	A	LD	0.55	0.00	0.00	0.00	0.00
Abutilon hirtum (Lam.) Sweet	Forbs	P	HD	6.61	4.68	7.31	10.96	13.02
Chlorophytum gallabatense	Forbs	A	D	3.31	4.68	2.31	10.09	13.02
Aristida kenyensis	Grass	A	D	10.47	10.70	10.38	19.74	23.44
Tetrapogon tenellus	Forbs	A	LS	1.38	2.01	5.38	1.32	0.00
Sporobolus pellucidus	Grass	A	D	2.48	7.02	5.38	1.32	3.13
Leptothrium senegalense	Grass	P	HD	7.71	4.35	2.69	3.07	6.25
Indigofera volkensii Taub.	Forbs	P	D	0.28	0.00	0.00	0.00	0.00
Chloris roxburghiana	Grass	P	HD	0.55	0.00	0.38	1.75	0.00
Setaria verticillata	Grass	A	D	4.68	2.01	1.54	2.63	2.60
Indigofera species.	Forbs	A	LS	0.28	0.00	0.00	0.88	0.52
Dactyloctynium	Grass	P	HD	0.55	2.34	1.92	3.95	3.13
Digitaria velutina	Grass	A	D	3.86	4.01	7.31	11.84	6.77
Osteospermum vailliantii .	Forbs	A	LD	0.28	0.00	0.00	0.00	0.00
Harpachne schimperi	Forbs	A	LD	0.28	0.00	0.00	0.00	0.00
Cenchrus ciliaris	Grass	P		0.83	0.67	1.54	0.44	0.52
Cyperus sp.	Grass	P	HD	0.00	1.34	0.00	0.00	0.00
Euphorbia crotonoides	Forbs	P	LD	0.00	0.67	0.00	0.00	1.04
Eragrostis species	Grass	P	HD	0.00	0.33	0.00	0.00	0.00
Bidens hildebrandtii	Forbs	A	LD	0.00	0.67	0.77	0.00	0.00
Macroculia species	Grass	P	HD	0.00	1.67	1.92	0.00	0.00
Chloris roxburghiana	Grass	P	HD	1.10	0.00	0.00	0.00	0.00
Bidens biternata	Forbs	A	LD	0.00	0.00	0.38	0.88	1.04
Sporobolus discosporus	Grass	A	LD	0.00	0.00	0.00	0.44	0.00
Partinium	Forbs	P	ND	0.00	0.00	0.00	2.19	0.00
Cladostigma hildebrandtioides	Forbs	P	D	0.00	0.00	0.00	0.44	0.00
Chenopodium opulifolium	Forbs	A	LD	0.00	0.00	0.00	0.44	0.00
Bothriochloa insculpta	Grass	P	HD	0.00	0.00	0.00	1.32	0.00

Response of Vachellia senegal to different bush control techniques within different season

The result indicated a significant difference observed between different seasons and treatments that applied on the control of *Vachellia senegal* bush species. The stump death of *Vachellia senegal* was highest for T1 (cutting at 0.15m above ground + 2, 4 D application) in dry season (78 %) followed by cool dry season (71 %). The result indicated that the stump death of *Vachellia senegal* was 78, 69, 63, and 61 % for T1, T2, T3 and T4 respectively in dry season while it was 71 54, 54 and 45 % in cool dry season for T1, T2, T3 and T4 respectively. But stump death of *Vachellia senegal* was showed lower response (less than 50%) for all treatments in both long and short rainy season except in T1.

This indicated that *Vachellia senegal* species could be controlled by using treatment 1 (cutting at 0.15m above ground + 2, 4 D) in the dry season as compared to the other treatments and seasons. A similar finding (Bikila *et al.* 2014) showed that probability of mortality among woody species is highest in dry season.



T1=cutting at 0.15m above ground + 2, 4 D application on stump, T2=cutting at 0.15m above ground + a mixture of 75 % 2, 4-D with 25% water application on stump, T3=cutting at 0.15m above ground + kerosene application on stump, T4=cutting at 0.5m above ground + debarking and T5=no cutting.

Figure 1: The response of *Vachellia senegal* to different treatments within four season

Response of bush control techniques on basal and litter cover, and soil erosion and compaction

Percentage of basal cover and litter cover were significantly ($P<0.05$) different between bush controlling techniques and the control treatments. The basal covers of the plots with treatment of different bush controlling techniques were increased by 10.25 % as compared to the control plots. The high basal cover in the treatment applied plots could be associated with reduced encroaching tree species densities which created a suitable condition for regenerate new grass and forbs species.

Table 2: Effect of bush controlling techniques on basal and litter cover, soil erosion and compaction

Treatment	Basal Cover	Litter Cover	Soil Erosion	Soil Compaction
T1	30.35a	16.95	12.25c	31.05b
T2	30.25a	14.30	14.25bc	33.55b
T3	31.60a	13.70	12.30c	40.56b
T4	29.88a	13.73	18.08b	38.30b
T5	12.80b	17.20	33.20a	52.85a
C V	39.67	85.29	72.86	51.29
P-values	0.0001	0.89	0.0001	0.0001

abc= Means in the same column without a common letter are different; T1=cutting at 0.15m above ground + 2, 4 D application on stump, T2=cutting at 0.15m above ground + a mixture of 75 % 2, 4-D with 25% water application on stump, T3=cutting at 0.15m above ground + kerosene application on stump, T4=cutting at 0.5m above ground + debarking and T5=no cutting.

The canopy gaps created by bush species removal are expected to result in increased herbaceous cover, diversity and abundance due to reduced competition for water and nutrients as well as increased availability of light (Savadogo *et al.,* 2008). In line with this finding, Karuaera (2011) found that non- encroached sites had a higher grass cover than the bush-encroached sites. The Percentage of soil erosions and compaction were significantly different between treatment plots and control plots. It showed that soil erosion and compaction was decreased by 18.98 % and 16.99 % respectively due to different bush controlling treatments as compared to the control treatment. The reductions in soil erosion and compaction might be due to the increment in basal cover of herbaceous species that support soil particles by their above and ground biomass. This in turn increases water infiltration rates into the soil and decreases runoff (Jiang *et al.* 2021).

Response of bush control techniques on species richness, diversity and evenness

The results indicated that the treatment applied plots were showed significantly difference in herbaceous species richness ($P < 0.01$) and evenness ($P < 0.02$) while no significat differences observed in species diversity. The differences was observed at treatment plots due to manual thinning of less desirable bush species in the encroached site, which reduce competition with herbaceous species, at the same time enhancing the chance of propagation than the control treatment. The result indicated that the herbaceous species diversity was not significantly different ($P > 0.05$) from the control treatment due to applying bush controlling techniques. In species richness, T1 and T4 have the highest values followed by T2 and T3 and the least for T5. These could be due to bush encroachment effects. This finding indicated that grass diversity is negatively correlated with woody plant density which is in accordance with Abule *et al.* (2007).

Table 3: Effect of bush controlling techniques on species richness, diversity and evenness

Treatment	Species richness	Species diversity	Species evenness_
T1	23a	2.78	0.38b
T2	20b	2.5	0.37b
T3	19b	2.49	0.50a
T4	23a	2.60	0.53a
T5	16c	2.20	0.31b
CV	22.3	35.6	25.3
P-values	0.01	0.69	0.02

abc= Means in the same column without a common letter are different; T1=cutting at 0.15m above ground + 2, 4 D application on stump, T2=cutting at 0.15m above ground + a mixture of 75 % 2, 4-D with 25% water application on stump, T3=cutting at 0.15m above ground + kerosene application on stump, T4=cutting at 0.5m above ground + debarking and T5=no cutting.

Response of bush control techniques on dry matter yield

Total dry matter yields of herbaceous species were influenced by the treatments with different bush controlling techniques and control treatment. There were highly significance ($P < 0.01$) differences between other treatments and control in grass and forbs species dry matter yield. The higher dry matter yield obtained except for the control treatment might be due to the increment in basal cover percentage related with regenerate herbaceous species. These herbaceous species are very important livestock feed resources in the rangeland ecosystem.

Table 4: Effect of bush controlling techniques on dry matter yield

Treatments	Grass (t/ha)	Forbs (t/ha)	Total (t/ha)
T1	0.145a	0.173a	0.318a
T2	0.110a	0.160a	0.268a
T3	0.136a	0.169a	0.305a
T4	0.105a	0.158a	0.263a
T5	0.026b	0.082b	0.108b
CV	32.35	32.35	32.35
P values	0.01	0.01	0.01

abc = Means in the same column without a common letter are different; T1=cutting at 0.15m above ground +2, 4 D application on stump, T2=cutting at 0.15m above ground + a mixture of 75 % 2, 4-D with 25% water application on stump, T3=cutting at 0.15m above ground + kerosene application on stump, T4=cutting at 0.5m above ground + debarking and T5=no cutting

Response of bush controlling techniques on socio-economic parameters

As listed in table5, the socio-economic parameters were evaluated to compare the bush controlling treatments. The result indicated that T1 was more effective in bush control, followed T2, T3 and T4 while the inverse is true for their socio-economic values. Among the chemicals used, 2, 4 D is herbicide which has toxicity to human beings, animals and soil macro and micro-organisms. Therefore, 2,4D required highly careful in handling during application than other bush controlling treatments. Moreover 2,4D and kerosene are not easily assessable and are expensive. On the other hands, debarking techniques has no cost, easily accessible and friendly to environment. However, compering to other treatments it was less effective in controlling bush and required high man power. Because of these facts debarking techniques was recommended as appropriate bush controlling techniques for pastoralist in the study area. But any sectors who have a capacity to afford (such as private sectors or investors) can use 2,4D and Kerosene carefully for its time saving and effective bush controlling.

Table 5: Responses of bush controlling techniques to socio-economic parameters

parameters used	Types of treatments			
	T1	T2	T3	T4
Cost of treatment per hectors	$19.8	$14.9	$8.5	No need
Accessibility of treatment	Has effort	Has effort	Has effort	effortlessly
Time of application per hectors	10 hours	10 hours	10 hours	48 hours
Man power needed per hector	One adult	One adult	One adult	Six adult
Action to be taken to prevent side effect	Good Mask and gloves	Good Mask and gloves	Any Mask and gloves	No need
Care to be taken during application	Very careful	Very careful	Careful	No need
Average amount of treatments needed per stump bush	25 ml	25 ml	25 ml	No
Effectiveness of the treatment per plots	Very higher	higher	higher	lower

T1=cutting at 0.15m above ground +2, 4 D application on stump, T2=cutting at 0.15m above ground + a mixture of 75 % 2, 4-D with 25% water application on stump, T3=cutting at 0.15m above ground + kerosene application on stump, T4=cutting at 0.5m above ground + debarking and T5=no cutting.

Conclusions and Recommendations

The result showed that different bush controlling techniques were found to improve the basal and litter cover, species richness, species evenness, and biomass yield of rangeland. The evaluated techniques were also decreased soil erosion and soil compaction. Among the tested bush controlling treatments, T1 was more effective in bush controlling in dry and cool dry seasons respectively as compared with the other treatments. Similarly in the dry season, the responses of T2, T3, and T4 in controlling *V. senegal* were 69 %, 63 % and 61 % respectively. However, 2, 4 D is herbicide that has chemical properties and toxic to human beings, animals and soil macro and micro-organisms. Moreover 2,4D and kerosene are not well assessable and are expensive. On the other hands, debarking techniques has no cost, easily accessible and friendly to environment. Compering to other treatments it was less effective in controlling bush and required high man power. Due to better accessibility, cost effectiveness and friendly to environment T4; cutting at 0.5m above ground and debarking was recommended for pastoralist to be used at dry season. But any sectors who have a capacity to afford (such as private sectors or investors) can use 2,4D and Kerosene carefully for its time saving and effective bush controlling. Besides, reseeding important herbaceous species with enough periods of resting shall be considered for improving degraded rangelands such as less recovery potential of bush cover areas

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Effects of Cultivation on Soil Seed Bank Flora of Borana Rangeland, Southern Ethiopia

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Abstract

The effects of cultivation on soil seed bank flora of Borana rangeland were examined under three land use systems (Enclosure land, open grazing land, and different ages cultivated land). Soil seed bank sampling was carried out at the end of the growing season from 0.25m2 quadrates at 30 mm deep. Of the total plant species identified in the soil seed bank, 34.6% were grasses and 65.4% were forbs species. From the nine grass species recorded, four were identified as highly desirable, two as desirable and three as less desirable. From the highly desirable group, Dactyloctenium aegyptium were found to be the common and dominant at the three land use systems of enclosure, open grazing and cultivated lands for less than five years, while highly desirable species perennial grass like Cenchrus ciliaris and Chloris roxburghiana were only found in enclosure lands. The species richness of enclosure, open grazing and cultivated 1-5 years land were higher respectively while cultivated land more than10 years was the least. There were significant differences ($P < 0.05$) in soil texture between land uses of the study sites. Due to cultivation pressure, more than ten years cultivated land has more sandy soil than the remaining rangeland of study areas. Generally long periods of cultivation and grazing pressure has negative impacts on Borana rangeland soil seed bank flora whiles enclosure land has higher values in restoring and sustaining the productivity of rangeland ecosystems. Therefore, rangeland land use policy is required to sustain productivity of rangeland through developing appropriate land use plan for each land use types of rangeland that used to reduces impact of rangeland ecosystems deterioration. Practices of rangeland managements like resting of rangeland through enclosure, moderate grazing with rotation grazing systems should be developed and strength in order sustain rangeland ecosystems and feed resource productivities.

Key words: Enclosure; Cultivation, Species composition, Species richness; Soil texture;

Introduction

In most areas of Ethiopia, where there is unpredictable rainfall, sustainable cropping system will produce more dry matter of nutritional value for ruminant forage than grazing areas. This factor overcomes the key negative impact of increased cropping with reduction of available areas for livestock grazing. In arid and semiarid areas, with crop failures to the extent of harvesting little animal feed is available especially in the face of climate change. In the Borana rangeland, cultivation is a recent phenomenon when compared to livestock production. Cultivated land quickly degrades the productivity of the rangeland. Cultivation can bring loss of many plants and animal, soil degradation and other losses. If the rangeland is cultivated for several years, it might affect rangeland biodiversity, resulting in disappearance of many important species that have paramount importance for pastoralism.

The sustainable use of Ethiopian rangelands by pastoralists depends on understanding the extent and degree of deterioration of rangelands (Tefera *et al.*, 2007). This study aims at complementing previous studies by contributing additional quantitative data for better understanding of the impact of different land use on rangeland productivity. Besides, these data may give important information to decision makers and development projects to improve the livelihood of Ethiopian pastoral communities. Therefore, the objectives of the study were to examine seedling density and floristic composition of cultivated and uncultivated land, to study the difference and similarities of grass species in cultivated and uncultivated land and to examine soil physio -chemical characteristics in the cultivated and non-cultivated land.

Materials and Methods

Study areas

The Borana rangeland was located in the southern part of Ethiopia. The altitude ranges from 1000 to 1500 m.a.s.l. (meters above sea level), with peaks in the mountains reaching above 2000 m.a.s.l. (Helland, 1982). It is dominated by arid and semiarid climate and characterized by general scarcity of water. The soil samples were collected from three districts of rangeland representatives (Yabello, Teltele and Dire districts). For each district, the field layout considered was five lands system; open grazing, enclosure land, cultivated land of 1-5 years, 6-10 years and cultivated land more than 10 years.

Soil sampling

Soil samples were carefully spooned from three separate soil depth, each 30 cm thick i.e. 0 – 10 cm, 10 – 20 cm and 20 – 30 cm using a sharp knife following the method used by Teketay and Granstroem (1995) and within each sample quadrat of 0.5 x 0.5 m. The samples were placed in labeled and tagged cheese cloth bags for immediate transportation to the lath house for germination. The pots were placed at random in the lath house with the assumption that the lath house’s is uniform.

Incubate of the soil samples

In the lath house, labeled plastic pots were filled with sterile sand. Before using the sterile sand, it was checked for possible seed contamination by keeping sterile sand moist over a clean flat floor. The soil sample was spread over the sand in each plastic pot to the depth of 100 mm. Each pot was hand-watered daily in the first week. Pots were examined every three to four days for the first two months and then periodically to keep soil moist. Except crop, all the germinated seedlings were counted over the experimental period.

Laboratory analysis

Soil was sampled and analyzed from samples collected and mixed for lath house experiment. The soil samples were properly labeled and packed in plastic bags for lab analysis. Samples were air dried ground and sieved with 2 mm mesh size. Soil parameters were analyzed for soil texture, pH, Available phosphorus, organic matter and organic carbon following standard procedures. Texture was determined by hydrometer method, pH by a pH meter in 1:2.5 soil: water ratio and soil organic carbon by the walkey-black method, the percent soil organic matter were calculated by multiplying the percent organic carbon by a factor of 1.724.

Data analysis

Plant diversity of germinated species from soil seed bank was analyzed using PAST version 3.10, Paleontological Statistical software (Hammer *et al,* 2001). The data generated from soil seed bank in the lath house was analyzed in randomized block design using general linear model by SAS software version 9,1. LSD (least significant differences) test with P<0.05 was used for means comparison

Result and discussion

Seed bank botanical composition

A total of 26 species representing graminoids flowering plants were identified (Table 1). Of these, 34.6% were grasses and 65.4% were non-grass plant species. The seed bank of botanical composition at enclosure, open grazing, cultivated <5 years and cultivated >10 years lands were dominated by *Aristida kenyensis* species respectively. In the enclosure land, *Aristida kenyensis* species made up 30% of the total botanical composition. Of the 9 grass species recorded, four were identified as highly desirable, two as desirable and three as less desirable. From the highly desirable group, *Dactyloctenium aegyptium* were found to be common and dominant at the three land use systems of enclosure, open grazing and cultivated <5 years, while highly desirable species like *Cenchrus ciliaris* and *Chloris roxburghiana* were only found in enclosure. In the less desirable grass species, *Aristida kenyensis*, was common and dominant in the enclosure, open grazing, cultivated <5 years and cultivated >10 years land. Among the desirable species, Cyperus species and ergrostis species were found to be common along the enclosure land. The productive types of rangeland grass species like *Cenchrus ciliaris* and *Chloris roxburghiana* occurred only at enclosure land.

