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**EFFECTS OF DIFFERENT FEED SUPPLEMENTS AND AGROECOLOGIES ON
HONEYBEE COLONY PERFORMANCES IN SOUTH WOLLO ZONE, AMHARA
REGIONAL STATE, ETHIOPIA**

PhD Dissertation

By

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Addis Ababa University, College of Veterinary Medicine and Agriculture

Department of Animal Production Studies

PhD program in Animal Production

**June. 2024
Bishoftu, Ethiopia**

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HONEYBEE COLONY PERFORMANCES IN SOUTH WOLLO ZONE, AMHARA
REGIONAL STATE, ETHIOPIA**

**A dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis
Ababa University in partial fulfillment of the requirements for the degree of Doctor of
Philosophy in Animal Production**

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Statement of the Author

I first, declare that this dissertation is my bonafide work and that all sources of materials used for this dissertation have been duly acknowledged. This dissertation has been submitted to the requirements for a Ph.D. Degree at Addis Ababa University, College of Veterinary Medicine and Agriculture, and is deposited at the University's Library to be made available to borrowers under the rules of the Library. I solemnly declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Dedication

I would like to express my heartfelt gratitude and dedication to the Almighty God, his mother (the revered Saint Virgin Mariam), and all the angels and Saints. It is through their blessings and divine guidance that I have been able to embark on this research work, effectively compile this manuscript, and reach this significant milestone. I am also deeply indebted to my cherished family for their boundless affection, unwavering encouragement, and unwavering support, which has propelled me to set my sights higher.

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List of Abbreviations

CAC	Codex Alimentarius Commission
CF	Crude Fat
CFi	Crude Fiber
CH	Carbohydrate
CP	Crude Protein
EA	Egg Area
Exrsw	Extent of swarming
FA	Free Acidity
GE	Gross Expenditure
GR	Gross Return
HA	Honey Area
HMF	Hydroxyl Methyl Furfural
HY	Honey Yield
LA	Larvae Area
Meq	Mill equivalent
Moist.	Moisture
NA	Nectar Area
NP	Net Profit
OM	Organic Matter
POA	Pollen Area
PA	Pupae Area
Qcc	Queen Cell Construction
Swate	Swarmed Tendency
Swcol	Swarmed Colony
TDF-m	Total Distance Followed to the Observer in Meters.
TNoS-B	Total Number of Stings on the Leather Ball
TNoS-G	Total Number of Stings on the Glove
TtfFs-sec	Total Time for the First Sting in Seconds
Tt-sec-mag	Total Time in Seconds for Mass Aggressiveness

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Effects of different feed supplements and Agro-ecologies on honeybee colony performances of in South Wollo Zone, Amhara Regional state, Ethiopia

Abstract

*A study was conducted to investigate the effects of different supplements on honeybee colony performances, evaluate farm honeybee performance and analyze honey quality and pollen. A study involved 30 *A. M. monticola* colonies in an apiary for treatments and across agro-ecology for on-farm evaluation. For honey quality and pollen analysis, 30 samples were taken from those hives which on farm performance evaluation was measured. The treatment diets included: **T1:** 50% sugar syrup, 14% roasted barley powder (beso), and 36% roasted spiced pea powder (Shiro). **T2:** 50% powdered sugar, 14% white sorghum powder, and 36% bakery yeast. **T3:** 50% powdered sugar, 14% white sorghum powder, and 36% skimmed milk powder. **T4:** 50% sugar syrup with an infusion of stinging nettle and 1% kerefa, along with 50% white sorghum powder. Feeds were provided at the entrance sides of the colonies. The study was conducted in two phases, the dry season (December 20, 2021, to March 22, 2022) and the rainy season (June 27, 2022, to September 7, 2022) which are dearth periods. While on-farm performance evaluation was done from September 10, 2021, to December 30, 2021, and March 27, 2022, to June 23, 2022 during active seasons of the year. Measurements of brood area, pollen, nectar, and honey space in the comb were taken using a transparent grid meter of frame size (cm²). Feed intake, profitability, honey stores, and total bee strength were also assessed. Honey yield, swarming tendency, foraging activity, defensive behavior, and absconding rate were measured during the active season. Honey quality and pollen were also analyzed. Notably, colonies that received T4 exhibited superior performance, with a feed intake of 98.3%, egg area of 464.9 cm², larvae area of 553.9 cm², pupae area of 627.1 cm², colony strength of 6.4 frames, and queen cell construction of 0.2, surpassing the other colonies. In addition, they had a nectar area of 54.3 cm², a pollen area was 68.7 cm², a honey area was 311.2 cm² honey yield of 7 kilograms. Colonies found in the highland showed the highest value on all performance measurement parameters and recorded a honey yield of 22.13 kilo grams. While, colonies found in the lowland were active foragers, starting their activities at 5:13 a.m. and returning to the hive at 7:04 p.m., and aggressive than others. Their response time after disturbances was approximately 53.29 seconds, and they followed up to 182.42 meters. The average values for free acidity, moisture content, ash content, pH, and hydroxyl-methyl-furfural*

(HMF) were measured as 19.14 meq/kg, 17.43%, 0.09%, 3.13, and 5.49 mg/kg, respectively. During pollen analysis, 41 and 23 plants were identified during the first and second honey harvesting seasons. There were unique plants for each agro ecology. During both harvesting seasons, Eucalyptus globulus accounted for the highest percentage. In general, the diets presented by T₄ performed better. A.m monticola in the highland showed better performance. The overall stated characteristics of the honey fell within the established ranges for honey quality standards of both the national and international and Eucalyptus globulus was the dominant plant which identified in the honey sample. But, further findings will be better regarding all these works.

Keywords: *Evaluation, Honeybee, Honey Quality, Performance, Pollen analysis, South Wollo, Supplementation*

CHAPTER 1: INTRODUCTION

The management of honey bee colonies for pollinating crops, honey, and other products is known as apiculture, or beekeeping (Bihonegn and Begna, 2021). Farmers and those without land use it serves as a non-farm, environmentally friendly business venture (Kassa and Regassa., 2020). This indicates that it is an intensive agricultural practice that is non-polluting, does not require a lot of land, and yields quick financial returns. Beekeeping is an ancient historical practice in Ethiopia and provides income to rural households (Legesse *et al.*, 2015; Teferi, 2018). Beekeeping generally benefits environmental stability and food security through the production of valuable goods like honey, beeswax, queens, and bee colonies, pollen, royal jelly, bee venom, and propolis. Almost one in ten rural households in Ethiopia keep honeybees, making it one of their most significant economic endeavors (Khoobakht, 2018).

Ethiopia's enormous biodiversity and afro-climatic variation allowed for a wide variety of honeybee flora and a large number of honeybee colonies (Gelalcha *et al.*, 2023). Beekeeping methods vary, with most hives being traditional (95.37%), followed by frame hives (3.33%) and transitional hives (1.30%) (Tesfaye *et al.*, 2017). Ethiopia leads in Africa and ranks ninth worldwide in honey production, with 7 million honeybee populations and 2 million households involved in the industry (Barik, 2017; Gelalcha *et al.*, 2023). Ethiopian honey is produced in practically every region. Nonetheless, Oromia, which is responsible for more than 51% of bee colonies and 38% of honey production, and Amhara, which is responsible for roughly 21% of colonies and 26% of honey production, are the two most significant honey-producing regions. Alternatively, about 18% of bee colonies and 18% of honey production are accounted for by the Southern Nations, Nationalities Peoples Regional States. While Benshangul-Gumuz and Tigray account for 8% and 7% of global honey production and 5% and 4% of all bee colonies, respectively (Gelalcha *et al.*, 2023).

Beekeeping growth is slow due to various challenges like incorrect harvesting, bee illnesses, pests, agrochemical poisoning, deforestation, the physical environment, and the genetic makeup of the bee itself (Dafar, 2018; Mossie and Biratu, 2019; Singh, 2019; Ahamad *et al.*, 2020; Paray *et al.*, 2021). Despite having the highest bee density, leading honey production the subsector's contribution to GDP does not reflect the country's abundant resources. Export performance has

been weak and declining due to stricter regulations and increasing fraud regarding its quality (Fenet and Alemayehu, 2016; Gelalcha *et al.*, 2023).