Table 1: Botanical composition of soil seed bank from different land types

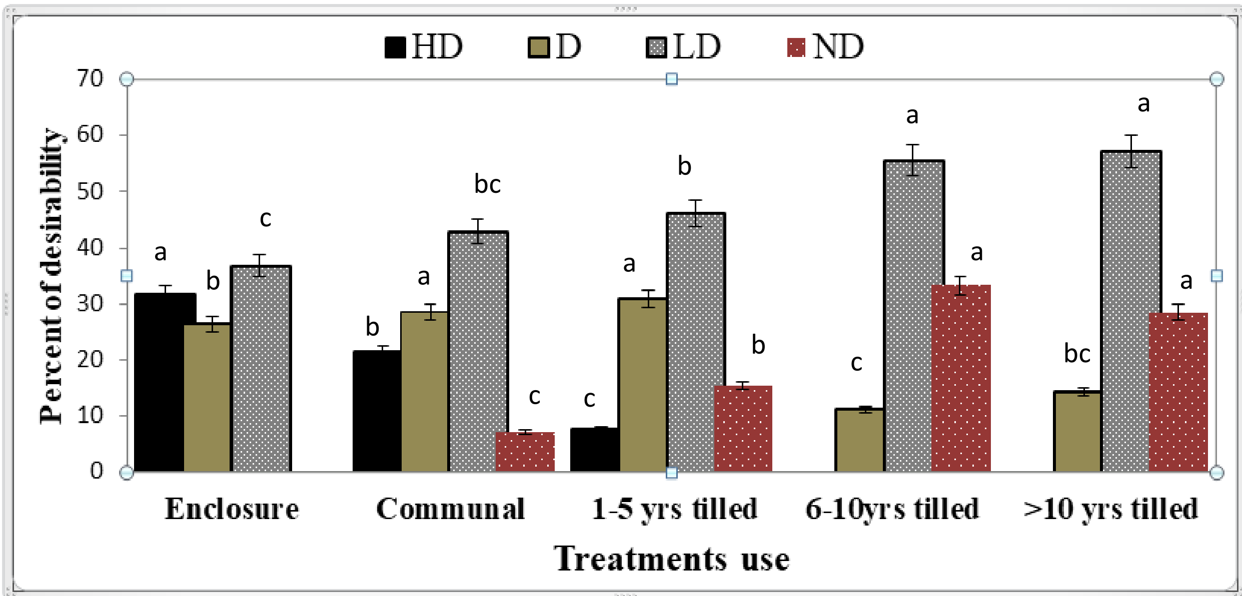
Scientific name	Local name	Growth form	Desirability	Enclosure	Land use type			
					Open grazing	Cultivated 1-5 years	Cultivated 5-10 years	Cultivated >10 years
Setaria verticillata	Raphuupha a	A	LD	2.29	0.00	3.77	0.00	0.00
Aristida kenyensis	biiilaa	A	LD	30.29	26.09	28.30	21.43	6.56
Cyperus sp.	Saattuu	A	HD	9.71	13.04	3.77	0.00	0.00
Digitaria species		A	LD	5.14	4.35	0.00	0.00	0.00
Commelina africana	Qaayyoo	A	HD	8.57	2.17	0.00	0.00	0.00
Ceratostigma species	gurbii	A	LD	1.71	2.17	0.00	0.00	0.00
Dactyloctenium aegyptium		P	HD	2.86	4.35	1.89	0.00	0.00
Barleria spinisepala	Qilxiphee	P	D	9.14	8.70	3.77	0.00	0.00
Tagetes minuta	sunkii	A	ND	1.14	2.17	0.00	0.00	1.64
Cenchrus ciliaris	Mata guddeessa	P	HD	1.14	0.00	0.00	0.00	0.00
Partinium	kuubaa	P	NO	0.00	2.17	7.55	25.00	24.59
Chloris roxburghiana	Hiddoo luucolee	P	HD	0.57	0.00	0.00	0.00	0.00
Eragrostis species	Eragorestic	P	HD	1.14	0.00	0.00	0.00	0.00
Abutilon hirtum	Gurbii	P	D	7.43	13.04	9.43	10.71	40.98

	daalatii							
Tribulus cistoides	mogoree	A	LD	0.57	0.00	3.77	14.29	1.64
Lippia carviodora	urgoo Loonii	P	D	2.29	0.00	0.00	0.00	0.00
Indigofera spinosa	shaana simpiree	A	LD	0.00	0.00	0.00	0.00	3.28
Amaranthus thunbergii	raafuu	A	LD	7.43	8.70	11.32	0.00	11.48
Sesamothamnus rivae	huuraa laafaa	A	D	0.00	4.35	0.00	0.00	3.28
Brachiaria eruciformis	birachria	P	HD	5.71	2.17	0.00	0.00	0.00
Acantospermum	keessaa ka'ii	A	ND	0.00	0.00	13.21	10.71	6.56
forb species	homoorafisaa	A	LD	0.00	0.00	5.66	10.71	0.00
Indigofera species		A	D	0.00	0.00	3.77	0.00	0.00
Lantana rhodesiensis	Midhaan durbaa	P	D	1.71	6.52	3.77	0.00	0.00
Bidens biternata		A	LD	0.00	0.00	0.00	7.14	0.00
Hibiscus crassinervius	Bungaala	P	HD	1.14	0.00	0.00	0.00	0.00

Of the total non-grass species identified, 65% were herbaceous annuals and 35% were semi-perennials. Peritoneum and Acantospermum species are invader species that were commonly found in cultivated land of study sites whereas the highly desirable species of herbaceous species were found in enclosure land. The differences observed in seed bank botanical composition along the five lands was due to the impact of levels of grazing intensity and duration of cultivation pressure. The higher seed bank of botanical composition was graminoids at the enclosure land reserves compared to the open grazing and cultivated areas of lands. The enclosure land has lower grazing pressures since it allow grazing only during dry season of the year and rested in both growing season and some time in cool dry season. While, the open grazing land had high grazing pressure with unlimited stock density in all season. Cultivated land had got higher chances to exposure for heavy erosion, which has been increasing in year round. Low graminoids seed bank compositions were measured at the cultivated land. It can be assumed that with continuous cultivation, the aboveground grass biomass has removed by root-off and destruction of germinated seed bank by repeatedly ploughing and weeding. Due to densely utilization grazing land, the low graminoids seed bank composition was measured at the open grazing areas because continuous overgrazing cause reduced the aboveground biomass and destruction of grass roots by trampling livestock (Brinkmann, 2020). Consequently, the production capacity of grasses and their ultimate contribution of seeds to the soil seed bank were reduced. Kinloch and Friedel (2005a) came to the conclusion that the impact of grazing on the standing herbage and seed bank depends on the severity of grazing over preceding decades and the coincidence with drought in that time. One finding also suggest that a decrease in total seed density at very high levels of grazing and an increase in density at intermediate levels due to a decrease in the number of animals (Kinloch and Friedel, 2005a). Therefore, the rangeland may have a capacity to rehabilitate the composition of the highly desirable and desirable species if only moderate grazing system is applied with sufficient rest period.

Desirability of species distribution

The proportional different groups of species desirability distributions for the study areas of soil seed bank were different across the five land field layout. The result indicated that at enclosure land, the less desirable species had the highest proportion followed by the highly desirable and desirable species (31.6, 26.3%) respectively, whereas the reverse held true for the 6-10 and more than 10 years cultivated land that dominate by less and none desirable species (less desirable species 55.6% and 57.1%, and also none desirable species 33.3% and 28.6% respectively). At the open grazing land, the less desirable species had the highest percentage (42.9%) followed by the desirable and highly desirable species (28.6 % and 21.4 %) respectively. For 1-5 years cultivated land, less desirable species had the highest proportion (46.2%) followed by desirable and none desirable species (30.8% and 15.4 %) respectively.



abc= Means in the same color without a common letter are different

Figure 2: Proportional species desirability of treatment use

Species richness and diversity

The individual herbaceous species that germinated from soil collected from the enclosure land (175) was higher (*P*<0.001) by 78.8 % and 76.7 % than germinated herbaceous species recorded from open land and cultivated land respectively (Table 2). The germinated individual species between all lands did show high marked variation. The herbaceous species that germinated in cultivated for 6-10 years was higher than land cultivated 1-5 years due to highly encroached and invaded by weeds species. There were highly significant differences in the germinated herbaceous richness for all land use types. The species richness of enclosure, open grazing and cultivated 1-5 years land were higher respectively while cultivated >10 years was the least (Table 2). This indicates that land cultivation decreased the species richness due to disturbed origin soil seed banks and exposed soil for wind and water erosion, the land was dominated by few weed species. Similarly, the species diversity and evenness of collected soil seed banks were highly significant (*P*<0.001) between all land use types. Therefore enclosure land of rangeland was the better practices of rangeland management which has higher values in restoring and sustaining the productivity of rangeland resources than other the remains land use types.

Table 2: Mean values of species richness and diversity of the soil seed bank under different land use types

Land use type	Numbers of species count	Species richness	Species diversity	Species evenness
Enclosure land	175a	19a	2.40a	0.58e
Open grazing land	47d	15b	2.36b	0.71c
Cultivated 1-5 years	53c	13c	2.27c	0.74b
Cultivated 6-10years	61b	9d	1.68e	0.59d
Cultivated >10 years	28e	7e	1.86d	0.92a
CV	9.1	9.1	9.1	9.1
P-values	0.001	0.001	0.001	0.001

abc= Means in the same column without a common letter are different

Soil physical properties

The soil physical properties of three district rangeland of study areas were presented in the table 3. The soil laboratory analysis result indicated that there were significant differences (*P*< 0.05) in soil texture between grazing and cultivation pressure. Due to cultivation pressure, ten years cultivated land has more sandy soil than remain rangeland of study areas. Enclosure rangeland type was less exposed to erosion by livestock trampling as that of open grazing rangeland (continuous grazing) during rainy season. Followed to enclosure land, cultivated land of 1-5 years has less sandy soil than others land type of study areas. There was no significance difference in soil texture classification among land of study sites. The soil texture of study sites of rangeland classified into sandy, clay, loam while slightly differed in their color within districts. Teltele site was brow color types of soil, while Yabello study site was red and Dire rangeland type was red-brown. This dissimilarity of soil texture and color were determined the soil properties while, they were affected by heavy grazing and cultivation pressure together with climatic factors through reduction of their soil parent materials. Similarly, Pimentel, D. (2006), Filp (2002) reported that environmental factors and anthropogenic activities affect soil living organism those maintain soil health and function.

Table 3: Mean values of soil physical properties of different land use type

Land use type	**Sand (%)**	**Clay (%)**	**Silt (%)**
Cultivated >10 years	**61.92a**	**23.08b**	**15.00b**
Open grazing land	52.77a	24.92ab	22.31ab
Cultivated 1-5 years	49.86a	27.92ab	22.22ab
Cultivated 6-10years	52.41a	33.58a	14.01b
Enclosure land	34.41b	34.15a	31.44a
CV	11.13	11.13	11.13
P-values	0.016	0.016	0.016

^{abc}=*Means in the same column without a common letter are different*

Soil chemical properties

The result of pH, organic matter, organic carbon and available phosphors of three land use of the study sites are indicated in table 4. The pH, organic matter and carbon and available phosphors were no significantly different (P > 0.05) between land use types. While enclosure land was higher in soil organic matter and carbon followed by 1-5 years cultivated land than open grazing, 6-10 years and > 10 years cultivated land because enclosure has better management than other land use while the recent rangeland that converted to cultivated land is a potential grazing land. Whereas, the percentage of soil organic matter and carbon were lower than the standard requirement of forage production i.e. medium average content of soil in their soil organic matter and carbon respectively (Frank, 1990). This is due to effect of overgrazing on both grazing land and also frequent cultivation that facilitator for degradation rangeland is the main factors complain with recurrent drought. The result agreed with Kumasi *et at.* (2010) and Zhan *et al.* (2020) that concluded the removal of vegetation by herbivores reduces ground covers and soil organic matters and nitrogen.

Table 4: Mean values of soil chemical properties of different land use type

Land use type	PH	EC	%OC	%OM	AvP
Enclosure land	6.96a	102.48ab	1.49a	2.5a	8.64a
Open grazing land	7.04a	93.66b	1.34a	2.30a	9.55a
Cultivated 1-5 years	7.04a	247.29a	1.17a	2.51a	10.07a
Cultivated 6-10years	6.69a	106.31ab	1.16a	2.02a	8.98a
Cultivated >10 years	7.34a	176.29ab	1.13a	1.94a	9.03a
CV	24.3	13.2	24.3	24.3	24.3
P-values	0.54	0.19	0.54	0.54	0.54

[abc] *=Means in the same column without a common letter are different; %OC = percentage of organic carbon, %OM = percentage of organic matter, and AvP = available phosphorous*

Conclusion and Recommendation

Differences in species composition among the land use type were manifest due to the variance in cultivation and grazing pressure. Long periods of cultivation and heavy grazing pressure have negative impact in changed the botanical composition of herbaceous species layer towards less desirable and none-desirable species and also size and composition in the soil seed bank. An enclosed land had higher botanical composition and desirability of species than open grazing and cultivated land of study areas. In Borana pastoralist, the enclosure land has been experienced in their traditional grazing system that ensured the restoration as well as maintenance of seed bank composition of desirable herbaceous species. The species richness of enclosure, open grazing and cultivated1-5 years land were higher while cultivated >10 years was the least. This indicates that land cultivation pressure decreased the species richness due to disturbed origin soil seed banks and exposed soil for wind and water erosion, and the land was dominated by few weed species. The size, species richness and species composition of seed bank of study areas are important indicators to reflect the impact of rangeland and better systems of rangeland ecosystem management. In Borana pastoralist, the enclosure land have been the common practices in their traditional grazing systems that used for stand hay preservation and ensured the restoration through maintain seed bank of desirable herbaceous species composition. The seed banks have been used to recover plants of threatened species for those species which have high palatable. Therefore, enclosure rangeland with light grazing was the better practices of rangeland management which has the higher values in restoring and sustaining the productivity of rangeland ecosystems. Now cultivation was expand and seen as options of livelihoods diversification. So rangeland use policy is required to shifting some specific areas marginal rangeland to crop land through developing appropriate dryland agriculture practices. Many clustering method with appropriate dryland agriculture practices such as little tillage, crop rotation, based on indigenous and modern rainfall forecasters, supplement irrigation method, and pure seed that free from weeds and has early age mature are used to reduce simple disturbing of rangeland ecosystems.

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Impact of Land Use land Cover Changes of Borana Rangelands, Southern Ethiopia

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Abstract

The study was aimed at understanding land use and land cover change on rangeland diversity, dynamics of land use land covers and to estimate the magnitude of different land uses. Time-series satellite images that included Landsat TM, ETM+, OLI and TIRS, which covered the time frame between (1991, 2001, 2011 and 2021), were used. Data for ground referencing (ground truth) were collect during field work by reading GPS and used for the land use/land classification. In addition, Key formant survey from selected elder was carried out to understand historical trends, Google earth and pre-processed (raw) imagery were used for accuracy assessment. Based on these result, mostly the traditional grazing landscapes of study areas were converted to sparse bush degraded land and dense bush land while grass land was extremely decline than any types of land use of Borana rangeland. The results were similar with pastoralist response on the main problems of Borana rangeland. Besides this pastoralist had been believed on cultivation is play a great role in reduction of grass land because most cultivated land are the bottom land which has good potentials for grazing. Generally to improve the rangeland condition of the study area, indigenous grazing systems; dry season grazing, wet season grazing and reserving grazing that used during hard time or drought periods could be strengthened in combination with government rangeland management plan. In general, land use planning and policy should be developed to separate land use based on its suitability for crop production, grazing lands and forest land.

Key words: Land use types, Land use cover change, Dense and sparse bush, Degraded land

Introduction

Conversions of land from one use to another have become recognized as major causes of global environmental changes (Camill, 2010). Conversion of rangelands into cultivated land is one of the main challenges affecting the management of rangelands in Ethiopia. Knowledge of the trends of land use changes are needed for local, regional, and global assessments because land use changes affect ecosystem processes (carbon stocks, biodiversity and vegetation composition) and the livelihoods of inhabitants are under threat. Earlier studies have shown that land use change in Africa’s rangelands is attributed to inefficient policies that govern natural resources, and that increasing anthropogenic activities such as grazing and cultivation. One of the dominant contemporary forms of land use change in Africa’s rangelands is the expansion of cultivation (Jonckheere, *et al*, 2017 and Safriel *et al*, 2005). Small-scale crop cultivation is regarded as livelihood diversification option to cope with economic hardships, but the process has negative impacts on ecosystem is veracity. In Ethiopia, the conversion of rangelands into cultivated land has been reported (Garedew, 2010; Flintan, 2011), but land use changes at its current situation and on-going processes have not been thoroughly investigated. Specifically, the Borana pastoralists of Southern Ethiopia have been appreciated for their superior systems of rangeland resource use and management for a long time (Coppock, 1994; Oba *et al.*, 2000). However, the prior open grazing management systems have been weakened over time. Alongside this, there has been increasing crop cultivation in some portions of the rangelands (Tache and Oba, 2010). Despite the fact that small-scale crop cultivation is an age-old practice of diversifying asset cases among pastoral communities inhabiting the precarious rangelands of Ethiopia (Mohamed, A. A. 2019). The negative trade-offs associated with its adoption, especially on contemporary scales, have adverse implications for the very livelihoods of these people and for the ecosystem.

A previous study by (Solomon *et al.,* 2007; Desta, 2006 and Tache and Oba, 2010) investigated the association between poverty and the participation of Borana herders in cultivation and analyzed current trends in cattle management, rangeland degradation, and the perceptions of pastoralists. It is, therefore, important to understand the trends influencing these conversions as a prerequisite for analysis of land use and land cover change processes. However, there is a lack of evidence regarding the trends and impacts of the current ongoing land conversions on vegetation diversity. Analyzing these variables could inform policy makers and rangeland managers of the extent to which land conversion affects the ecosystem in terms of vegetation diversity. Hence, the study was conducted with the objectives to determine the dynamics of land use land covers and to estimate the magnitude of different land uses of Borana rangelands.

Materials and Methods

Description of the study area

The study was conducted in Borana rangeland, southern Ethiopia. Based on the vegetation or ecological description, five PAs; Borbor, Dubuluk, Magado, Utallo and Harboro PAs were selected respectively from Dhas, Dubuluk, Dire, Yabello and Gamole districts. Discussion was made with pastoralist elders and community as well as PDO experts to get the general information of land uses of the study sites. In each selected PAs of Borana rangeland; open grazing, forest and cropland were selected as a treatment with five traditional grazing landscapes.

Acquisition of satellite imageries

Landsat satellite images of four Periods (1991, 2001, 2011 and 2021) were obtained from United States Geological Survey (USGS) data portal (https://earthexplorer.usgs.gov/ assessed in 17-27 July, 2022). Images were taken during the dry season to avoid cloudy, in December and/or January in conjunction with field surveys (Table1). A sufficient quantity of reference data was very important for quality assessment. Data for ground referencing (ground truth) were collected during field work by reading GPS Garmin 72H and used for the land use/land classification. In addition, interview from selected households, Google earth and pre-processed (raw) imagery were used for accuracy assessment.

Table 1: Satellite characterization

Index	Sensor	Path row	Spatial resolution (m)	Acquisition date	Producer	Use bands
1991	TM	166-57, 167-57, 167-58, 168-56, 168-57, 168-58, 169-56, 169-57	30x30	28-Dec-1991	USGS	1-5, 7
2001	ETM+	166-57, 167-57, 167-58, 168-56, 168-57, 168-58, 169-56, 169-57	30x30	24-Jan-2001	USGS	1-5, 7
2011	ETM+	166-57, 167-57, 167-58, 168-56, 168-57, 168-58, 169-56, 169-57	30 x30	22-Dec-2011	USGS	1-5-7
2021	OLI/TIRS	166-57, 167-57, 167-58, 168-56, 168-57, 168-58, 169-56, 169-57	30x30	30-Jan-2021	USGS	1-5, 7

Data Analysis and Preparation

Landsat image preprocessing such as geometric correction, layer stacking/composite, image mosaicking were applied to all images using Arc GIS version 10.3. All images were stacked into a multilayer image to combine the different bands of each image. All images were extracted (clipped) to the study area of Borana rangeland. Supervised image classification using maximum likelihood algorithm methods was used. Google earth and GPS points were used for ground referencing (ground truth).