The goal of management strategies in apiculture is to enhance or improve a variety of elements required for the colony's development and growth as well as improve product quality (Abera and Alemu, 2023). The harvesting season, associated climatic factors, and postharvest handling procedures are what cause honey quality fluctuations (Million and Desalegn, 2020). Identifying the cause of colony decline (Asrat *et al.*, 2023), evaluating of honey bee colony performance at on-farm and on-station (Merssa *et al.*, 2016; Aleme *et al.*, 2017; Mossie and Biratu, 2019; Aleme and Nwankwo, 2021; Peter *et al.*, 2023) and assessing flowering calendar (Bareke and Addi, 2018; Betelhem *et al.*, 2019; Bareke and Addi, 2019; Wolditsadik *et al.*, 2022; Clara *et al.*, 2023; Ofjan and Etenesh, 2023) of that specific area is important for achieving the management strategies. The challenges of beekeeping have called on the attention of beekeepers, researchers, and other stakeholders to advance knowledge to improve their status and output of hive products. This can be done by improving beekeeping technology through honey bee breeding (Plate *et al.*, 2019) or feeding of qualified and quantified supplements during dearth periods (Ahamad *et al.*, 2020; Hoover *et al.*, 2022; Topal *et al.*, 2022; Timuroğlu *et al.*, 2022) to mitigate beekeeping challenges and increase the chances of good pollination services.

One of the major concerns for pollinator populations is impaired nutrition due to reduced forage availability. Adequate nutrition is essential for the long-term health and survival of honey bee colonies (Avni *et al.*, 2014; Wright *et al.*, 2018; Amro *et al.*, 2016; Pain, 2017; Arien *et al.*, 2020; Topal *et al.*, 2022). Inadequate nutrition may cause a gradual failure of workers, which reduces colony fitness. While supplementary feeding has proven valuable during resource scarcity and stressful conditions, available supplements may not always fulfill seasonal colony requirements, potentially due to nutritional deficiencies (DeGrandi-Hoffman *et al.*, 2016; DeGrandi-Hoffman *et al.*, 2018). To address this issue, different mixtures and ratios of micronutrients can be explored as potential remedies (Bonoan *et al.*, 2018).

Numerous countries have conducted feeding studies on honey bees and bumblebees, as evidenced by research studies in various nations (Kösoğlu *et al.*, 2019; Eshbah *et al.*, 2018a; Mcaulay *et al.*, 2018; Nicolson *et al.*, 2018; Sena *et al.*, 2017). Furthermore, scientific models have been devised

to explore the impact of these nutrients on colony growth, providing insights into the future outlook for bee colonies (Paiva *et al.*, 2016). During the pine honey season in autumn, when pollen sources are scarce, colonies tend to weaken following the honey harvest. Therefore, it becomes crucial to implement appropriate feeding strategies for wintering to overcome this challenge (Topal, *et al.*, 2022). The provision of pollen and nectar supplements or substitutes helps save weaker colonies and maintain enough bee population to derive benefits of the future non-dearth period. However, the practice of protein-source feeding is not followed. Beekeepers in Ethiopia mostly feed their honeybee colonies with sugar syrup, *beso* (roasted and grounded barley flour), *Shiro* (roasted spiced pulses flour), and honey (Beyene *et al.*, 2015; Gebremeskel *et al.*, 2015; Kiros and Tsegay, 2017 and Lango and Lomba, 2020).

1.1. Statement of the Problem

There are other feed types in addition to *Shiro* and *beso* reported by researchers for example, skimmed milk powder, bakery and brewery yeast, egg yolk powdered, sorghum powder, and stinging nettle infusion (Al Mārghitaş *et al.*, 2010; Haleem *et al.*, 2015; El-Wahab *et al.* 2016; Agarwal *et al.* 2023). However, there is no finding on the impact of these feeds on our country's honeybee race because supplementing honeybees with qualified and quantified feeds during this period is a key factor in sustaining the colony and boosting hive products. On the other hand, assessing the potentiality of the races (local honeybees) and laying the foundation for future selection and improvement of the local honeybees in their habitat is very important. Though the color, size, and distribution of races in the country are documented, their performance is not well studied so far, especially *Apis mellifera monticola*.

The harvesting season, associated climatic factors, and postharvest handling procedures are what cause honey quality fluctuations (Million and Desalegn, 2020). But as of right now, no information is recorded about the characteristics of honey collected in eastern Amhara, especially in certain parts of the South Wollo zone. And it is very beneficial to be aware of the honey production area's quality standards. The data can be used by interested parties and policymakers to guide future sector development initiatives. In addition, there is also a dearth of knowledge regarding the type of flora and floral calendar of bee forages in the Eastern Amhara, South Wollo Zone, Ethiopia which is very important in management strategies.