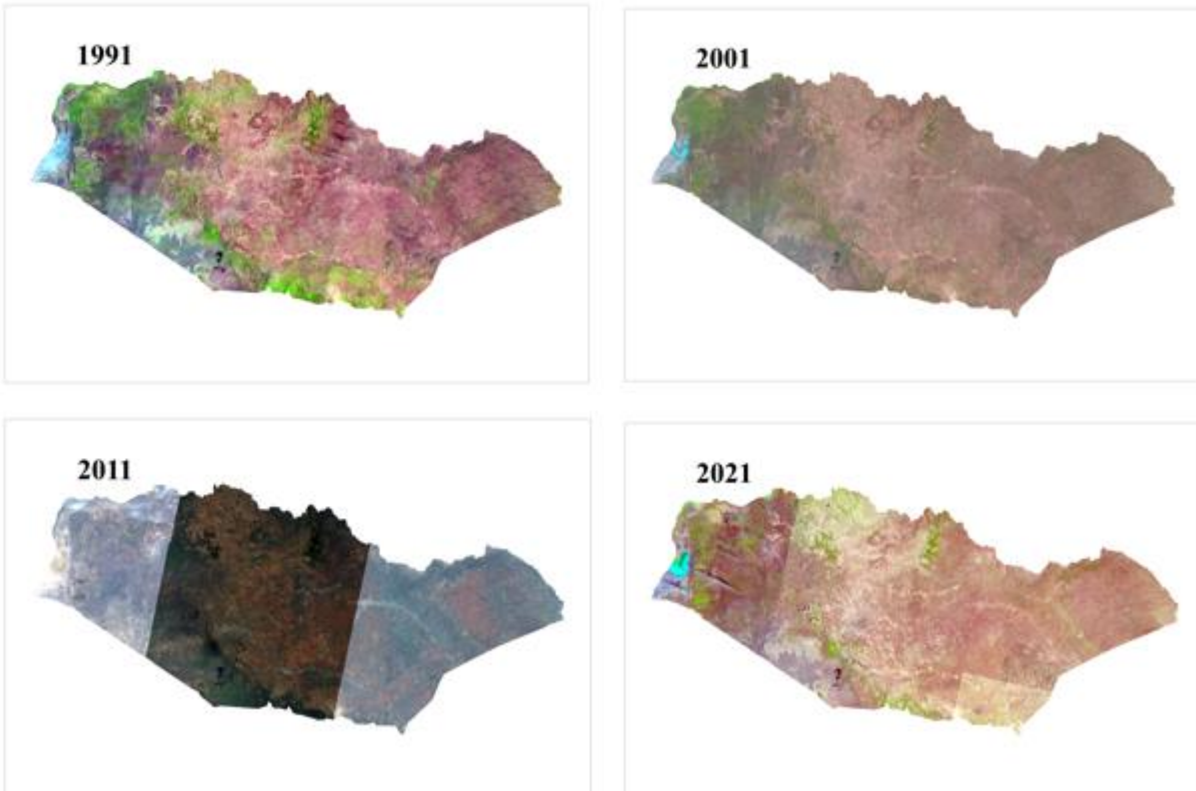

Figure 1: Band combination of images that use for analysis land use land cover

Result and Discussion

The land use land cover (LULC) classifications images were presented for six land use categories, including grass land, dense bush land, sparse bush degraded land, degraded land, forest land and crop land. The grass land and forest land rapidly changing in Borana rangelands. The results of the land cover classification maps generated for each of these four periods of the study sites were presented as follows.

Land use land covers of the study area for the year 1991

The result of LULC classification in the year 1991indicated that the largest areas of the land use and land cover was dense bush land class with land size of 2987096.99 ha (53.60 %) followed by grass land 1526095.4 ha (27.39 %) and forest land 472533.74 ha (8.48 %) respectively (Table 1). Whereas, the aerial coverage of degraded land and crop land were the least coverage of the study area which occupied about 135467.88 ha (2.43%) and 30458.06 ha (0.55%), respectively. As primary information collected from key formant of pastoralist this period was the time of bush encroachment start to increase on Borana rangeland due to interfere of Derge regime on indigenous ways of rangeland management of pastoralist. Since that time, the government proclamation was ban burning of rangeland due to management of natural resources specially forest while prescribed burning is tools of rangeland management. Pastoralist believed that prescribed burning is the best options while it's difficult to apply due to the absence of ground herbaceous fuel loads. According to the pastoralists, fire has multi-dispel uses in controlling bush encroachment through drying/killing of small bushes and seedling, make infertility of large bush and avoid germination of dropped seed bush with improving the quality and quantity of herbaceous biomass than any applied treatments.

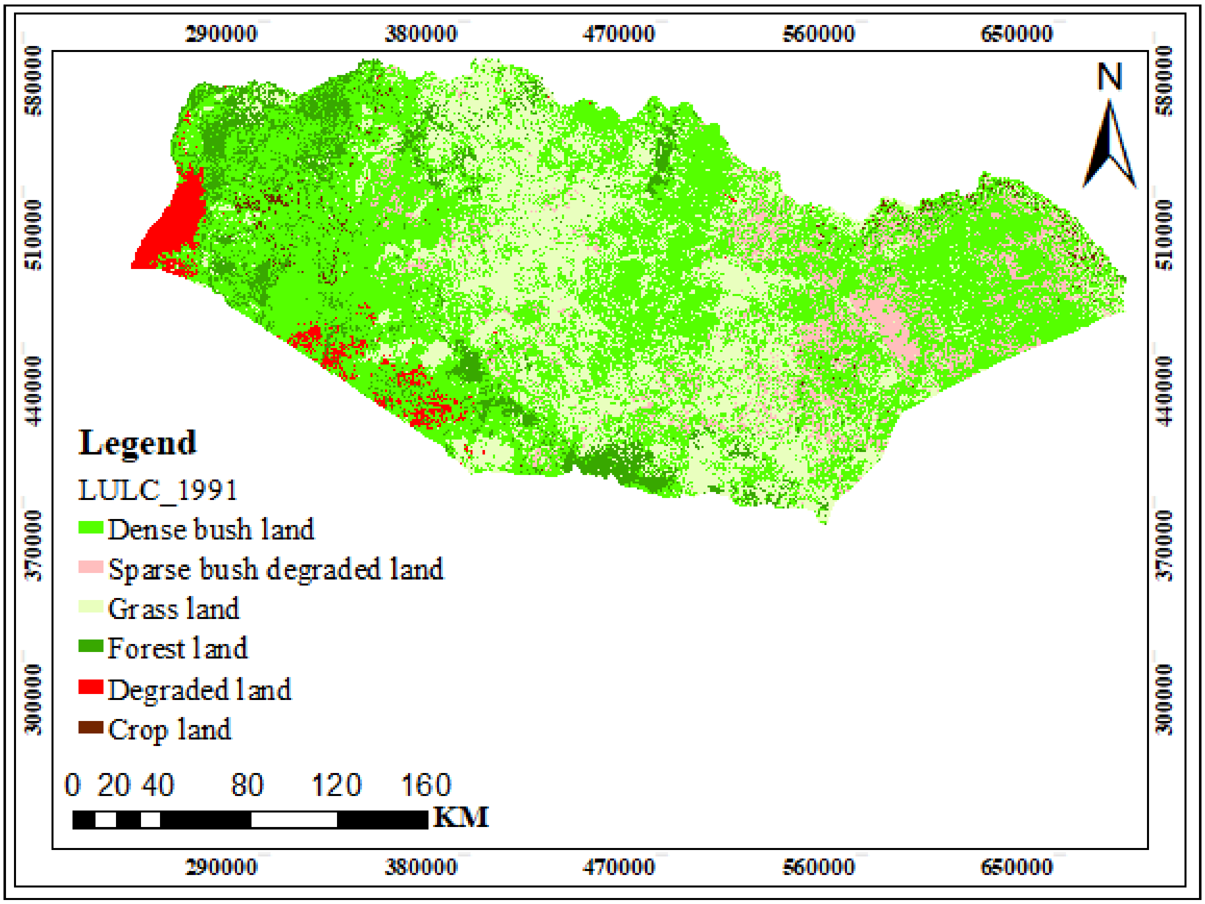

Figure 1. LULC map for the year 1991 of the study area

Table 1 Land use land cover classes and their corresponding area for the year 1991 of the study area

Land use land cover	**Area (ha)**	**Area (%)**
Dense bush land	2987096.99	53.60438
Sparse bush degraded land	420834.38	7.552004
Grass land	1526095.42	27.38626
Forest land	472533.74	8.479765
Degraded land	135467.88	2.431013
Crop land	30458.06	0.546579

Land use land covers of the study area for the year 2001

As show in table 2, the largest areas of the land use and land cover was dense bush land class with land size of 2160408.05 ha (38.77 %) followed by sparse bush degraded land 1465582.04 ha (26.30 %) and grass land 800705.83 ha (14.37 %) respectively. Whereas, the aerial coverage of forest land and crop land were the least coverage of the study area which occupied about 332084.85 ha (5.96 %) and 65203.31 ha (1.17%) respectively. As pastoralist said, in this periods rangeland management had been stated through manual bush clearing methods by different development organization while it's gradually improved into bush thinning.

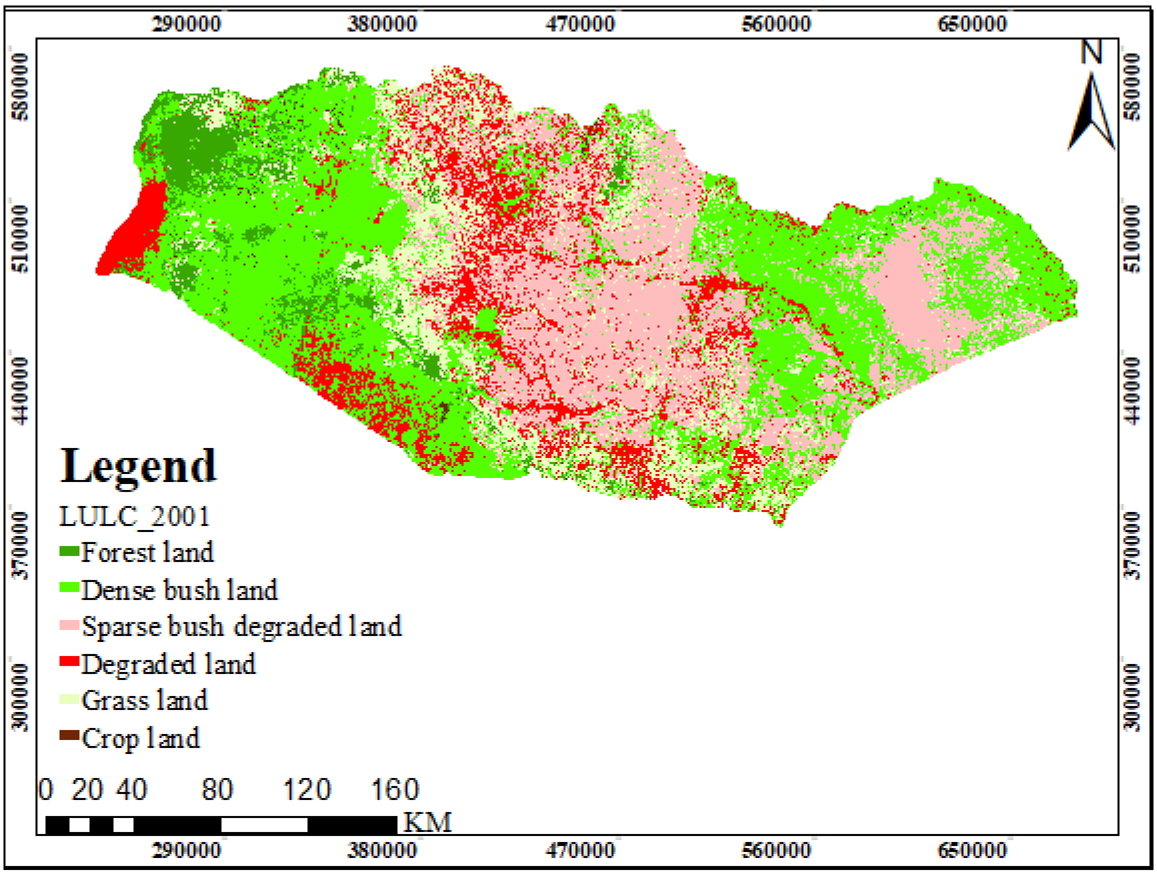

Figure 2. LULC map for the year 2001 of the study area

Table 2. Land use land cover classes and their corresponding area for the year 2001

Land use land cover types	Area (ha)	Area (%)
Dense bush land	2160408.05	38.77
Sparse bush degraded land	1465582.04	26.30
Grass land	800705.83	14.37
Forest land	332084.85	5.96
Degraded land	748005.63	13.42
Crop land	65203.31	1.17

Land use land covers of the study area for the year of 2011

As result show in table 3, the largest areas of the land use and land cover was dense bush land with land size of 3316896.1ha (59.52%) followed by sparse bush degraded land 1465395.41 ha (26.30%) and degraded land 368979.72 ha (6.62%) respectively. Whereas, the aerial coverage of grass land and crop land were the least coverage of the study area which occupied about 65771.75 ha (1.18%) and 374.05 ha (0.01%) respectively. Based on these result, mostly Borana rangeland was converted to dense bush land and sparse bush degraded land while grass land was extremely decline than any types of land use. The results were similar with pastoralist response on the main problems of Borana rangeland. The encroachment of bush after bush control actions that under took by different development organization were very high than prior due to miss management that is resting and prescribed burning.

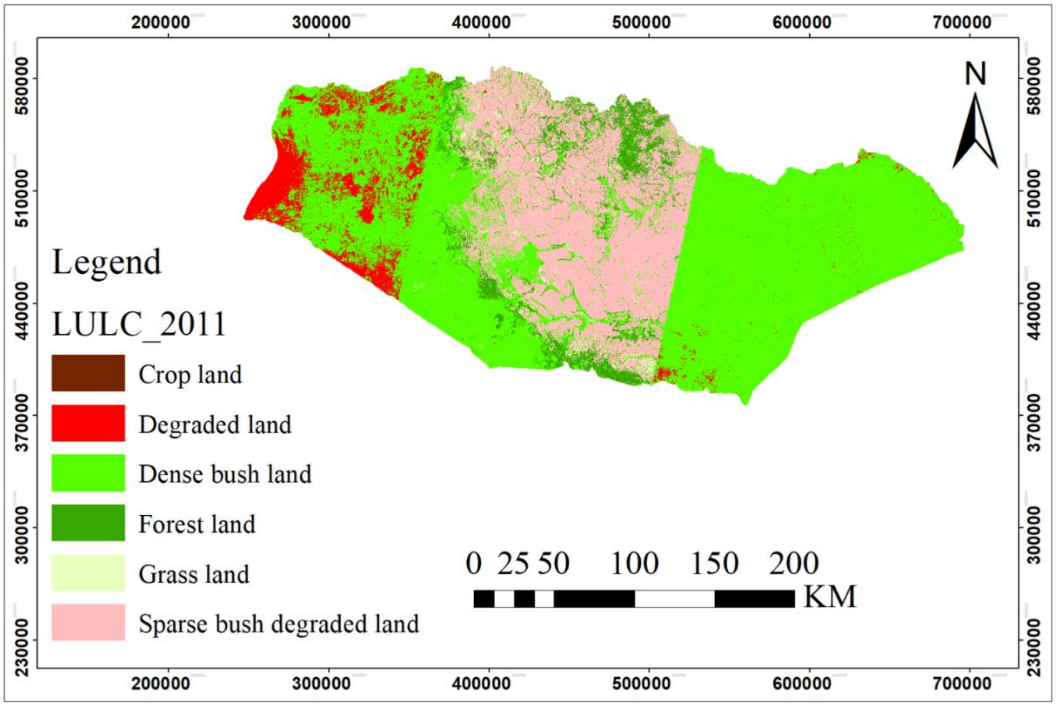

Figure 3. LULC map for the year 2011 of the study area

Table 3: Land use land cover classes and their corresponding areas for the year 2011

Land use land cover types	Area (ha)	Area (%)
Dense bush land	3316896.1	59.52
Sparse bush degraded land	1465395.41	26.30
Grass land	65771.75	1.18
Forest land	277419.8	5.0
Degraded land	368979.7	6.6
Crop land	78051.66	1.4

Land use land covers of the study area for the year of 2021

The largest area of the land use and land cover class in year 2021 was for sparse bush degraded land 2625430.35 ha (47.11 %) followed by dense bush land class 2323473.13 ha (41.70 %) and degraded land

341645.84 ha (6.13 %) respectively. Whereas, the aerial coverage of crop land and grass land were the least coverage of the study area which occupied about 114847.04 ha (2.06) and 6226.89 ha (0.11 %) respectively. Based on these result, mostly the traditional grazing landscapes of study areas were converted to sparse bush degraded land and dense bush land while grass land was extremely decline than any types of land use.

The result was similar to the responses of the pastoralist who indicated that increasing livestock population was the cause for weakening of rangeland management systems and increasing frequency of recurrent drought outbreaks and declining of the grass land in the study area. Besides this pastoralist had been believed on cultivation would be play a great role in reduction of grass land because most cultivated lands are the bottom land which has good grass development potential. Whereas total bush cover areas were increased due to improper bush control action application like lack of post managements. Both traditional burning and prescribed burning were not totally forgotten in Borana rangeland while it's the best methods of bush control techniques in rangeland areas. Resting of rangeland was not practices for both bush controlled and uncontrolled areas.

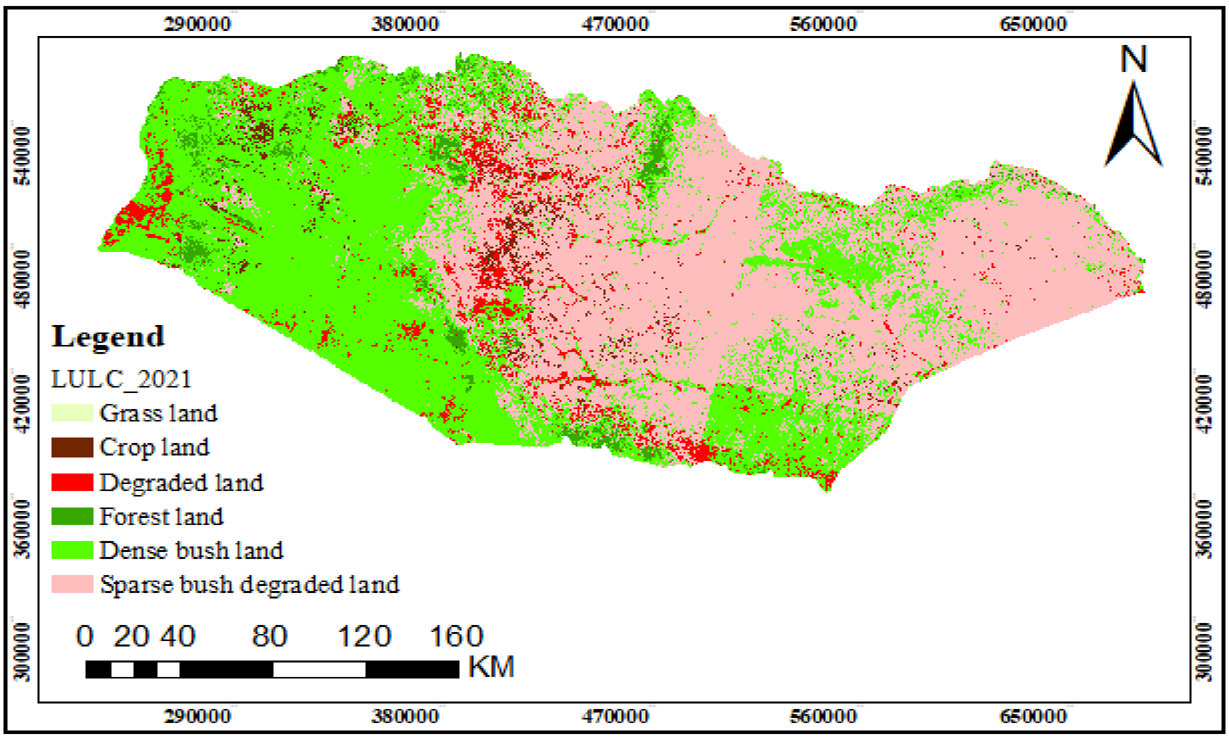

Figure 4. LULC map for the years 2021 of the study area

Table 4: Land use land cover classes and their corresponding areas for the year 2021

Land use land cover types	Area (ha)	Area (%)
Dense bush land	2323473.13	41.70
Sparse bush degraded land	2625430.35	47.11
Degraded land	341645.84	6.13
Grass land	6226.89	0.11
Forest land	160866.25	2.89
Crop land	114847.04	2.06

Trends of land use land cover classes with in the study periods

Land use land cover change of the study period indicated that dense bush land was declined from 53.60 % in 1991 to 38.77 % by year 2001 and increased again to 59.5 % in the year 2011and then become declined to 41.7 % in the year of 2021. The cover of dense bush land reduced from the year of 1991 to 2001 periods might be due to bush control interventions through bush thinning and again the dense of the bush become increased in the year of 2011due to lack of appropriate bush control techniques and then decline due to better bush management. The denser bush land area was dominated by Vachellia species like *V. mellifera, V. refeciens, V. oerfota and V. senegale.* .