1.2. Objectives

1.2.1. General objective

To evaluate effects of different feed supplements and agro-ecologies on performances of honeybees in South Wollo Zone Amhara Regional state, Ethiopia

1.2.2. Specific objectives

1. To investigate the effect of different feed supplements on honeybees' performances at on-station
2. To evaluate on-farm honeybees' performance under varied agro-ecologies and
3. To analyze honey quality and pollen from honey samples under varied agro-ecologies in South Wollo zone, Amhara regional state, Ethiopia

CHAPTER 2: LITERATURE REVIEW

2.1. Effects of Feed Supplementations on the Performances of Honeybees

Various factors like climate change, agricultural intensification, alterations to landscapes, and atmospheric pollutants can impact the abundance and productivity of plant populations (*Şahin et al.* (2015; Pasquale *et al.* 2016; DeGrandi-Hoffman *et al.*, 2016; Topal, *et al.* 2019; Topal *et al.*, 2021). According to research conducted by Ghosh *et al.* (2020), it was discovered that colonies of bees have varying preferences for pollen-based on its protein content. Bees tend to favor floral sources that offer higher nutritional value. Further studies conducted by Brodschneider and Crailsheim, (2010), Radev *et al.* (2014) as well as Zheng *et al.* (2014) indicate, a definitive link between the nutritional value of pollen and the overall development, reproduction, and productivity of bee colonies.

In the field of beekeeping, pollen, and honey-based supplementary feeding are employed to combat hunger and promote greater population growth within bee colonies during the spring season. This practice primarily focuses on facilitating brood formation among young worker bees and supporting the proliferation of queens and drones. The objective behind these efforts is to ensure the overall health of the colonies, reducing the risk of bee loss resulting from the application of agricultural chemicals in plant production (Wheeler and Robinson, 2014; kösoğlu *et al.*, 2019). It is crucial to note that the egg-laying capacity of queen bees, longevity of honey bees, and vulnerability to diseases prompt some individuals to abandon the colony, and permanent mortality is significantly influenced by the composition and proportion of nutrients they receive (Topal *et al.*, 2019; Amro *et al.*, 2020).

Because the flora of interest to bees is not available in sufficient quantities all year round, efforts have been made to maintain the strength of the colony by increasing brood survival and length of adult age through the provision of pollen substitutes and supplements. Many diets containing major ingredients such as soybean flour, brewer's yeast, parched gram, guar meal, egg yolk powder, skimmed milk powder, casein, pea powder, rice bran, and fish meal have been developed and tested on honeybees. Numerous investigators who achieved success under particular evaluation conditions have developed and documented a variety of diet combinations (Ahamad *et al.*, 2020).

Researchers looked into the connection between the development of the hypopharyngeal gland in bee workers and their consumption of diets high in protein. When worker bees were fed bee bread, their hypopharyngeal glands displayed the largest acinal surface area. This was followed by the yeast-gluten mixture, pollen loads, Nectapol, and conventional substitutes, in that order (Ahamad *et al.*, 2020). The impact of proline as a nutrient on *Apis mellifera* hypopharyngeal gland development was ascertained by (Smodiš and Gregorc, 2015). Proline had the effect of increasing the acini's diameter. When the colonies are fed artificial diets, there is an increase in all activities in honey. Depending on the severity of lean periods in various countries, diets have been fed during both the summer and the winter (Kumari and Kumar, 2020). Nonetheless, regardless of the bees' geographic location, it has been reported that feeding them during lean periods has a positive impact on the colony parameters (Ahamad *et al.*, 2020).

According to Mohammed *et al.* (2019), Agarwal *et al.* (2023), and Al Mārghitaş *et al.* (2010), white sorghum powder and syrup of nettle infusion have higher attractiveness, respectively. Findings of Haleem *et al.* (2015), El-Wahab *et al.* (2016) and Agarwal *et al.* (2023) support the attraction of yeast and skimmed milk powder to honey bees. The result of Ricigliano *et al.* (2022) revealed that sugar-negative feed is less acceptable by honey bees than sugar-positive ones. Nettle infusion can increase brood surface area (Al Mārghitaş *et al.*, 2010). Yeast also has high amount of protein levels and generally provides a more balanced set of amino acids and is also more attractive than soya bean flour which is confirmed by (Jach *et al.*, 2022). Proteineous supplemental diets have been shown to have a positive effect on the efficiency of queen egg laying (Szawarski *et al.*, 2019; Palmer-Young *et al.*, 2021). Proteins in nettle infusion (Al Mārghitaş *et al.*, 2010) and omega-3 polyunsaturated fatty acids (PUFAs) and the presence of leucine, isoleucine, and valine in white sorghum (Mohammed *et al.*, 2019; Pezzali *et al.*, 2020; Masresha and Belay, 2020; Pontieri *et al.*, 2022) and high amount of protein level in yeast (Jach *et al.*, 2022) have a great impact on honeybees colony performances. This is consistent with Zheng *et al.*, (2014), who claimed that ten of the 21 amino acids the proteins' building blocks are necessary for the development of worker bees and the rapid growth of larvae.

According to Brodschneider and Crailsheim (2010), colony development is restricted by a lack of at least one of the essential amino acids in the food protein and the highest requirements are l-leucine, l-isoleucine, and l-valine. The importance of supplementing honey bees with essential

amino acids was also noted by (Hendriksma *et al.* 2019). According to Shumkova and Balkanska, (2020), it is anticipated that foods like Apimix and Apipasta, which are high in vitamins and amino acids, will encourage bee colonies to produce more bee brood. For honey bees to lay eggs, improve brood rearing, increase yield, and avoid diseases and stress, food quality and availability are essential (Branchiccela *et al.*, 2019; Topal *et al.*, 2022; Timuroğlu *et al.*, 2022). Research from Lamontagne-Drolet *et al.* (2019), Islam *et al.* (2020) and Hossaini *et al.* (2020) stated that honey bees increased the surface of sealed brood areas after consuming various supplements and pollen substitutes. Supplemental diets increased the population density in comparison to the non-supplemented control group (Islam *et al.*, 2020; Moumeh *et al.*, 2020; Kumari and Kumar, 2020; Ahmad *et al.*, 2021; Sarioğlu-Bozkurt *et al.*, 2022; Topal *et al.*, 2022).

There was a strong correlation between colony strength and food quantity and quality as well as brood area. El-Wahab *et al.* (2016), Branchiccela *et al.* (2019), Ahamad *et al.* (2020), and Eshbah *et al.* (2018) also stated giving a mixture of food with sugar syrup improves brood and adult colonies of honey bees. Similarly, Topal *et al.* (2022) indicated that most protein supplementations affect the health and longevity of honey bee colonies. Hoover *et al.* (2022) Islam *et al.* (2020), Ahamad *et al.* (2020), and Ahmad *et al.*, (2021b) revealed that supplemental diets increased the population density in comparison to the non-supplemented control group. The existence of a complex network between nutrition, gut microbiota, immunity, and pathogen infection was stated by (Castelli *et al.*, 2020; Hesham *et al.*, 2022; Mortazavi *et al.*, 2020). Hristov *et al.* (2020) stated adult worker bees' metabolic health is influenced by the nutritional quality of their larval diets which are better for survival and have higher metabolic rates per unit of body mass.

Supported research from Boes (2010) and Stürup *et al.* (2013) that suggested a colony might modify queen production depending on the season (day length (long, short) and temperature (cold, warm, humid)), as well as other environmental cues like colony size and food availability. The nutrition of honey bee larvae influences the development of new queens favorably (Czakońska *et al.*, 2015). Protein-rich supplemental diets have an advantageous effect on the production cells of queens (Estegamat and Gholami, 2010; Haleem *et al.*, 2015; Szawarski *et al.*, 2019; Cengiz *et al.*, 2019). The quality of royal jelly, which is secreted by the hypopharyngeal glands, depends on the source of honey bees' food and the time of year (Sherif *et al.*, 2018; Hu *et al.*, 2019; Shakeel *et al.*, 2020). For instance, the amount and structure of essential Royal Jelly components like amino

acids, carbohydrates, and vitamins are significantly altered when bees are fed sugar syrup (Shi *et al.*, 2018; Khan and Ghramh, 2022).

Table 1: Summary of different supplemental feeds and their effect on the performances of honeybees.