The land use and land cover classes of sparse bush degraded land was gently increased from periods 1991 to 2021 year. This indicated that the sparse bush degrade land cover was increased due to overgrazing and increased frequency of recurrent drought. As the result the major rangeland of herbaceous biomass become reduced and encouraged bush encroachment. Even if governments aware the diversification of crop production improves the livelihoods of the pastoralists, the land use land cover change of crop land was increased slightly because of the climate condition of Borana rangeland. Similarly, In Ethiopia the conversion of rangelands into cultivated land has been reported (Garedew 2010; Flintan 2011), but the driving factors behind the on-going processes have not been thoroughly investigated.

On the other hands, in the study periods, the Borana rangeland of grass land cover change was gently decreased. In the year 1991, the proportion of grass land cover had the second rank (27. 39 %) and the grass lands were found in all areas of the study area. But within 10 years the grass land use land cover was decreased in half percent and become extremely decline in the year of 2011 and almost difficult to get pure grass land cover in the year of 2021. Only few area of original grass land of rangeland is left at Dida Harbala site in Eloye district in the year of 1991.

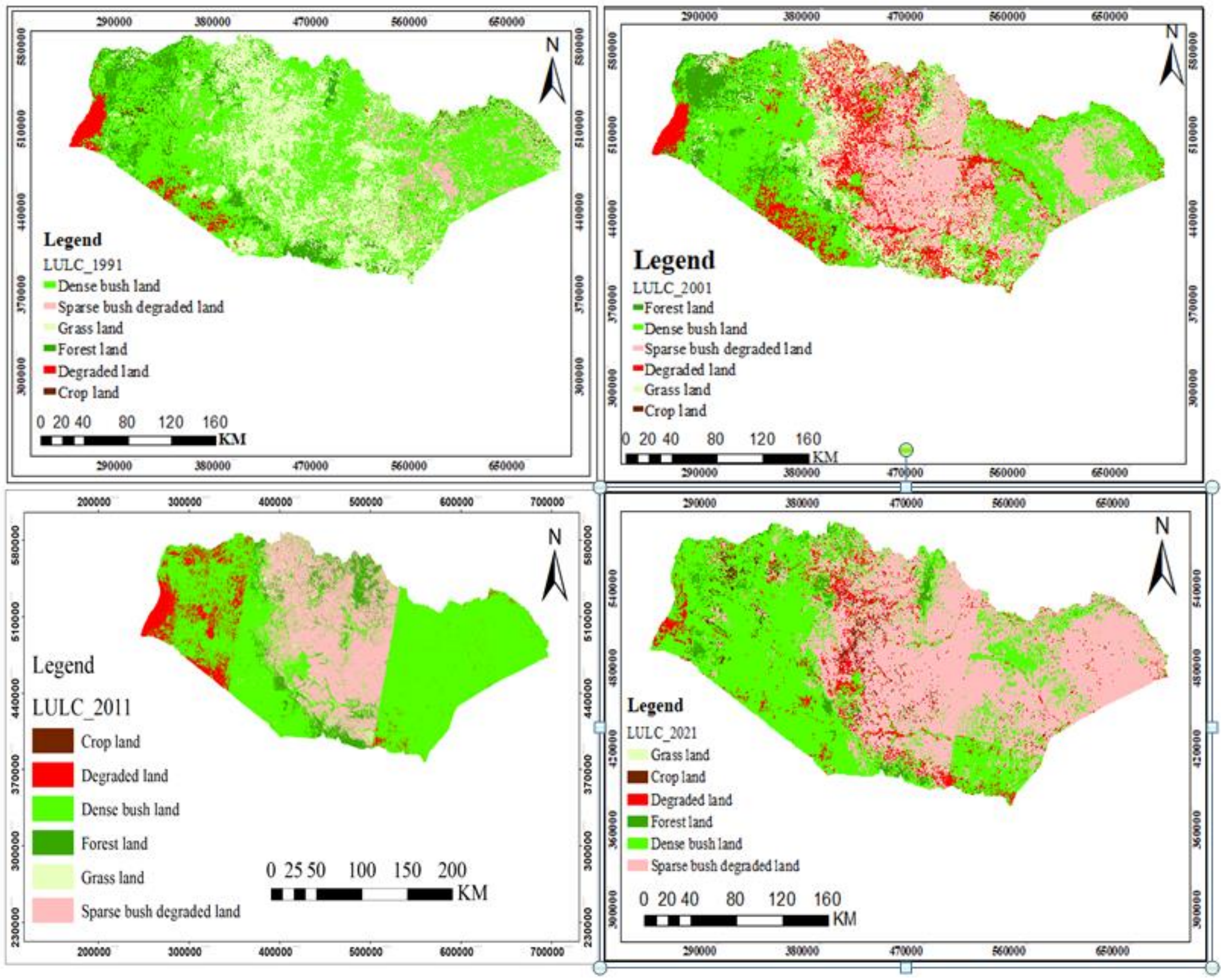

Figure 5. LULC map for the years 1991, 2001, 2011 and 2021 of the study area

Table 5: Land use land cover classes and their corresponding areas of all periods

Land use land cover types	1991		2001		2011		2021	
	Area (ha)	%	Area (ha)	%	Area (ha)	%	Area (ha)	%
Dense bush	2987096.9	53.6	2160408.1	38.8	3316896.1	59.5	2323473.1	41.7
Sparse bush degraded land	420834.4	7.6	1465582.0	26.3	1465395.4	26.3	2625430.4	47.1
Grass land	1526095.4	27.4	800705.8	14.4	65771.8	1.2	6226.89	0.11
Forest land	472533.8	8.5	332084.9	5.9	277419.8	5.0	160866.3	2.9
Degraded land	135467.9	2.4	748005.6	13.4	368979.7	6.6	341645.8	6.1
Crop land	30458.1	0.5	65203.3	1.2	78051.66	1.4	114847.1	2.1

This result was similar to pastoralist point of view response as they confirmed that the grass land was gently reduced due to heavy grazing pressure, recurrent drought outbreak and traditional rangeland burning management principle. Rangelands are experiencing rapid transformation due to continuous encroachments by alternative land users (influenced by the increased demand for land for both crop farming and settlements) and are also threatened by degradation and climate change (Berg et al., 2016; Godde et al., 2020; Herrick et al., 2013; Liao et al., 2018. These reduced the recovery of perennial grass species and increasing degraded and bush encroachment covers. Similarly forest land cover change was decreased from the year 1991 to 2021 mainly because of deforestation for the purpose of construction, crop expansion and animal feeds. The long dry season and drought outbreaks also aggravated the situation. Sparse bush degraded land was also increased as most of rangeland areas were dominated by *Acacia mellifera* , *Acacia reficiens* and species which degraded ground covers and coupled by heavy grazing and *Acacia mellifera* and *Acacia reficiens* bush encroachment covers.

Conclusion and Recommendation

Different land use land cover types in the study area of Borana rangelands includes bush encroachment land, bush degraded land, forest land, grass land and crop land. The identified land use categorized based on direction with timelines, some are declined whereas the others increased. Grass land and forest land were declined in study periods (1991, 2001, 2011 to 2021 years) while crop land, land degradation and bush land were increased. These resulted in shrinkage grazing land that lead to land degradation via over grazing and finally to feed scarcity in pastoralists' areas. Climate Variability (drought) and land management are the major factors that have effect on land use change. To improve the rangeland condition of the study area, indigenous grazing systems; dry season grazing, wet season grazing and reserving grazing that used during hard time (drought periods) could be strengthened in combination with government rangeland management plan. In general, land use planning and policy should be developed to separate land use based on its suitability for crop production, grazing lands and forest land.

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Adaptation trial of Oat (*Avena sativa*) Varieties in Two Agro-ecologies of Buno Bedele and Ilu Abba Bor Zones, South Western Oromia, Ethiopia

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Abstract

Shortage of feed is a critical problem for livestock production in Ethiopia. Thus, production of oat varieties that can be performed better at different agro-ecologies is very important to mitigate the feed shortage problem in study areas. The experiment was conducted to evaluate oat varieties for their herbage dry matter yield, seed yield and other agronomic traits under two agro ecologies of Buno Bedele (Chora & Gechi) and Ilu Aba Bor (Hurumu & Mettu) zones, South Western Oromia, Ethiopia. The trial was carried out at Gechi and Hurumu districts and Chora and Mettu districts which were purposely selected to represent highland and midland agro-ecologies, respectively for two consecutive years (2020 and 2021 G.C.) of the main cropping season. Seven oat varieties (Lamptom, CI-2806, Bonsa, Bate, CI-2291, Jassery and CI-8251) were laid out in randomized complete block design with three replications. Data on stand and vigor, plot cover percentage, dry matter yield, plant height, leaf to stem ratio, disease score, maturity date and seed yield were collected and subjected to general linear model procedures of Genstat software version 18.1 and least significance difference for data analysis and mean separation were employed respectively. The varieties had responded differently ($P<0.05$) at both highland and midland areas in dry matter yield, leaf to steam ratio, plant height and disease score but the seed yield and maturity date were not significantly difference in highland and midland areas respectively. The dry matter yield of seven oat varieties ranged 10.6-15.4 t ha^{-1} at highland and 9.5-13.8 t ha^{-1} at midland areas. The highest mean seed yield was recorded for varieties CI-2291 followed by CI-2806 and Bonsa in both agro ecologies. Generally, better dry matter yield and seed yield performances were recorded from varieties Bonsa, 2291, and 2806. Therefore, these oat varieties were recommended for the study areas and similar agro-ecologies.

Keywords: Dry matter yield, Leaf to stem ratio, Oat, Plant height, Seed yield, Varieties

Introduction

The livestock sector is a significant contributor to Ethiopia's economy at the national and household level. In Ethiopia, livestock is an integral component of the agricultural sector with a large bovine population which includes 59.5 and 60.9 million cattle and shoat, respectively (CSA, 2017). In addition to direct income benefits, livestock provides indirect benefits, such as fuel and fertilizer from animal manure and draught power for farm production. In spite of its significant contribution, the country's livestock productivity is low. In addition to animal health problems, lack of adequate quantity and quality of feed is a major factor in poor livestock productivity. Animal feed shortage remains the main constraint on herd size and productivity in both mid and highlands. The farmers face fodder deficiency in winter when they have only dry stalks of cereal fodder or dry summer grasses. Therefore, there is a direct need to maximize fodder production per acre which could be increased by adopting improved varieties and agronomic practices

Substantial efforts have been made so far to resolve the feed shortage problem in the Ethiopian highlands, aiming at improving feed availability and thereby improve livestock productivity. The available fodder supply is 1/3 less than the actual needs of animals (Younas and Yaqoob 2005). So that more nutritious and high yielding fodder varieties are needed to run an efficient livestock industry. Utilizing improved forage varieties like fodder oat has several advantages. Oat (*Avena sativa* L.) is one of the most important well-adapted fodder crops grown in the highlands of Ethiopia. It is dual purpose forage in many parts of the world (Mekonnen Y. and Ali S., 2013). The improved varieties of oats have potential to produce three-fold green fodder i.e. 60-80 t ha^{-1} and could feed double the number of animals per unit area as against the traditional fodder crops (Haqqani et al 2003). With the introduction of new high yielding oat varieties, the farmers have recognized oat as important fodder crop for filling the fodder gap (Habib et al 2003). On average, it contains around 10-12% protein, 5% fat, 12-14% fiber and 64% carbohydrate. Many oat cultivars have high feeding value if cut at its best harvesting time (50% flowering stage) and can meet the demand of rapidly growing livestock industry of Ethiopia. Hussain et al (2002) also found that oats variety fatua harvested at 50 percent flowering stage produced the highest green fodder and dry matter yields. It is produced by some pre-urban dairy cattle producers and smallholder farmers who own crossbred dairy cows. It is early maturing, palatable, succulent and energy rich crop. It can also be used as bedding for animals. Therefore, the current study was conducted with the objective to identify the adaptable and superior oat varieties under mid and high altitudes of Buno Bedele and Ilu Abba Bor Zones of South Western Oromia, Ethiopia.

Materials and methods

Description of the study areas

The experiment was conducted under rain-fed conditions during 2020 and 2021 of main cropping season in highlands and midlands of Buno Bedele and Ilu Aba Bor zones, South Western Oromia, Ethiopia. Two districts were selected purposively based on altitude, Gechi and Humuru districts from highland areas and Chora and Mettu were selected from midland areas. Gechi district lies at an altitude of 2014 m above sea level, whereas Hurumu district lies 1796-2580 m above sea level. Annual precipitation Gechi district ranges from 1500 to 2200 mm with 6 to 9 months of rainfall and daily temperature of the district varies from 12 to 35°C. The mean annual rainfall of Hurumu district is 1698.3 mm with an average temperature of 23 °C. Chora and Mettu districts are situated at an altitude of 1962 m above sea level and 1605 m above sea level respectively.

Experimental treatments and design

The study was designed to identify the best adapted and superior oat varieties. The experiment consisted of seven oat varieties namely: Lamptom, CI-2806, Bonsa, Bate, CI-2291, Jassery and CI-8251. The experiment was laid out in randomized block design with three replications on well prepared and leveled field. All the treatments were randomly allocated to different plots in each replication. A plot size of 3 m x 1.8 m (5.4 m^2) was used. . The spacing between rows and blocks were 30 m and 1 m, respectively. Each plot had 6 rows and the rows were 3 meters long and data was collected from the middle four rows and the two rows were served as a border. Drill method was used for seed sowing at a rate of 100 kg/ha. NPS fertilizer was uniformly applied at the rate of 100 kg ha^{-1} at sowing and urea was also uniformly applied once at the rate of 100 kg ha^{-1} after the establishment.

Agronomic Data Collection

Data was collected from two central rows for all parameters including emergency date, stand and vigor percentage, days to 50% flowering, plant height, falling percentage, dry matter yield, plot cover percentage, leaf to steam ration, disease/insect (1-5 score), maturity date and seed yield.

Statistical Data Analysis

The data recorded were analyzed using the Genstat software version 18.1and significant means separated by using least significance difference (LSD) at 5% probability level.

Results and Discussions

Combined analysis of variance for Dry matter yield of seven oat varieties tested at two agro-ecologies of Buno Bedele and Ilu Aba Bor Zones is given in Table 1. As the interaction between the varieties and the locations was significant, separate analysis for the factors is given the subsequent tables.

Table 1: Combined analysis of variance for Dry matter yield of seven oat varieties

Source of Variation	DF	Sum of square	Mean squares	F-value	p-value
Replication	2	170.22	85.11	7.10	
Varieties	6	310.23	51.71**	4.31	0.001
Location	3	437.51	145.84**	12.17	0.001
Varieties *Location	18	91.26	5.07	0.42	0.981
Error	138	1654.18	11.99		
Total	167	2663.41			

Dry Matter Yield (t/ha)

The result of combined analysis showed that herbage dry matter yield was significantly ($p<0.05$) affected by oat varieties. Bonsa and CI-2291varieties produced the maximum dry matter yield of 15.4 t ha^{-1} and 14.4 t ha^{-1}, respectively at highlands of the study areas (Table 2). Yet again, Bonsa and CI-2291 oat varieties produced optimum dry matter yield of 13.8 t ha^{-1} and 12.7 t ha^{-1} at midlands of the study areas (Table 3). Jassery variety was produced lower herbage dry matter yield at both highland and midland agro ecologies of the study areas.

Leaf to stem Ratio

The result of this study revealed that oat varieties significantly ($p<0.5$) affect leaf to stem ratio at both agro-ecologies. Nawaz et al (2004) also reported significant differences among the oat cultivars regarding green forage yield. The maximum leaf to stem ratio was recorded for CI-2291 (0.90), Bonsa (0.88) and followed by CI- 2806 (0.85) whereas minimum leaf to stem ratio was recorded for Bate variety (0.56) at midland of the study areas (Table 2). Plant leaves play a great role in growth and development of plants thereby influence forage biomass yield. This result agrees with (Gebremedhn *et al*., 2015).

Plan Height)

Plant height is a major factor contributing towards forage yield of different crops. There was significant variation recorded for plant height among oat genotypes in 2020 and 2021 cropping seasons (Table 2 and 3). The result obtained was in agreement with findings of Chohan *et al* (2004) that reported significant differences among the oat varieties regarding plant height. The differences among varieties in plant height were due to differences in genetic makeup and it is a foremost feature contributing towards forage biomass yield (Gebremedhin *et al.,* 2015). Lamptom variety showed the highest plant height (128.3 cm) followed by Bate variety (127.7 cm) while the lowest plant height (109.2 cm) was recorded by Jassery variety at highland areas of Gechi and Hurumu districts. Lamptom was also produced the highest plant height (111.2 cm) followed by Bate variety (109.6 cm) while the lowest plant height (94 cm) was recorded by Jassery at midland areas of Chora and Mettu districts.

Occurrence of disease and pests

The occurrence of disease was measured in 1-5 scale during the experimental period.. Leaf rust was occurred on Bate, Jassery and Lamptom varieties. Based on 1-5 scale, Bate, Jassery and Lamptom varieties scored a mean of 2.8, 2.6 and 2, respectively for leaf rust disease at highland areas of Gechi and Hurumu districts (Table 2). Therefore, Bonsa, CI-2291 and CI-2806 varieties scored 1 and showed to be relatively tolerant to leaf rust disease in the study areas (Table 2 and 3).

Seed Yield

The result of combined analysis showed that seed yield did not significantly ($p>0.05$) affected by oat varieties at highland areas (Gechi and Hurumu districts), while the tested varieties showed a significant ($P<0.5$) differences on seed yield at midland areas of Chora and Mettu districts (Table 2 and 3). Even though the seed yield was not differ significantly ($p>0.05$) among the oat varieties at highland areas, optimum seed yield of 31.02, 30.14 and 28.58 were recorded for variety CI-2291, CI-2806 and Bonsa, respectively at highland areas of Gechi and Hurumu districts (Table 2). Similar trends were observed at midland areas of Chora and Mettu districts, in that optimum seed yield of 26.01, 23.76 and 21.82 qt/ha were recorded in that order (Table 3). The lowest seed yield was recorded by Bate (15.15 qt/ha), Lamptom (16.23 qt/ha) and CI-8251 (16.3 qt/ha) varieties at midland areas of Chora and Mettu districts (Table 3). The mean seed yields of CI- 2806 variety obtained in highland areas of Gechi and Hurumu districts were higher than the 24.5 qt/ha obtained by Atumo Tessema and Kalsa Getinet (2020).

Table-2: Combined mean performance of forage yield and yield related components of Oats at Highland areas of Gechi and Hurumu Districts in 2020 and 2021

Varieties	Stand and vigor (%)	Plot cover (%)	Dry matter yield (t/ha)	Leaf stem ratio	Plant height (cm)	Disease incidence (1-5)	Maturity date	Seed yield (qt/ha)
Bonsa	83.7[ab]	87.1[abc]	15.4[a]	0.85[abc]	124.3[a]	1[c]	132.2[ab]	28.58
Bate	80.6[ab]	84.7[bc]	12.5[ab]	0.65[c]	127.7[a]	2.8[a]	126.6[b]	25.33
Lamptom	83.3[ab]	89.4[abc]	12.9[ab]	0.84[abc]	128.3[a]	2.0[b]	130.2[ab]	26.97
CI-2806	88.2[a]	92.0[a]	13.9[ab]	0.87[ab]	118.9[ab]	1[c]	132.6[ab]	30.14
CI-2291	87.2[a]	92.9[a]	14.4[a]	0.78[abc]	109.4[b]	1[c]	133.2[a]	31.02
Jassery	78.8[b]	83.2 [c]	10.6[b]	0.66[bc]	109.2[b]	2.6[a]	136.0[a]	24.71
CI-8251	87.2[a]	90.4[ab]	14.1[ab]	0.88[a]	124.8[a]	1[c]	132.3[ab]	28.53
Mean	84.2	88.5	13.4	0.79	120.4	1.62	131.87	27.90
CV (%)	11.9	9.7	33.7	31.9	12.4	20.9	5.8	30.7
LSD (5%)	8.14	7.01	3.67	0.20	12.17	0.27	6.23	6.96
P- value	*	**	*	*	**	**	*	NS

Table 3: Combined mean performance of forage yield and yield related components of Oats at Midland areas of Chora and Mettu Districts in 2020 and 2021

Varieties	Stand and vigor (%)	Plot cover (%)	Dry matter yield(t/ha)	Leaf stem ratio	Plant height (cm)	Disease incidence (1-5)	Maturity date	Seed yield (qt/ha)
Bonsa	80.2[ab]	83.2[ab]	13.8[a]	0.94 [a]	106.2[ab]	1.0[c]	133.1	21.82[ab]
Bate	82.8[ab]	86.8[ab]	10.8[bcd]	0.63 [b]	109.6[a]	2.3[a]	129.2	15.15[c]
Lamptom	84.8[ab]	88.5[a]	10.1[cd]	0.82[ab]	111.2[a]	1.9[b]	131.7	16.23[c]
CI-2806	87.2[a]	88.2[ab]	11.6[abc]	0.89 [a]	109.3[a]	1.1[c]	131.8	23.76[a]
CI-2291	85.4[a]	87.7[ab]	12.7[ab]	0.97 [a]	101.6[ab]	1.0[c]	133.4	26.01[a]
Jassery	76.4[b]	79.4 [b]	9.5[d]	0.79[ab]	94.0[b]	2.3[a]	133.8	18.25[bc]
CI-8251	85.2[a]	88.9[a]	11.9[abc]	0.87 [a]	110.7[a]	1.0[c]	133.3	16.13[c]
Mean	83.2	86.1	11.41	0.85	106.1	1.52	132.32	19.62
CV (%)	12.5	12.7	21.8	30.2	14.3	28.6	5.3	34.5
LSD (5%)	8.48	8.92	2.03	0.21	12.34	0.35	5.71	5.51
P- value	*	*	**	*	**	**	NS	**

Stability of Performance/Adaptability

The forage yield stability parameters for tested oat varieties foe two years at highland areas of Gechi and Hurumu districts and midland areas of Chora and Mettu districts were studied. Analysis of result of the dry matter yield using the comparison GGE Biplot shows that Bonsa and CI-2291 varieties were the most stable and desired varieties as compared to the other varieties at highland areas (Gechi and Hurumu districts) (Fig.1). Bonsa and CI-2806 varieties were mostly stable and desired varieties in dry matter yield as compared to the other varieties at midland areas (Chora and Mettu districts) (Fig.2). This resulted in a good adaptability compared to the remaining tested varieties in the test environments and similar agro-ecologies (Fig.1 and 2).

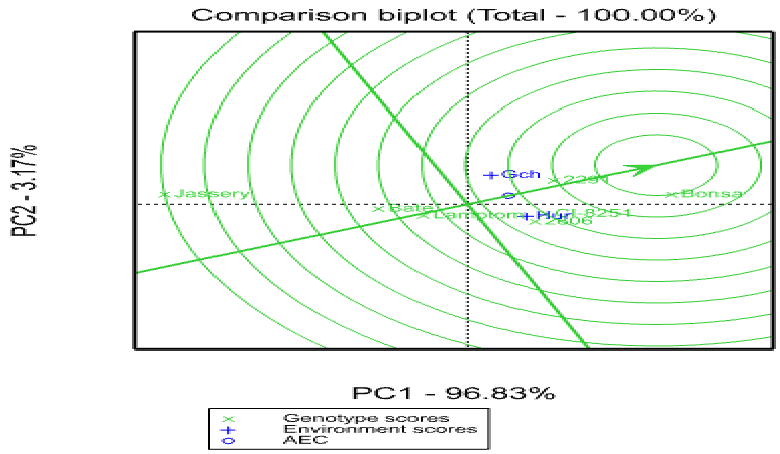

Fig.1: GGE Biplot analysis of Dry matter yield (t/ha) at highland areas Gechi and Hurumu districts

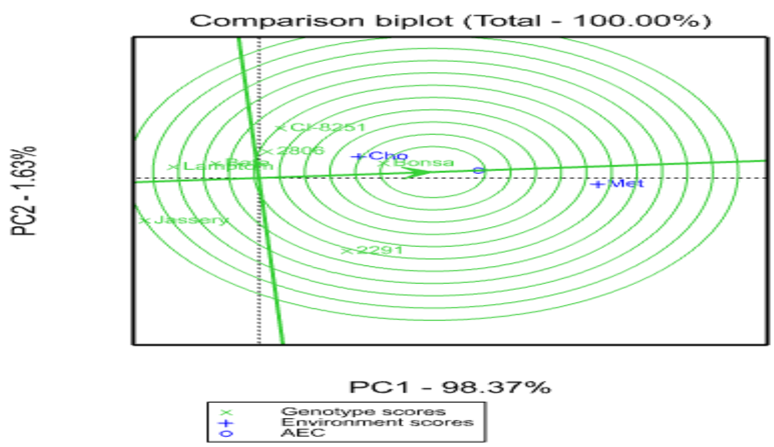

Fig.2: GGE Biplot analysis of Dry matter yield (t/ha) at Midland areas Chora and Mettu districts

Conclusions

Seven oat varieties were evaluated for herbage yield and yield related components during 2020 and 2021main cropping season in highlands and midlands of Buno Bedele and Ilu Aba Bor zones, South Western Oromia, Ethiopia.The result of combined mean performance shows that the tested oat varieties were varied significantly ($p<0.05$) on dry matter yield, leaf to steam ratio, plant height and disease score in both highland and midland agro ecologies of study areas. The mean value of seed yield in the highland agro ecologies of Gechi and Hurumu districts shows not significantly difference. The result of analyzed mean value shows that Bonsa, CI-2291 and CI- 2806 varieties records the maximum dry matter yielder than the other oat varieties at highland areas of Gechi and Hurumu districts and midland areas of Chora and Mettu districts.The maximum leaf to stem ratio was recorded for CI-2291, Bonsa and followed by CI-

2806 whereas minimum leaf to stem ratio was recorded for Bate variety at midland of the study areas. The highest plant height was recorded by Lamptom and Bate variety while the lowest plant height was recorded by Jassery variety at both highland and midland of the study areas. Leaf rust has occurred on the varieties of Bate, Jassery and Lamptom. Bonsa, CI-2291 and CI-2806 varieties were found to be more tolerant to the major diseases at highland and midland agro ecologies of the study areas. Results of the combined mean value of CI-2291, CI- 2806 and Bonsa varieties showed that these varities are high seed yielders when compared to the other oat varieties in both highland and midland agro ecologies of study areas. The lowest seed yield was recorded by Bate, Lamptom and CI-8251 varieties at midland areas of Chora and Mettu districts.

The result of this study revealed that Bonsa, CI-2292 and CI-2806 varieties proved to be the highest dry matter and seed yielders which reflects that they are better fodder producing varieties. The are proved to be superior varieties with respect to getting higher dry matter and seed yields in the study areas. The seed yield variation among the tested oat varieties that were grown in similar environments may be due to genetic potential of the varieties and their adaptability. Therefore, Bonsa, CI-2291, and CI-2806 varieties were recommended as the best options for green fodder, dry matter and seed yields in the study areas and similar agro-ecologies of Buno Bedele and Ilu Abba Bor Zones.

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Adaptation Trial of Alfalfa (*Medicago sativa*) Varieties in Selected districts of West Hararghe Zone, Oromia, Ethiopia

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Abstract

*Ethiopia has the largest livestock population in Africa. The sector supports a million people in the country. But, the sector is primarily challenged by animal feed in terms of quality and quantity. So, it is important to introduce different improved forage at the smallholder farmer level. The experiment was conducted in West Hararghe Zone, Oromia, Ethiopia. The trial was conducted at mid to highland agro-ecologies for two consecutive major cropping seasons, 2020 to 2022 years aimed to evaluate, identify and recommend the best adaptable, high biomass yielders and drought tolerance alfalfa varieties for the study area. Five alfalfa varieties were used as experimental treatments in a randomized complete block design (RCBD) with four replications. All agronomic and yield parameters (*50% flowering date, disease incidence, pest incidence, stand vigor, plant height, Green biomass yield, Leaf stem ratio, dry matter yield and plant height*) showed statistically significant ($p<0.05$) variation among varieties except plot cover. The highest mean dry matter yield was recorded for Arba Rakate FTC (5.51 t/ha), Kuni Segeria (3.62 t/ha), and on station (3.42 t/ha) with a combined mean of 4.18t/ha. The highest combined mean dry matter was recorded from the variety Hair Peruvian (4.82 t/ha) followed by Magna#788 (4.16 t/ha) and the lowest dry matter was recorded from Magna#801 (3.9 t/ha) and the fresh biomass yield varied from 14.02 to 17.34 t/ha with the average of 15.16 t/ha that the highest mean fresh biomass yield recorded from Hair Peruvian (17.34t/ha) followed by hunter river (15.30 t/ha) and the lowest recorded from Magna#801 (14.02 t/ha). The combined mean plant height ranged from 68.6 to 82.65 cm with a mean of 78.95 cm. The highest combined leaf-to-stem ratio was recorded from Magna#801 (60.73) followed by Magna#788 (54.06) and the lowest was recorded from Hunter river (49.53). The mean disease occurrence varied from 1.18 to 1.88. The most susceptible varieties were Magna#788 (1.88) and Magna#801(1.81) while the tolerance varieties were werer (1.18) and Hair Peruvian (1.25). From this study, it is concluded that Hair Peruvian followed by Hunter River were found more promising in terms of agronomic traits, DM yield, and fresh biomass yield than others during the main rainy season and popularized as an alternative feed resource under smallholder conditions in the study areas and other places of similar climatic and edaphic conditions*

Kew words: *Alfalfa varieties, Dry matter yield, fresh biomass and west Hararghe*

INTRODUCTION

Ethiopia has the largest livestock population in Africa, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels and 49 million chickens (CSA, 2020a). The national herd supports, at least in part, the livelihoods of more than 11.3 million rural households, including 27– 35% of the highland livestock keepers, and a large proportion of the lowland herders, who live below the Government of Ethiopia established poverty line (Shapiro *et al.*, 2017). The sector contributed up to 40% of agricultural Gross

Domestic Product (GDP), nearly 20% of total GDP, and 20% of national foreign exchange earnings (World Bank, 2017). The productivity of this sector is constrained by several factors, including poor quality and varying seasonal availability of feed, poor genetics, low reproductive performance, high disease incidence and parasite challenges, and low accessibility to services and inputs (Zeleke and Harris, 2021). Green pasture (55.2%) and crop residues (30.8%) are the main feed types available in the country (CSA, 2020a). The available feed resources in the mixed crop-livestock production areas are natural pastures, crop residues, and to a lesser extent, improved forage, concentrates, and nonconventional feeds (CSA, 2020a). Animals are fed on crop stubble during harvesting seasons. In some places, improved forage is cultivated and fed to dairy cows to increase milk production, but this practice is not widespread (Dawit *et al.,* 2012).

Among the improved forage alfalfa is the most influential in animal production industry. It is the most widely cultivated forage legume worldwide (Neima and Mustafa, (2016) and is widely known as the "queen of the forages" due to its ability to consistently produce high-yielding, high-quality, persistent, profitable potential if given adequate management and adaptability to different climatic conditions (Turan *et al.,* 2009; Lacefield and Lacefield (2004) and the most important forage legume in the world, grown in more than 30 million hectares (Annicchiarico, 2015). It is a valuable crop because of numerous agronomic and environmental advantages in terms of preserving soil fertility and biodiversity, soil erosion protection, mitigation of climate change impacts, reduction of groundwater nitrate pollution, fossil fuel consumption, greenhouse gas emissions (Vasileva *et al*., 2015; Shi *et al.,* 2017). Alfalfa is one of the most palatable forages, providing high energy and protein for dairy and beef cows as well as other types of livestock. It is an "engine of human food production," eventually transformed into milk, cheese, and meat, wool and honeys (Neima and Mustafa, 2016).

Livestock production is one of the major components of agriculture in Ethiopia, and it is highly dependent on the quantitative and qualitative adequacy of feed resources (Alemayehu *et al.,* 2017). Feed shortage has been a chronic problem for animal production in pastoral area of Ethiopia (Denbela, 2015). To reduce the nutritional constraints on livestock productivity, the use of adaptive, high yielding, and drought-tolerant improved forages of high quality is important (Alemayehu *et al.,* 2017). The major livestock feed resources in Ethiopia are crop residues, natural pasture and crop aftermath. West Hararghe feed shortage is accounts 75.7%, Animal health (4.8%) and feed cost (3%) (Abdi *et al.,* 2013). Previous studies in various parts of West Hararghe Zone Fekede *et al.* (2016) and Fikadu and Asfaw (2017) reported that the major constraints of cattle keepers showed that feed shortage is ranked the first.

Hence, introducing different improved forage through adaptation is the quick and inclusive mechanisms in the study area. Among the improved forage crops alfalfa (Medicago sativa L.) could play an important role in providing a significant amount of quality forage, both for the smallholder farmer as well as intensive livestock production systems with appropriate management practices. So, this activity was initiated with the objective of to evaluate, identify and recommend the best adaptable, high yielder and drought tolerance alfalfa varieties for the study area

MATERIALS AND METHODS

Description of the study area

The study was conducted in West Hararghe zone mid to high land parts which include Chiro, Gemechis from high land and Daro Lebu from mid land districts as alfalfa has wide range of agro ecologies adaptation (Turan *et al.,* 2017). These districts are describes as follow:-

Chiro district is located at 9°05′N and 40°52′E. with an average altitude 1826 above sea level. Chat is an important cash crop of this district. Coffee is another important cash crop, with over 5,000 hectares is planted with this crop Fikadu and Asfaw Zewdu (2017). The district is mainly characterized by steep slope and mountains with rugged topography, which is highly vulnerable to erosion problems (Fekede *et al.,* 2018). It has maximum and minimum mean rainfall of 1800 and 900 mm respectively (Gosa, 2016). The rainfall type is bimodal and erratic. Main rainy season is from June to September for the high land areas and from March to April for midland and that of lowland around July. The amount of rainfall is relatively adequate the highland and midland than the lowland (Fekede *et al.,* 2018).

Daro Labu district is located at latitude of 40°30′ E and 8°10′ N and Mecahara Agriculture Research Center found at 08° 35' E longitude and 40° 19' latitude with an altitude of 1,700 m above sea level. The district is located at 434 km and 111km to East of capital city of the country, Addis Ababa and Chiro, capital city of the zone respectively. The major soil texture of the center is sandy loam with reddish color. The ambient temperature of the district ranges from 14 to 26°C with the average of 20°C with average annual rainfall of 1094 mm/year

Gemachis district is also found in West Hararghe Zone, Oromia National Regional State, and eastern part of Ethiopia. The district is located about 343 km southeast of Addis Ababa and 17 km from Chiro town, the capital of West Hararghe Zone. It is located at 8°10′N latitude and 40° 45′E longitude longitudes in the East. The district covers an area of 77,785 ha and it has 35 rural kebeles and 3 urban administrative towns (GDoANRO, 2016).

Experimental design and layout

The experiment was conducted under rain fed conditions during the main cropping season for two consecutive (2020 – 2022) years in selected districts of West Hararghe zone. Accordingly, three districts, which includes Gemechis, Chiro and Daro Lebu were selected and used to conduct this experiment. From Gemechis: Kuni segeria FTC, from Chiro: Arba Rakate FTC and from Daro Lebu: Mechara Agricultural Research Center, on station was used as experimental sites. The planting material used for this was five alfalfa varieties which include Werer, Magna#788, Magna#801, Hair Peruvian and Hunter river varieties collected from Werer and Adami Tulu Agricultural Research Center. After the sites had been selected, land preparation was started from April to June through tractor for first time and oxen at second and third times. The sowing date was at the first week of July with plot size of 2*2m using a randomized complete block design (RCBD) with three replications. The space between block, plot and row was 1m, 1m and 0.25m respectively. 8kg/ha seed rate was used through hand drilling with fertilizer rate of 100 kg/ha NPS and 50 kg/ha UREA at the time of sowing. All other crop management practices like weeding, hoeing and guarding were done uniformly to all plots as required.

Data collection

The collected data for the trial was included 50% flowering date, plot cover, stand vigor, leaf to stem ratio, herbage yield, dry matter yield, disease occurrence, pest infestation and plant height. From each plot, 220 gram samples of Alfalfa varieties were taken and dried in a forced draft oven dry at 105°C for 24 hours to get constant weight of dry matter yield.

Model and Statistical analysis

Agronomic data was analyzed using ANOVA by the general linear model procedure of SAS, 2002 version 9.0. Means were separated using Least Significant Difference (LSD) at 5% significant level.