Feedstuff	EA (cm ²)	PA (cm ²)	TB (cm ²)	TB (cm ²)	NA (cm ²)	HA (cm ²)	POA (cm ²)	CS (frames)	HY (kg)	References
Protein+ carbohydrate +vitamin and mineral	-	7495	-	7495	-	9800	252	18	11.3	Sihag & Gupta, (2013)
Wheat gluten	18235	-	-	-	-	-	-	-	-	Irandoost & Ebadi (2013)
Pea flour	-	-	1274.3	-	-	-	-	9.7	26.8	Gemeda (2014)
Soy flour-1part, brewer's yeast-1part, soy protein hydrolysate1part, sugar-1part, glucose-1part	-	66	-	76.2	-	59.7	-	13.7	-	Kumar & Agrawal (2014)
Bee-sup	-	1938.3	-	1121	-	1963.5	-	8.3	-	Kumari & Kumar (2020)
Soya bean diets	-	-	300	-	258.9	-	219.9	9.4	11.5	Hunde (2022)
45 g soybean flour + 15 g Brewer's yeast + 75 g powdered sugar + 7.5 g skimmed milk + 7.5 g date palm pollen + 200 ml sugar syrup supplement with Vitamin C)	-	2277.3	-	-	-	-	-	14.5	13	Saboor <i>et al.</i> (2021)
Sucrose solution	-	2153	-	-	-	-	-	-	-	Khan <i>et al.</i> (2022)
Soybean flour (traditional pollen substitute), sugar cane syrup	-	1489.27	-	-	-	-	-	8	8.7	Ullah <i>et al.</i> (2021)
30 g soybean flour + 15 g Brewer's yeast+ 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup	-	1562.0	-	-	-	-	-	10	7.5	Islam <i>et al.</i> (2020)
3 kg of Feed bee/colony	-	773.5	-	-	-	-	-	-	-	Sena <i>et al.</i> (2012)

EA: Egg Area; **PA:** Pupae Area; **TB:** Total Brood; **TAB:** Total Un-Sealed Brood; **NA:** Nectar Area; **PO:** Pollen Area; **HA:** Honey Area; **HY:** Honey Yield; **CS:** Colony Strength; **QC:** Queen Cell

Table 1. *continued ...*

Feedstuff	PA (cm ²)	TB (cm ²)	TAB (cm ²)	NA (cm ²)	HA (cm ²)	POA (cm ²)	CS (frames)	HY (kg)	References
Soybean flour (60g) + Honey (35g) + Yeast (5g) + Vitamins (1 g/kg)	-	790.70	-	-	-	-	5.8	-	Kumar <i>et al.</i> (2021)
10 brewery yeast+ 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A,D and E vita-mins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup)	285.8	-	-	-	-	-	5.3	3.7	El-Wahab <i>et al.</i> (2016)
Pollen supplemental gram diet 250 g per colony + brewer's yeast + sugar	-	4945	-	-	3820	-	-	-	Mahmood <i>et al.</i> (2013)
soya bean flour and wheat gluten	-	22680	-	-		143.5		8.9	Irاندoust & Ebadi (2013)
Defatted soya flour = 150g;Wheat flour = 150g; Deactivated yeast = 100g; Sugar = 266g; Water = 134ml (Total = 800 g + 40 ml Rum)	-	4945	-	-	602.2	-	6	-	Dogra <i>et al.</i> (2023)

PA: Pupa Area; **TB:** Total Brood; **TAB:** Total Un sealed Brood; **NA:** Nectar Area; **PO:** Pollen Area; **HA:** Honey Area; **HY:** Honey Yield; **CS:** Colony Strength; **QC:** Queen Cell

2.2. Performance of Local Honeybees in Ethiopia

Ethiopia, which is located in Northeast Africa at close proximity to the Middle East, is an important region for honeybees. The country is known for its diverse agroecological zones (AEZs), ranging from humid to arid, and elevations from > 100 m below sea level to 4500 m above sea level (masl) (Teweldemedhn *et al.*, 2020). Honeybee races in Ethiopia are *A.m. monticola*, (Guguftu in south Wollo up to Woko (west of Woldeya) and the highland areas of east of Debre Tabor, the plateau of Korem (around Lake Ashenge) and south of Mekele. These bees are distributed to north Gonder near Milligebsa in Debark, and the Semen Mountains areas.) *A.m. scutellate* (western humid mid-land parts of the country,), *A.m. bandansii* (central highlands up to western parts of south Gonder, west and east Gojjam, north Showa and west Showa up to Nekemte and Shambu as well as southern parts of the central highlands of Ethiopia in areas like Wolliso and Hossana) , *A.m. jementica*, (northwest parts of the country along the Sudan border areas above Kumruk, west of Manbuk, west of Aykel up to Metema, west of Lay Armacho (all areas of the Angereb lowland); in the north, the western Tigray zone places like Humera and Shiraro. In the east, the distributions of these bees include all areas of the Afar lowland plains and the associated valleys and escarpments), *A.m. woyi gambela* (Gambela regional state) (Gupta *et al.*, 2014; Pirk *et al.*, 2015; Tesfu and Abebe 2016).