The model: Yijk = μ + Gi + Ej + Bk(j) + (GE)ij + eijk

Where, Gi = Variety effect, Ej = Environmental effect, Bk(j) = Block effect, GEij = Variety and Environment interaction, μ = the overall mean and eijk = random error

RESULTS AND DISCUSSIONS

The results of the analysis of variance indicated significant effects of alfalfa varieties on most of the tested variables (Table 1). The interaction effect of Varieties, locations and years showed highly significant ($p<0.001$) variation for 50% flowering, disease incidences, pest incidence, stand vigor, plant height, green biomass yield, Leaf stem ratio and dry matter yield. However, no significant ($p>0.05$) differences were observed for plot cover. Varieties were highly significant ($p<0.001$) variations between the years for all parameters. This might be due to the variations of rainfall distribution during the experimental years. Similarly, highly significant ($p<0.001$) variations observed between the years for almost all parameters except plot cover and green biomass yield which was showed non-significant difference. The mean square of location also showed a significant ($p<0.05$) variation on all parameters except plot cover and dry matter yield.

Table1: Combined Analysis MSS of ANOVA for agronomic parameters

Source Variation	DF	FD	DI	PI	PC	SV	PH	LSR	BMtha	DMtha
Varieties	4	7.39^{NS}	1.58^{***}	0.82^{***}	1762.61^{***}	8.29^{***}	989.75^{***}	313.17^{**}	50.85^{*}	2.81^{*}
Replication	3	4.97^{NS}	0.35^{NS}	0.21^{NS}	128.81^{NS}	0.15^{NS}	136.95^{NS}	143.91^{NS}	25.27^{NS}	0.52^{NS}
year	1	884.5^{***}	24.2^{***}	21^{***}	610.51^{NS}	7.81^{**}	1230.09^{**}	17282.2^{***}	21.82^{NS}	76.4^{***}
Location	2	4774^{***}	31.25^{***}	30.01^{***}	918.01^{NS}	7.81^{**}	8366.09^{***}	1169.15^{***}	1895.6^{***}	0.79^{NS}
Variety*loc.	8	15.98**	0.49NS	0.76***	169.77^{NS}	0.99^{NS}	108.13^{NS}	45.41**	82.97NS	1.83^{NS}
Variety *year*loc	14	132.16^{***}	1.36^{***}	1.03^{***}	249.98^{NS}	1.26^{*}	600.67^{***}	427.42^{***}	54.09^{*}	3^{***}

*NS = Non-significant, * = (p<0.05), ** = (p<0.01), *** = (p<0.001), DF = degree freedom, 50%F = 50% flowering date, DI= disease Incidence, PI = pest incidence, PC= plot cove, SV= stand vigor, PH= plant height in cm, GBMYtha = Green biomass yield tone/ha, LSR = Leaf stem ratio, DMtha = dry matter yield tone/ha and PH = plant height in cm.*

Dry matter and Fresh biomass Yield

Forage dry matter yield and fresh biomass yield did not showed significant (P>0.05) variation among the tested alfalfa varieties across the locations except the Kuni Segeria FTC (Table 2). The result indicated that the highest mean dry matter yield recorded from on station was (3.42 t/ha), Arba Rakate FTC (5.51 t/ha) and Kuni Segeria (3.62 t/ha). Combined analysis indicated that dry matter yield varied significantly (P<0.05) among the tested varieties. The significant varieties differences observed for dry matter yield of alfalfa in this study is in line with reports of (Gezahagn *et al.*, 2017; Tessem *et al.*, 2021; Denbela, 2015). From the present findings, dry matter yield ranged from 3.9 to 4.82 t/ha with a mean of 4.18 t/ha. This finding is lower than the mean yield of 6.46 t/ha reported by Gezahagn *et al.* (2017), 6.5 t/ha reported by Denbela, (2015), 7.01 t/ha reported by Tessem *et al.* (2021) and Alemu *et al.,* (2022) 20.46. The reverse, dry matter yield ranged from 1.78-3.23 t/ha Afsharmanesh, (2009) and from 0.67-2.16 t/ha were reported Awad and Bakeri, (2009) which were indicate lower than the present findings. Generally, Hair Peruvian gave the highest dry matter yield and fresh biomass and fresh biomass yield (t/ha) yield. The wide range of dry matter yield observed in different research findings could be attributed to varietal and environmental differences, their interactions, harvesting stage, soil types, management practice and other biotic and abiotic factors.

Combined analysis indicated that fresh biomass yield varied significantly (P<0.05) among the tested varieties. The highest mean fresh biomass yield recorded for Hair Peruvian (17.34t/ha) followed by Hunter river (15.30t/ha) and the lowest mean fresh biomass was recorded for Magna#801(14.02t/ha with the average of 15.16 t/ha. Generally, Hair Peruvian gave the highest fresh biomass yield. The present finding is lower than the mean yield 37.95t/ha reported by Turan, (2017); 29.83 t/ha reported by Denbela, (2015); 66.18 t/ha reported by Tucak *et al.* (2020); and 78.16t/ha reported by Alemu *et al.* (2022). This high forage production difference from other findings indicated that other potential varieties are must be evaluated for the study area.

Table 2: Mean Dry matter and Fresh biomass yield of Alfalfa varieties tested across locations

Varieties	**Dry matter yield (t/ha)**				**Fresh biomass yield (t/ha)**			
	On station	A/Rakate FTC	K/Segeria FTC	Combined mean	On station	A/Rakate FTC	K/Segeria FTC	Combined mean
Werer	3.38	5.25	3.57^{b}	4.07^{b}	8.98	16.7	17.8^{bc}	14.49^{ab}
Magna#788	3.36	6.32	2.81^{b}	4.16^{b}	9.15	20.6	14.2^{c}	14.65^{b}
Magna#801	3.53	4.85	3.33^{b}	3.9^{b}	9.48	15.4	17.19^{bc}	14.02^{b}
Hair Peruvian	3.54	6.03	4.9^{a}	4.82^{a}	9.13	19.1	23.8^{a}	17.34^{a}
Hunter river	3.31	5.09	3.51_{b}	3.97^{b}	8.48	16.55	20.88^{ab}	15.30^{ab}
Mean	3.42	5.51	3.62	4.18	9.04	17.67	18.78	15.16
CV	23.65	25.48	23.83	24.32	24	23.9	29.24	31.38
LSD	0.83	2.16	0.89	0.86	2.23	6.51	5.63	3.79
P level	NS	NS	***	***	NS	NS	*	***

FTC = farmer training Center, NS = Non-significant, * = (p<0.05), ** = (p<0.01), *** = (p<0.001), CV= CV= Coefficient variation, LSD = Least Significant difference, A/Rakate= Arba Rakate, K/segeria=Kuni Segeria

Plant height and Leaf stem ratio

Mean plant height of alfalfa varieties were significantly (P<0.05) different across all testing locations (Table 3). The result showed that the tallest mean plant height recorded for on station (56.32 cm), Arba Rakate FTC (78.95 cm) and Kuni Segeria FTC (76.77 cm). Hair Peruvian variety produced the tallest plant height (66.48 cm) at on station while Werer produced the tallest plant height at Arba Rakate FTC (82.65 cm) and Kuni Segeria FTC (85.65cm). On the other hand, Magna#801 produced the shortest plant height at on station (47.7 cm) and Arba Rakate FTC (68.6 cm) while Hunter river produced the shortest at Kuni Segeria (73.61 cm). Combined analysis for plant height also differed significantly (P<0.05) which ranged from 68.6 to 82.65 cm with a mean of 78.95 cm. The mean plant height of the present result is lower than the report of Teshale and Ketema (2021) 89.7 cm, Turan, (2017) 80.4 cm but higher than the mean report of Tucak *et al.* (2020) 63.94 cm, and Alemu *et al.* (2022) 52.61 cm This variation could be due to the differences in moisture content and soil fertility condition of the testing locations. Generally, variety Magna#788 gave the highest mean plant height followed by Werer while Hunter river gave the lowest plant height.

The mean leaf stem ratio were no showed significantly (P>0.05) variation at two locations (on station and Arba Rakate FTC) but significance (P<0.05) variation were recorded from Kuni Segeria FTC. But the combined mean of leaf stem ratio of alfalfa varieties were showed significantly (P<0.05) different (Table 3). The highest combined leaf to stem ratio was recorded from Magna#801 (60.73%) followed by Magna#788 (54.06%) and the lowest was recorded from Hunter river (49.53%). The present finding is in line with the findings of Teshale and Ketema, (2022) which reports from 0.54 to 0.7 ratios.

Table 3: Mean plant height (cm) and Leaf stem ratio of Alfalfa varieties tested across locations

Varieties	Plant height (cm)				Leaf stem ratio			
	On station	A/Rakate FTC	K/Segeria FTC	Combined mean	On station	A/Rakate FTC	K/Segeria FTC	Combined mean
Werer	59.5^{ab}	82.65^{a}	85.65^{a}	82.65^{a}	55.63	48.75	45.63^{bc}	50.63^{b}
Magna#788	50.28^{cd}	81.55^{a}	75.1^{ab}	81.55^{a}	56.25	46.25	51.88^{b}	54.06^{b}
Magna#801	47.7^{d}	68.6^{b}	64.4^{a}	68.6^{b}	62.5	48.75	58.96^{a}	60.73^{a}
Hair Peruvian	66.48^{a}	81.05^{a}	85.08^{a}	81.05^{a}	55.63	47.5	48.75^{bc}	52.19^{b}
Hunter river	57.63^{bc}	80.9^{a}	73.61^{b}	80.9^{a}	56.25	46.25	42.81^{c}	49.53^{b}
Mean	56.32	78.95	76.77	78.95	57.25	47.5	49.6	53.43
CV	13.13	9.49	14.28	9.49	16.98	11.29	13.12	16.09
LSD	7.59	11.54	11.25	11.54	9.97	8.26	6.68	6.09
P level	***	*	**	*	NS	NS	***	***

*FTC = farmer training Center, NS = Non-significant, * = (p<0.05), ** = (p<0.01), *** = (p<0.001), CV= CV= Coefficient variation, LSD = Least Significant difference, A/Rakate= Arba Rakate, K/segeria=Kuni Segeria*

Diseases and Pest Infestation

Mean diseases occurrence and pest infestation of alfalfa varieties were showed significantly (P<0.05) different at all experimental sites except at Arba Rakate FTC but the combined mean disease occurrence for both diseases occurrence and pest infestation showed significantly (P<0.05) variation for all research sites (Table 4). The diseases recorded were Lepto leaf spot and Stemphylium leaf spot. The mean diseases occurrence varied from 1.18 to 1.88. Relatively susceptible varieties were Magna#788 (1.88) and Magna#801(1.81) while the tolerance varieties were werer (1.18) and Hair Peruvian (1.25). Teshale and Ketema, (2021) reported that Magna- 801-FG and Peruvien DZF- 406 were resistant to diseases that showed Peruvien are most tolerant that similar with the present findings. The findings of Teshale and Ketema, (2021) also indicated that disease occurrence of alfalfa varieties ranged from 0.7 to 2.17.

Lepto leaf spot

Stemphylium leaf spot

Pest infestation is the most factors that reduce both forage quality and quantity. The mean pest infestation varied from 1.06 to 1.56. Relatively varieties attacked by pest (aphids) were Magna#801 and Magna#788 (1.56) followed by Hunter river (1.44) while the lowest pest infestation were recorded from Hair Peruvian (1.06) variety which almost free of pests that similar results with the findings of Putnam *et al*. (2001) all alfalfa varieties were less affected by pests as alfalfa has tremendous genetic resistance to many pests.

Table 4: Mean disease occurrence and Pest Infestation of Alfalfa varieties tested across three locations/environments

Varieties	**Diseases Occurrences**				**Pest Infestation**			
	On station	A/Rakate FTC	K/Segeria FTC	Combined mean	On station	A/Rakate FTC	K/Segeria FTC	Combined mean
Werer	1.88^{c}	1.25	0.5^{b}	1.18^{c}	1.88^{c}	1	0.5^{c}	1.19^{bc}
Magna#788	2.75^{a}	1.25	1^{ab}	1.88^{a}	2.63^{a}	1	0.5^{c}	1.56^{a}
Magna#801	2.5^{ab}	1.5	1.13^{a}	1.81^{ab}	2.13^{b}	1	1^{ab}	1.56^{a}
Hair Peruvian	1.63^{c}	1.5	0.88^{ab}	1.25^{c}	1.5^{c}	1	0.63^{bc}	1.06^{c}
Hunter river	2b^{c}	1.5	1^{ab}	1.5^{bc}	1.75^{c}	1	1.15^{a}	1.44^{ab}
Mean	2.15	1.4	0.9	1.53	1.98	1	0.75	1.36
CV	24.18	23.51	56	34.14	21.29	0	55	33.14
LSD	0.53	0.51	0.52	0.37	0.43	0	0.42	0.33
P level	***	NS	*	***	***	NS	*	***

FTC = farmer training Center, NS = Non-significant, * = ($p<0.05$), ** = ($p<0.01$), *** = ($p<0.001$), CV= Coefficient variation, LSD = Least Significant difference, A/Rakate= Arba Rakate, K/segeria=Kuni Segeria

Seed Yield at Different Harvesting year

There were a significance ($p<0.05$) variation in terms of seed yield for two year harvesting at on station. The mean maximum seed yield (737.9 kg/ha) was recorded from Werer alfalfa variety (702.6 kg/ha) followed Hair Peruvian variety whereas the lowest mean seed yield (65.8 kg/ha) was recorded form Magna#801 variety (Table 5). This result is higher than the reports of Saprykin *et la*. (2021) at first and second (83 -122 Kg/ha) and (180 -250 kg/ha) harvesting year respectively. Alfalfa seed yield setting is the major problems in the country as a general that only one location (on research station) gives the seeds both research years.

Table 5: Mean seed yield (Kg/ha) of Alfalfa varieties tested on research station

	Harvesting Time		
	First year harvesting (kg/ha)	Second year harvesting (kg/ha)	Combined mean yield(kg/ha)
Werer	98.31[a]	1377.5[a]	737.9[a]
Magna#788	**50.75[ab]**	**188.1[c]**	**119.4[c]**
Magna#801	32.75[b]	98.8[c]	65.8[c]
Hair Peruvian	77.63[ab]	1327.5[ab]	702.6[ab]
Hunter river	80.50[ab]	597.5[bc]	339.0[bc]
Mean	67.98750	717.88	392.93
CV	60.96416	66.12	61.49
LSD	63.857	731.27	372.28
P level	*	**	**

NS = Non-significant, * = ($p<0.05$), ** = ($p<0.01$), *** = ($p<0.001$), CV= Coefficient variation, LSD = Least Significant difference

Seed production is increasing from first year to second year harvesting (fig. 1). The yield of perennial crops increases.

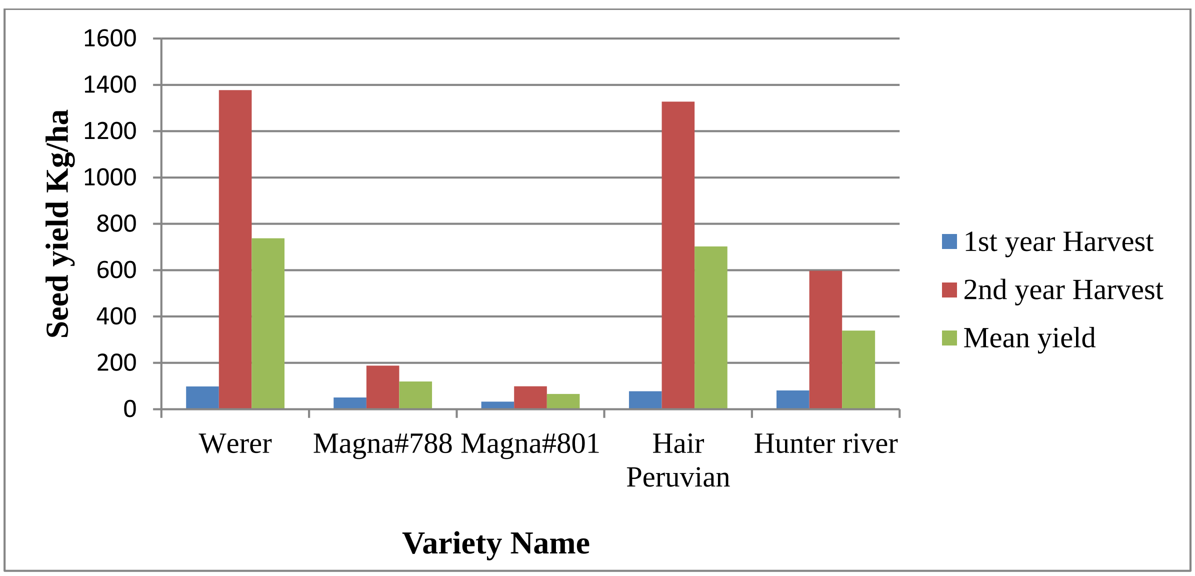

Figure 1: - Seed yield (Kg/ha) of Alfalfa Varieties for two harvesting year on station

Conclusions and Recommendations

Livestock production is challenged by animal feed both in terms of quality and quantity in West Hararghe. To solve these challenges, it is important to introduce different improved forage to the study area. Five alfalfa varieties were introduced to evaluate the arability. The highest mean dry matter was recorded from Hair Peruvian (4.82 t/ha) followed by Magna#788 (4.16 t/ha) and the lowest dry matter was recorded from Magna#801 (3.9 t/ha). The fresh biomass yield varied from 14.02 to 17.34 t/ha with the average of 15.16 t/ha that the highest mean fresh biomass recorded from Hair Peruvian (17.34t/ha). The mean diseases occurrence varied from 1.18 to 1.88 that the most susceptible varieties were Magna#788 (1.88) and Magna#801(1.81) while the tolerance varieties were werer (1.18) and Hair Peruvian (1.25). From this study, it is concluded that Hair Peruvian followed by Hunter River were found more promising in terms of agronomic traits, DM yield, and fresh biomass yield than others during the main rainy season and popularized as an alternative feed resource under smallholder conditions in the study areas and other places of similar climatic and edaphic

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On-station Evaluation of biomass Yield and Nutritive Value of Perennial Grasses intercropped with Multipurpose Tree Forages

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Abstract

The experiment was conducted at Adami Tulu Agricultural Research Center with the objectives to determine the effect of integration of grasses and legumes on the growth, total forage biomass yield, nutritive quality and identify compatible forage varieties in their integrations with rain fall and supplementary irrigation at dry season. Factorial combination of three forage grasses (Panicum maximum, Chloris gayana and Cenchrus ciliaris) and two multipurpose tree forages (Cajanus cajan and Leucaena leucocephala) were arranged in Randomized Complete Block Design with three replications. Plant heights for both forage showed significant (p<0.001) difference among treatments and cuttings. From MPTFs and grasses the maximum plant height was recorded from Leucaena sole and Chloris gayana integrated with cajanus cajan with values of 186.14 and 124.50cm, respectively. The highest forage biomass yield (7.39 t/ha) was obtained from cajanus cajan integrated with Rhodes gras. In combined result also this integration of Rhodes grass and cajanus cajan gave high (29.20 t/ha) forage biomass yield. The highest LER (2.05) was obtained from the integration of Rhodes grass and cajanus Cajun. Ash and organic matter contents showed significant (P< 0.05) difference except for CP contents of grasses united with MPFLs. Among the tested forage components, Chloris gayana integration with Cajanus cajan was recommended for the study areas and similar agro-ecologies due to its high total forage biomass yield and other agronomic performances

Key words: - Alley farming, Biomass yield, Multipurpose Tree Forage, Nutritive quality and Perennial grass

Introduction

The major problem facing livestock production in the study area is insufficient quality pasture due to seasonal fluctuation and expansion of cropping land (Assefa *et al.*, 2013). The main feed resources for livestock are natural pasture, crop residues and crop aftermath, which are low in quantity and quality for sustainable animal production throughout the year (Husein and Diriba, 2021). In order to solve the shortage of feed and improve livestock productivity, it is necessary to produce and cultivate high-quality forages with high yielding ability.

Among the improved and the adaptable forage grass in the study area, *Panicum maximum, Chloris gayana and Cencheris ciliaris* grasses could play an important role in providing high biomass yield with high quality when associated with legume forage. Forage grass mainly grows in pure stands; it can be cultivated in association with multipurpose tree forage legumes. Such associations have higher nutritional value than alone and can produce higher dry matter yields (EARO 2001; Alemayehu 2004). *Panicum maximum* + *Cajanus cajan* combinations produce 19 t/ha and pure *Panicum maximum* stands produce for 9.8 t/ha dry matter (Alalade *et al*., 2014). Their association increases forage productivity and quality in terms of crude protein content 11.22% and pure stand produce 7.11% (Alalade *et al*., 2014). Legumes improve the nutritive value of lower quality native pastures grown with them and are important components of farming system since they have high nutritive value and able to rehabilitate nutrient depleted from the soil. Multipurpose Tree Forages (MPTFs) provided high quality fodder in the dry season (Alalade *et al*., 2014). This has produced interest in their use in pastures leading to the development of tree based forage production systems such as alley farming system.

MPTFs are pruned and subsequently managed through periodic pruning of the re-growth so that inter planted grasses get enough light. Legume-grass mixtures have reduced weed encroachment and erosion and have led to greater stand longevity than legume or grass monoculture farming (Sanderson *et al*., 2012). Usage of commercial concentrates as livestock supplements are limited due to inability of farmers to purchase them (Husein and Diriba, 2021). A farmer who buys concentrates can reduce costs by combining grass forage with forage legumes. Grass forage is low in crude protein though high in carbohydrates, there is a need for integrating it with forage legumes which rich in crude protein (Roque, 2015).

Forages used for the trial were perennial types that were suitable for the farmers for increasing availability with quality for livestock feed in the study area throughout the year. Forage legumes were also multipurpose types (used for construction, fuel, animal feed, improve soil fertility, fence and bee forage). After the end of this research, the farmer can easily utilize this technology for livestock by planting on board line, road side, backyard and margin land. As a result, this experiment was designed with the intention to control soil erosion and increase green feed availability throughout the year in study areas. Therefore, the objectives of this study are to determine the effect of integration and cutting frequencies of grasses-legumes on the growth, total forage biomass yield and nutritive quality and to identify compatible forage varieties in their integrations.

Materials and Methods

Description of study areas

The study was conducted at Adami Tulu Agricultural Research Center (ATARC) from 2019 to 2021. ATARC is located at 174 km from the capital city of Ethiopia, Addis Ababa. The altitude of ATARC ranges 1650 meters above sea level and has semi-arid type of climate. The mid rift valley has erratic, unreliable and low rainfall, averaging between 500 and 900 mm per annum. The rain fall is bi-modal with the long rain June to September.

Experimental Materials

Forage grasses used for the trail were Guinea grass (*Panicum maximum (*Degun geziya*)*); Rhodes grass (*Chloris gayana (*Massaba*)*) and Buffel grass (*Cenchrus ciliaris* and MPTFs were Pigeon pea (*Cajanus cajan* (DZ16527) and Leucaena (*Leucaena leucocephala*).

Treatments and Experimental Design

The trial was designed with factorial arrangement in randomized complete block design with eleven treatments in three replications and their treatments were grasses sole, MPTFs sole and their combination. Size of plot, distance between each block, plot and plants (grass) row spacing were 3 m*****4 m**,** 1m, 50 cm and 20 cm; respectively. Forage legumes were sown at row spacing of 1m keeping 40 centimeters distance from grass and grass forages at spacing 20 cm from grass to grass. The recommended ratio between grass and legume in a plot were 2:1 (2 row grass: 1 rows legume) (Ebeid *et al*, 2011).

Lists of treatments

1. *Panicum. maxim* + *Leucaena leucocephala*
2. *Chloris gayana* + *Cajanus cajan*
3. *Cenchrus ciliaris* sole
4. *Panicum maximum+Cajanus cajan*
5. *Chloris gayana* sole
6. *Cajanus cajan* sole
7. *Chloris gayana+ Leucaena leucocephala*
8. *Leucaena leucocephala* sole
9. *Cenchrus ciliaris* + *Leucaena leucocephala*
10. *Panicum maximum* sole
11. *Cenchrus ciliaris* + *Cajanus cajan*

Experimental Procedures

The sole seeding rate of MPTFs and grasses were 4 and 10 kg per hectare, respectively. While seeding rates for the mixed forages was half of pure stands, because of the area was taken up by both forages. The study was conducted under rain fed at the beginning of rainy season in the study area and supplementary irrigation was provided at dry season two times in a week. Germination test was done for both seed types before sowing in order to determine the quality.

Collected Data

Coverage (%), Plant height (both forages), length of branch and number of branch grown per plant for MPTFs, Date of 50% and 30-40% flowering of grass and MPTFs, respectively, Biomass yield and Frequency of cuttings/harvesting

Land equivalent ratio (LER) is the most commonly used to indicate the biological efficiency and yield per unit area of land as compared to mono-cropping system. When LER is greater than 1, mixing improves the productivity of the mixed species. In contrast; when LER is lower than 1, mixing negatively affects the growth and yield performance of plants (Dhima *et al*., 2007). LER was calculated as:

$$\text{LER} = \frac{\text{Yield of MPTS in mixture}}{\text{Yield of MPTS alone}} + \frac{\text{Yield of grass in mixture}}{\text{Yield of grass alone}}$$

Forage Sampling Procedures

Forage samples were taken at 50% and 30-40% flowering for forage grasses and MPTFs legumes, respectively by taking from the two middle rows per plot. The average plant height was measured from five plants starting from the ground to the tip of the main stem. The length of branch was measured from the main stems of MPTFs. Number of branches grown per plant was also counted from the main stem and twigs with the diameter ≤ 6 mm were considered as edible parts for MPTFs total forage biomass yield determination. On the other hand, in order to measure grass dry matter yield at 50 % flowering, the two middle rows per plot was harvested by using hand suckles and fresh sample was measured in field by spring weight balance and 250 g subsample per plot was brought to ATARC animal feed laboratory and chopped in to pieces for further chemical analysis.

Forage Biomass Yields Determination

The representative subsamples were put in oven at 105°c for overnight for total dry matter determination (DM). Accordingly, DM yield (t/ha) estimation was calculated by using recommended formula by **(Tarawali** *et al.*, **1995)**. The final DM yields were reported in tons per hectare and calculated **as:**

10 x TotFW x (DWss /HA x FWss)

Where, TotFW = Total fresh weight, DWss = Oven dried subsample, FWss = Fresh weight subsamples and HA = Harvesting area.

Chemical Analyses

The dried subsamples were ground to pass through a 1mm sieve for chemical analyses. CP was calculated as N × 6.25 (Kjeldahl methods) and NDF and ADF were determined according to (Van Soest *et al*., 1991). Hemicelluloses and cellulose were calculated as NDF minus ADF, and ADF minus acid detergent lignin (ADL), respectively ATARC.

Data Analysis

The data collected on DM yield, plant and branch height, number of branches, number of tiller per plant, coverage and nutritional quality parameters using the Generalized Linear Model of SAS 9.1 (SAS Institute, 2004). Where a significant effect of treatments was found, a Tukey HSD test was employed to separate means at $P \leq .05$. Proportional data were arcsine transformed to meet the assumptions of normality and homogeneity of variance. The following model was used for the analysis.

$$Y\mathrm{ijk}=\mu+Ai+B\mathrm{j}+A\times B\mathrm{ij}+E\mathrm{ijk}$$

Where, Yijk is the measured response, μ is the overall mean; Ai is the fixed effect of treatment, Bj is the fixed effect of cut, A × Bij is the interaction effect of treatment and cut, and Eijk is the error term.

Results and Discussion

Performance of MPTFs legumes and grasses associations tested for determination of agronomic qualities and compatibility at on station were presented in Table 1. The interaction effect of treatments and cuttings showed significant effect on the collected parameters. Plant heights for both forage types varied significantly ($p<0.001$) famong the treatments and cuttings. The highest mean values (186.14 cm) for plant height was recorded for *L. leucocephala* sole followed by *L. leucocephala* associated with *Panicum maximum, Cenchrus ciliaris,* and *Cajanus cajan* sole with respective values of 183.72, 175.58 and 171.78 cm. The current result was lower than the 193.4 cm reported by Tolera *et al*. (2021) from a study conducted at Fedis Agricultural Research Center on *Cajanus cajan*. This difference might be due to the difference in soil moisture and rainfall availability of the study area. The shortest plant height (122.92 cm) was obtained for *Cajanus cajan* intercropped with *Chloris gayana.*

From the tested grass varieties the maximum plant height (124.50 cm) was recorded for *Chloris gayana* integrated with *Cajanus cajan* followed by Rhode sole and *Panicum maximum* sole with respective values of 121.71 and 118.75 cm. The minimum plant height (79.72 cm) was obtained for *Chloris gayana* combined with *L. leucocephala*. The current finding was similar with the report of Dawit *et al*. (2022) in which plant height of 103.2 cm was obtained for *Chloris gayana* mixed with alfalfa at on station and Shashemene district. The current study indicated that, as cutting frequencies of both forages increase plant heights also increased. This was happened due to the advanced development of stems and leaves which could have easily utilized soil and other surrounding resources. Increasing in leaf length and number stimulates better capturing of light for photosynthesis thus improving yield. This agrees with the findings of (Muinga *et al.*, 2007).

The result of combined analysis indicated that, the branches length recoded for MPFLs was significantly ($p<0.0001$) varied. The maximum mean value of branch length (65.37 cm) was recorded for *L. leucocephala* sole followed by *Cajanus cajan* sole, the combination of *panicum maximum* and *L. leucocephala* and *Cajanus cajan* and Rhodes grass combinationwith respective values of 60.74 , 54.23 and 34.99 cm.

With regard to the forage biomass yield, significant variations among the treatments and cuttings frequencies were noted. From MPTFs, the highest forage biomass yield (7.39 t/ha) was obtained from *Cajanus cajan* integrated with *Chloris gayana.* The combined mean values of total forage biomass yield was also high (29.20 t/ha) for this integration of Rhodes grass and *Cajanus cajan.* The current results are in line with the finding of Dawit *et al*. (2022), Karadau (2003) and Babayemi *et al*. (2006) in which the grass-legume integration was reported to improve forage productivity as compared with monocultures without any other inputs. On the other hand, the current result is inverse to the work of Cook *et al.* (2005) who reported forage biomass yields of 10 to 17 t/ha for pure stand and 1-7 t/ha for integrated forages. This dissimilarity could probably be due to the difference in harvesting stage, season of harvest, forage combination condition and management practices.

From the results of the combined analysis, the lowest total forage biomass yields were recorded from both sole MPTFs (*Cajanus cajun* and *L. leucocephala*). *Cajanus cajan* intercropped with *Panicum maximum* produced the lowest biomass yield (3.6 t/ha). Among forage grasses tested in the current research, maximum forage biomass yield (24.39 t/ha) was recorded from *Chloris gayana* alone followed by Rhodes grass integrated with *cajanus cajan* which gave 21.81t/ha. The minimum mean value of forage grass biomass yield was obtained from *Cenchrus ciliaris* intercropped with *L. leucocephala* and *Panicum maximum* with *L. leucocephala* with values of 15.05 and 15.10 t/ha, respectively.

The highest total forage biomass yield (26.26 t/ha) was harvested during the 4th cutting followed by the 3rd cuttings (23.68 t/ha), while the lowest (21.41 t/ha) was collected during 1st cuttings. The average values obtained in the present finding is higher than the report of (Alalade *et al*., 2014) in which 19 t/ha was reported. Total forage biomass yield increased as cutting interval increased. This might be due to the additional tiller, leaf formation, leaf and branches elongation and stem development. The increase in total forage biomass yield with advancement in age agrees with the findings of Amole *et al*. (2015) in which it is stated that, at advanced age, both grass and legumes relatively establish more and utilize more soil resources for optimum growth. Also, Njarui *et al*. (2007) confirmed that early harvest produces lower yield due to the fact that forages are yet to develop longer roots for competing for nutrients and water.

Number of tillers per plant of the tested forage grasses were significantly different ($p<0.001$) among treatments and cuttings. The highest number of tiller per plant (73.59) was recorded from the integration of *Chloris gayana* and *Cajanus cajan.* This may be due to the growth characters of *Chloris gayana* which is semi erected/prostrate. This results in more efficient use of resources such as light when grew together than separately (Atis *et al*., 2012). The lowest mean value (29.8) was obtained from *Panicum maximum* intercropped with *Cajanus cajan*. The highest number of tiller per plant (52.23) was counted from 4th cutting and the lowest from the rest cuttings as indicted in (Table 1). Due to cutting interval, additional formation of tillers might have been occurred. This result is also similar with the finding of (Amole *et al.*, 2015).

There were significant variations in number of branches grown per plants of MPTFs ($p<0.0001$) among treatments and cuttings. The largest number of branches grown (32.58) were obtained from the integration of *Cajanus cajan* intercropped with *Chloris gayana* and from the 4th cutting (40.71). The lowest mean value was obtained from *L. leucocephala* integrated with *Panicum maximum* treatment and from the 1stcutting. Average mean value of number of branches per plan in the current work is lower than previously reported value of 45.83 by (Denbela *et al*., 2020). The 1st cutting provided the lowest number of branch grown per plants (17.88). Increasing number of branches grown per plants with increasing age at harvesting is supported with the finding of (Amole *et al.*, 2015).

As indicated in the Table 1, the integration of MPTFs and forage grasses produced greater than 1.0 value of LER. Integrations of legumes and grasses produced more DM yield as compared to the yield of pure stands of both forages. The highest LER (2.05) was recorded for integration of *Chloris gayana* and C*ajanus cajun* while the lowest value (1.27) was recorded for the combination of *panicum maximum* and *Cajanus Cajun.* The highest LER observed for the integration of *Chloris gayana* and *Cajanus Cajun* could be because of the benefit the grasses get from the fixed N as a result of integration of *Cajanus canas*. A study by Erkovan (2005) also reported the advantage of N transfer from the fixed N by legumes to the grasses in the legume-grass integration

Table 1: The combined mean of agronomic parameters and biomass yield of MPTFs perennial grasses combination

Treatments	**Plant height(cm)**		**Branches(** MPTF)		**Biomass yield (t/ha)**		**Grasses**		**LER**
	MPTFs	**Grasses**	**BL(cm)**	**NBP**	**MPTFs**	**grasses**	**NTPP**	**Cover**	
P. maxim + L.leucoce	183.72^{a}	110.20^{b}	54.23^{abc}	24.75^{b}	4.99bc	15.10^{b}	49.30^{c}	78.58^{d}	1.51
C. gayana + C.cajan	122.92^{d}	124.50^{a}	34.99^{d}	32.58^{a}	7.39^{a}	21.81^{a}	73.59^{a}	92.00^{a}	2.05
C. ciliaris sole	-	94.40^{c}	-	-	-	15.28^{b}	57.68^{b}	87.87^{b}	-
P.maximum+C.cajan	146.74^{c}	85.98^{d}	45.00^{cd}	30.92^{a}	3.60^{c}	15.41^{b}	29.80^{e}	75.74^{d}	1.27
C.gayana sole	-	121.71^{a}	-	-	-	24.39^{a}	49.00^{c}	92.83^{a}	-
C.cajan sole	171.78^{ab}	-	60.74^{ab}	33.25^{a}	6.42^{ab}	-	-	-	-
C. gayana+L.leucoce	157.73^{bc}	79.72^{e}	49.65^{c}	25.92^{b}	5.67^{b}	16.31^{b}	42.54^{cd}	87.03^{b}	1.83
L.leucocephala sole	186.14^{a}	-	65.37^{a}	25.33^{b}	6.11^{ab}	-	-	-	-
C. ciliaris+ L.leucoce	175.58^{ab}	84.69d^{e}	53.07b^{c}	26.50^{b}	5.26^{b}	15.05^{b}	37.01^{de}	82.75^{c}	1.85
P. maximum sole	-	118.75^{a}	-	-	-	21.87^{a}	43.65^{cd}	82.83^{c}	-
C. ciliaris + C.cajan	136.42^{cd}	97.15^{c}	47.40^{c}	31.08^{a}	5.39^{b}	15.34^{b}	36.98^{de}	87.92^{b}	1.84
CV	25.78	7.24	29.13	11.81	23.78	25.45	18.99	5.41	
P-value	***	***	***	***	**	**	***	***	
Cuttings									
1	112.65^{b}	95.74^{b}	43.13^{b}	17.88^{d}	5.20	16.21^{b}	43.27^{b}	83.41^{b}	
2	100.34^{b}	101.97^{a}	40.29^{b}	25.38^{c}	5.64	16.80^{b}	43.97^{b}	83.84^{b}	
3	163.93^{a}	105.43^{a}	48.8^{b}	31.21^{b}	5.77	17.91^{ab}	46.98^{b}	86.88^{a}	
4	163.59^{a}	104.46^{a}	73.02^{a}	40.71^{a}	5.81	20.45^{a}	52.23^{a}	87.01^{a}	
LSD(0.05)	**	**	**	**	**	*	*	*	
Significance									
trt	<.0001	<.0001	<.0001	<.0001	0.0060	0.0004	<.0001	<.0001	
cut	<.0001	0.0061	0.0002	0.0041	0.0008	0.0492	0.0414	0.0247	
Trt*cut	0.0073	<.0001	0.3202	0.0034	0.9966	0.6578	0.1843	0.6074	

[a, b, c, d, e] =Means in a column within the same category having different superscripts differ (from P<0.05 to P<0.001), MPTFs-Multipurpose Tree Forage, BL- Branch Length, NBP- Number of branch per plant, NTPP- Number of Tiller per plant, p.maximum- panicum maximum, L. leucoce- L. leucocephala, C.gayana- Chloris gayana, C.canus- Cajanus canus and C. ciliaris- Cenchrus ciliari

Chemical composition

The nutritional content of the perennial grasses integrated with MPFLs are indicated in Table 2. CP content was not significantly ($p>0.05$) different among the treatments. Numerically the highest CP content (18.15%) was recorded from *Panicum maximum* integrated with *Cajanus cajan,* where as sole *Panicum maximum* produced the lowest CP content. Result of the current study was supported by the finding of Ahmed (2010) in which the grass-legumes integration was reported to improve fresh fodder yield and protein contents. The overall mean CP concentration observed from the current result was higher the 11.22% reported by Alalade *et al*., (2014).

Ash contents of grass forages integrated with MPTFs varied significantly ($p<0.05$). The maximum ash content (13.12%) was obtained from *Chloris gayana* integrated with *L. leucocephala* followed b*y the* 12.77% obtained for *Panicum maximum* associated with *L. leucocephala* and *Chloris gayana and* 11.83% for *Cajanus cajan. T*he lowest ash content (9.45%) was *observed* from *panicum maximum* and *Cajanus cajan* integration.

Organic matter content of the grass integrated with MPTFs of the current work exhibited significant ($p<0.05$) difference. The highest average mean value was recorded for *Chloris gayana* and *Cajanus cajan* integration and the lowest was recorded from the integration of *Chloris gayana* with *L. leucocephala* and *panicum maximum* with *L. leucocephala* treatments.

Table 3: Combined mean values of Chemical composition of perennial grasses integrated with MPTFs

Treatments	**Parameters**		
	CP%	**Ash%**	**OM%**
P. maxim + L.leucoce	16.89	12.77^{ab}	74.82^{b}
C. gayana + C.cajan	17.06	11.83^{ab}	77.733^{a}
C. ciliaris sole	15.37	11.18^{abc}	75.77^{ab}
P.maximum + C.cajan	18.15	9.45^{c}	76.13^{ab}
C.gayana sole	15.19	11.48^{abc}	76.13^{ab}
C. gayana + L.leucoce	16.87	13.12^{a}	74.27^{b}
C. ciliaris + L.leucoce	16.09	11.72^{abc}	75.43^{b}
P. maximum sole	14.62	11.00^{abc}	75.68^{ab}
C. ciliaris + C.cajan	15.77	10.68^{bc}	76.18^{ab}
Overall mean	16.22	11.47	75.79
CV (%)	24.11	17.50	2.49
LSD(0.05)	4.55	2.33	2.1926
P- value	ns	*	*

a, b, c =Means in a column within the same category having different superscripts differ (from P<0.05 to P<0.001), trt- treatments MPTFs-Multipurpose TreeForage, BL- Branch Length, NBP- Number of branch per plant, NTPP- Number of Tiller per plant, p.maximum- panicum maximum, L. leucoce- L. leucocephala, C.gayana- Chloris gayana, C.canus- Cajanus canus, and C. ciliaris- Cenchrus ciliaris

Conclusions and Recommendations

Results of the current study showed that legume introduction to the grass had positive effects on agronomic performances of the forages. It had an effect on plant height, total forage biomass yield, number of branches grown per plant and length of branches. The highest plant height was measured from the integration of *Panicum maximum* with *L. leucocephala* and the lowest average mean value was observed from the integration of *Chloris gayana* with *Cajanus cajan*. From forage grass the highest plant height was recorded for *Chloris gayana* integrated with *Cajanus cajan* and the lowest value was observed from Rhodes grass combined with *L. leucocephala*. The maximum total forage biomass yield was obtained from the integration of *Chloris gayana* and *Cajanus cajan* and the lowest mean value was from the sole *Cajanus cajan* and Leucaena. As cutting interval increases, plant heights, numbers of branches per plant, length of branches and number of tiller per plants were also increased.

The integration of MPTFs legumes and grasses produced greater than 1.0 value of LER. The maximum LER was recorded from the integration of *Chloris gayana* and cajanus Cajun and the minimum value was observed for the combination of *Panicum maximum* and *Cajanus cajun*. Integrations treatments improved the productivity of the mixed species as the obtained values of LER were greater than 1.0. Numerically, the maximum CP content was recorded from *Panicum maximum* integrated with *Cajanus cajan* and the minimum was from sole *Panicum maximum*. *Chloris gayana* integration with *Cajanus cajan* was recommended for the study areas and similar agro-ecologies due to its high total forage biomass yield and other agronomic performance as compared to the other tested tratments.

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Adaptation Trial of Oat Varieties to Moisture Stress Areas of Central Rift Valley of Oromia

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Abstract

The experiment was conducted at Adami Tulu Agricultural Research Center (ATARC), Dugda and Lume districts with the objective of evaluating adaptability, agronomic performance, yield and quality of oat varieties. Six oat varieties (Bonsa, Bate, ***CI-****8251, Bona-bas, Jasari and Cl-8237****) were laid out in a*** *Randomized in Complete Block Design (RCBD) with three replications.* The results showed that *plant height, total forage biomass yield and seed yields were significantly (p<0.001) influenced by oat varieties. The highest mean plant height of 122.34 cm was recorded from Bate variety whereas the lowest (90.96 cm) was recorded from CI-8251variety. The highest mean value (13.03 t/ha) of total biomass yield was obtained from Bate and the lowest (8.87 t/ha) from Bonabas. The maximum seed yield (21.95 quant/ha) was produced by Bonsa, followed by CI-8251 (20.8 quant/ha) and Bate (20.07 quant/ha) in that order. The lowest seed yield (17.72 quant/ha) was recorded from CI-8237.* With regard to *maturity at 50% flowering, late (80 days) and early (69 days) maturing varieties were CI-8237 and Bonabas, respectively. Bate and Bonsa varieties performed well in total forage biomass yield, seed yield and nutritive quality. Bonabas was better in attaining maturity to 50% of flowering earlier than other varieties in those moisture stress area. Thus, Bate, Bonsa and Bonabas oat varieties were recommended for the end users of the study areas and similar agro ecologies*.

Key words: Adaptation, feed shortage, fodder, moisture stress and Oat variety

Introduction

Shortages of animal feed resources have been identified as one of the major factor limiting the production and productivity of livestock. In mid rift valley, livestock feed is based on natural pastures, fallow and stubble grazing and crop residues (Assefa *et al.*, 2013). However, natural pasture and crop residues are poor in quantity and quality. Thus, the existing feed resources do not meet the nutrient requirements for growth and reproduction of animals (Ramana *et al.*, 2015). One approach for alleviating the problem is identification and development of forage species suitable for the existing climatic condition. Hence, production of adaptable forage species with high herbage yield and quality are very important for tackling the problem of feed shortage.

Oats are important in feeding ruminant animals for their high dry matter production and low cost. Oats are very palatable (softness), good in protein when compared to the other grains and considered as an excellent feed for all livestock (Alemayehu, 2002). Oat are mainly cultivated in high land of Ethiopia than low land (Gebremedhn *et al*., 2015), however other cereals crops are cultivated in this moisture stress area. The adaptability of fodder oat was not evaluated in study area. The adaptability of wheat, barley and others were evaluated in this study area. Fodder oat is harvested earlier; because of it is harvested at 50% of flowering for livestock feeding and not delayed for maturity as other cereals crops. This indicated fodder oat is not take prolonged time for forage production as compared with other cereals crops. Other cereal crops are took a long time until maturing for seed providing, oat is harvested before maturity for quality feed production at recommended flowering stage. Regardless of the importance of this fodder, adaptable and high yielding oat varieties have not been identified for forage production in moisture stress areas of central rift valley. Therefore, this study was initiated with the objective of evaluating the adaptability, yield performance and nutritive qualities of fodder oat varieties under mid rift valley conditions.

Materials and Methods

Description of the study area

The study was conducted at Adami Tulu Agricultural Research Center (ATARC), Dugda and Lume Districts under rain fed condition for two consecutive years (2020 to 2021). ATARC is located at 174 km from the capital city of Ethiopia, Addis Ababa. The altitude of the center is 1650 meters above sea level and the area has a semi-arid type of climate. The mid rift valley has erratic, unreliable and low rainfall, averaging between 500 and 900 mm per annum. The rain fall is bi-modal with the long rain June to September.

Dugda district is located at 135 km from the capital city of Ethiopia, Addis Ababa and 90 km from East Shewa Zonal Capital, Adama. The district covers 5.2 % of East Shewa Zone with an area of 751 km^2. Dugda has 18 Kebele's among which one kebele was used for this study. The district has an average 636 mm annual rainfall and 26 c^o average temperature.

Lume district is located at 88 km from Addis Ababa and 25km from zonal capital, Adama town. The district covers 9.8% of East Shewa Zone with an area of 870 km^2. Lume has 38 Kebeles among which one kebele was used for this study. The annual rainfall of the district ranges from 500-1200 mm and its temperature ranges from 18 to 28^0c. The major crops grown in the area include tef, wheat, chickpea and lentil.

Experimental Materials

Six oat varieties (Bonsa, Bate, **CI-**8251, Bona-bas, Jasari and Cl-8237) were used for the study. **Some Descriptions of the varieties are given in Table1.**

Table 1. Descriptions of fodder oats used for the trial

SN	Varieties	Fodder yield (t/ha)	Year of release	Breeder /maintainer center
1	Bonsa	10.3	2011	SARC/OARI
2	Bate	8.56	2018	Bako ARC/OARI
3	**CI-**8251	9.34	2013	HARC/EIAR
4	Bona-bas,	10.1	2011	SARC/OARI
5	Jasari	8.76	1982	Kenya
6	Cl-8237	8.61	1976	HARC/EIAR

Treatments and Experimental Design

Six oat varieties (Bonsa, Bate, **CI-**8251, Bona-bas, Jasari and Cl-8237) were laid out in Randomized Complete Block Design with three replications. The gross plot comprised of 3 m x 3 m (9 m^2). The distance between plots and blocks were 50cm and 1m, respectively. All other management practices were applied as per the recommendations.

Experimental Procedures

The seeds were planted at a spacing of 20 cm between rows and the seeds were drilled within rows. All recommended field management practices and packages such as land preparation, weeding and fertilizer applications (100 kg/ha NPS and 50 kg/ha urea at the time of planting) were applied in a similar manner for all the plots in each of the treatments. Germination test was done for varieties before sowing in order to determine the quality of seeds.

Collected Data

Plant height, Coverage, Numbers of tiller per plants, Leaf to Stem Ratio, seed yield, Total forage biomass yield and Leaf length.

Forage Sampling Procedures

Total forage biomass yield was determined by harvesting from the two middle rows of each plot at its 50% flowering stage, at a height of 5 cm near the ground. After harvesting, the total fresh weight of the forage sample from each plot was measured immediately for total biomass yield determination by using a sensitive balance at field and 250 g sub-sample per plot was brought to ATARC Animal feed laboratory and chopped in to pieces for further chemical analysis**.**

Forage Biomass Yields Determination

The representative subsamples were dried in oven at 105°c for overnight for total dry matter determination. Accordingly, DM yield (t/ha) was calculated by using recommended formula by **(**Tarawali *et al.*, 1995**)**. The final total dry matter yields were reported in tons per hectare and calculated **as:**

$$\mathbf{10 \times Tot\ FW \times (DWss\ /HA \times FWss)}$$

Where, Tot FW = Total fresh weight, DWss = oven dried subsample, FWss = Fresh weight subsamples and HA = Harvesting area.

Chemical Analyses

The dried subsamples were ground to pass through a 1 mm sieve for further chemical analyses. Ash was determined by igniting the samples in a muffle furnace at 550^{0}C for 3 hours in ATARC. CP was calculated as N × 6.25 (Kjeldahl methods) in the Ziway Soil Research Center, and ADL and ADF were determined according to (Van Soest *et al*., 1991) at ATARC Animal feed laboratory.

Data Analysis

The collected data on total dry matter yield, plant height, and leaf length, number of tiller per plant, coverage and nutritional quality parameters were analyzed using the Generalized Linear Model of SAS

9.1 (SAS Institute, 2004). When there was a significance difference, mean separation was tested by using LSD at a significance level of 5%

Results and Discussion

Table 2: Mean squares of ANOVA for total dry matter yield of six oat varieties tested in different sites

Source of Variation	DF	Mean squares	F-value	P-value
Replication	2	28.1563815	3.55	0.0340
Variety	5	46.1398059	5.82	0.0002
Site	2	442.5555787	55.79	0.0001
Variety*Site	10	15.3992731	1.94	0.0536
Error	70			
Total	89			

Where: DF= degree of freedom

Agronomic parameters

Combined mean values for the different parameters of the tested varieties are given in Table 3. From the results the mean value of plant height was significantly ($p< 0.001$) different among oat varieties. The highest mean value of plant height (122.34 cm) was recorded for Bate whereas the lowest (90.96 cm) was recorded for CI-8251. This variation might be due to the genetic difference of the varieties (Tamirat *et al*., 2020). The overall mean plant height (108.9cm) of the oat varieties in the current finding is almost similar with the value of 106. 5 cm report by Yehalem (2012), higher than the 60.7cm reported by Tewodros and Amare (2016) and lower than the 131.23 cm report by Mekonnen *et al.,(*2020). These differences might be due to the difference in soil factors, soil moisture and management practices. Leaf length was significantly ($p<0.001$) different among the tested varieties. The highest leaf length (43.89 cm) was recorded for CI-8237 followed by Bate (42.78 cm) and Bonabas (22.93 cm) varieties.

Dry matter yield

A significant ($p<0.05$) difference was observed among the six oat varieties in total dry matter yield. The highest dry matter yield (13.03 t/ha) was obtained from Bate variety followed by Bonsa variety (12.06 t/ha) while the lowest biomass yield was recorded from Bonabas variety (8.87 t/ha). The overall mean of dry matter yield in the present study is higher than the report of Mekonnen *et al.,* 2020) (8.03 t/ha).

Seed yield

There were significant ($P<0.001$) differences in the seed yield of oat between varieties. The mean seed yield of the varieties was ranged 13.25-21.95 qt/ha. The maximum seed yield (21.95 qt/ha) was produced by Bonsa variety and followed by CI-8251 and Bate varieties, with respective seed yields of 20.8 and 20.07 qt/ha. The lowest seed yield (17.72 qt/ha) was recorded from CI-8237. The mean seed yield of the current work is within the range of mean seed yields (15.6-28.85 qt/ha) reported by Tamirat *et al*. (2020), but it is lower than the 21.70 to 29.80 qt/ha found by Dawit and Mulusew (2014) for oat varieties. This difference might be due to the difference in agro ecology of the study areas, since the former research was conducted at Bale high land and the current was at low land areas.

The analyzed data indicated that significant ($p<0.001$) difference was observed among the tested oat varieties in days taken to reach 50% of flowering. The highest day to 50% flowering (80 days) was recorded for CI-8237 and shortest (69 days) was recorded for Bonabas. Days to maturity at 50% flowering were higher than the mean figures of 64.94 days reported by Tamirat *et al*. (2020). This variation might be due to the difference in agro ecology of the study area. In contrasting the study sites, the maximum days to attained 50% of flowering was observed at Dugda site followed by at ATARC site, whereas the shortest days was recorded at Lume site. This difference might be due to the difference in soil moisture contents of the experimental sites. Varieties and sites interaction effects were found to be significant for the collected parameters.

Table 3. Combined mean values for herbage yield, grain yield and other agronomic parameters

Parameters	Varieties								
	Bate	**Bonabas**	**Jasar**	**Bonsa**	**CI-8251**	**CI-8237**	**Overall mean**	**CV**	**LSD(0.05)**
PH(cm)	122.34^{a}	121.7^{a}	118.92^{a}	92.03^{c}	90.96^{c}	107.92^{b}	108.97	10.6	***
LL(cm)	42.78^{a}	22.93^{c}	41.93^{a}	32.72^{b}	34.09^{b}	43.89^{a}	36.39	12.7	***
TBMY(t/ha	13.03^{a}	8.87^{c}	9.64^{c}	12.06^{ab}	10.44^{bc}	9.67^{c}	10.62	27.5	**
NTPP	21.56^{a}	21.17^{a}	17.07^{b}	17.10^{b}	20.82^{a}	17.13^{b}	18.60	22.5	*
SY(q/ha)	20.07^{abc}	13.25^{d}	18.18^{bc}	21.95^{a}	20.80^{ab}	17.72^{c}	18.30	22.9	***
LSR	1.94^{c}	2.067^{ab}	2.054^{ab}	2.30^{a}	2.24^{ab}	2.12^{ab}	2.12	23.9	**
CO	91.50^{a}	85.67^{c}	88.56^{b}	89.00^{ab}	88.50^{b}	87.33^{bc}	88.43	4.4	**
50% F	74.11c	69.33^{d}	75.17^{bc}	79.67^{a}	76.39^{b}	79.89^{a}	75.76	4.09	***

*$^{a, b, c, d}$ = Means in a column within the same category having different superscripts differ (from $P<0.05$ to $P<0.001$), * = significant, ** = very significant, = *** highly significant, PH- Plant height, LL- Leaf Length, TDMY- Total Dry Matter Yield, NTPP- Number of Tiller per plant, CO- Coverage, SY- seed Yield, Quantal/ha, LSR- Leaf Stem Ratio and 50%F- 50% of flowering*

Chemical composition

Nutritive value of the evaluated oat varieties is presented in (Table 4). The result indicated that Crude protein was significantly affected ($p<0.05$) by oat varieties while other parameters were not varied ($p>0.05$) for the tested oat varieties. The highest CP (10.41%) was recorded for Bate and this was statistically at par with Bonsa, CI-8251 and Jasar for which respective values of 9.14, 8.21 and 8.19%, were recorded. The lowest CP (7.22%) content was recorded for CI-8237. The current result was higher than the work of Usman *et al.* (2018) and Fekede *et al.* (2008) who reported 3.0-7.9 and 4.8-7.6 %, respectively. This dissimilarity might be due genetic variability of the tested materials as well as the differences in soil fertility and soil moisture content of the experimental sites and stage of harvesting. The CP content of the current study was in the range of quality roughage (Bezabih *et al.*, 2013).

As the analyzed data indicated, ash content of the oat varieties didn't vary significantly ($p>0.05$). The overall mean obtained in the current study was higher than the mean of 8.63% found by (Mekonnen *et al.*, 2020). ADF and ADL contents of the oat varieties also didn't show significant difference ($P>0.05$). The fiber content in the current result is in the normal range that would not limit dry matter intake of ruminants as, according to the findings of Van Soest (1994) and Kellems and Church (1998), less than 40% fiber is categorized as high quality and above 40% is categorized as low quality.

Table 4: Combined Chemical composition (%) of Oat varieties sown at different sites

Varieties	Parameters			
	CP	Ash	ADF	ADL
Bate	10.41^{a}	13.35	24.15	10.35
Bonabas	8.02^{b}	12.90	22.35	9.47
Jasar	8.19^{ab}	12.50	20.78	10.82
Bonsa	9.14^{ab}	13.55	21.54	11.39
CI-8251	8.21^{ab}	13.7	18.71	8.34
CI-8237	7.22^{b}	12.45	20.65	13.20
Mean	8.53	13.08	21.36	10.59
CV (%)	10.43	5.61	11.85	26.82
LSD (0.05)	2.29	1.89	6.51	7.30
SL	*	ns	ns	ns
P- value	0.0115	0.4137	0.5624	0.5014

$^{a, b}$ *=Means in a column within the same category having different superscripts differ (from P<0.05 to P<0.001), * = significant, ns= none significant. CP=Crude protein, ADF= Acid detergent fiber, ADL= Acid Detergent Lignin, SL= Significance level*

Conclusions and Recommendations

The results of the current study showed that there were significant variations in plant height, leave length, total forage biomass yield and seed yield among the tested oat varieties. The highest mean value of plant height was recorded from Bate and the lowest from CI-8251. The highest mean value for forage biomass yield was obtained from Bate followed Bonsa varieties and the lowest from Bonabas variety. From the recorded data; the maximum seed yield was obtained from Bonsa variety followed by from CI-8251 and Bate varieties in that order. The lowest seed yield was recorded from CI-8237. The longest and shortest maturity date at 50% flowering was recorded for CI-8237 and Bonabas varieties, respectively. The highest average mean value of CP content was recorded for bate and the lowest for CI- 8237 variety.

Among the tested oats varieties bate and Bonsa varieties performed well in total forage biomass yield, seed yield, nutritive quality and other agronomic parameters. Bonabas was better than the other tested oat varieties in maturing early to attain 50% flowering in moisture stress area. Therefore, Bate, Bonsa and Bonabas oat varieties were recommended for the end users in the study areas and for similar agro ecologies.

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