Weather parameters play a major role in influencing brood rearing, pollen area, nectar area, bee strength, and honey production as well as the activity of pollen foragers (Manoj *et al.*, 2017). Colony performance was impacted by the beehive type and honey bee race (Abou-Shaara *et al.*, 2013). According to Alqarni *et al.* (2014), pollen harvesting was impacted by solar radiation, but brood-rearing activities were connected with monthly pollen storage. Based on climatic conditions, vegetation cover, altitude, and other abiotic and biotic factors, honey bee races are dispersed throughout the nation for each region's agro ecology (Aleme and Nwankwo, 2021).

Foraging activity by honey bee colonies depends on gathering pollen, nectar, honey, and its yield (Hristov *et al.* 2020). The main tendency of nectar and pollen storage activity does coincide with the pattern of brood rearing of the investigated colonies in agro ecologies (Divyaet *et al.*, 2017; Hoover *et al.*, 2022). The distance of honey bee forages, colony size, food availability, month, and time of day may all play a role in the difference between the active and dearth seasons of foraging

activity and all are affected by weather conditions (Topal *et al.* (2022). Adult worker bees' metabolic processes are influenced by the nutritional quality of their larval meals, with diets that are better suited for survival having a greater metabolic rate per unit of body mass (Crone and Grozinger, 2021).

According to Sihag and Kaur (2018), there is mounting proof that high-quality nutrition can reduce the negative effects of several stresses on honey bees (*Apis mellifera*). For the development of more resilient phenotypes, pollen, the main source of protein and fats in bee diets, is particularly important. According to Manoj *et al.* (2017), diet is one element that influences how much nectar is present in honey bee colonies' hives. According to El-Wahab (2016) and Gabka (2014), colony strength has an impact on the pollen, nectar, and honey areas. Pollen and nectar stores were also identified by Shehata (2016), Saini *et al.* (2018), and Tawfik *et al.* (2020) as being positively correlated with brood and colony strength. Due to repeated queen cell growth and advanced markers of swarm preparation, the race produced more queen caps per hive in October, demonstrating a strong desire to swarm (Hunde and Hora, 2022; Tarekegn *et al.*, 2022). The expression of these behavioral traits can be strongly influenced by environmental factors and beekeeping management techniques (Büchler *et al.*, 2013; Delaplane *et al.*, 2013).

Table 2: Summary of performance of local honeybee races documented in Ethiopia

Races	PA (cm ²)	TB (cm ²)	TAB (cm ²)	NA (cm ²)	HA (cm ²)	POA (cm ²)	Hy (Kg)	Ef (am)	Lf (pm)	Ab (%)	Qc	References
<i>A.m. scutellata</i>	-	216	-	65.6	-	29.3	-	-	-	27	2	Alemu <i>et al.</i> (2014)
<i>A.m. bandansii</i>	-	252.5	-	-	-	-	12.33	6:25	6:40	-	-	Mossie and Biratu (2019)
<i>A.m. scutellata</i>	-	148	-	131.9	-	97.5	-	-	-	63.3	5.2	Negash and Argaw (2022)
<i>A.m. wayi gambela</i>	-	-	127	32	144	63	-	6:25	6:25	26	-	Aleme <i>et al.</i> (2017)
<i>A.m. scutellata</i>	127	-	54	35	-	2	-	6:10	6:29	30	3.42	Tarekegn <i>et al.</i> (2022)
<i>A.m. bandansii</i>	-	6114.1	-	3399	-	487	11.8	-	-	54	2.2	(Hunde & Hora, 2022)
<i>A.m. monticollata</i>	2546	-	20.4	254	-	24.4	18.2	5:55	7:04	37.5	30	Merssa <i>et al.</i> (2016)
<i>A.m. bandansii</i>	-	-	-	-	-	-	12.5	-	-	-	-	Zewudu <i>et al.</i> (2012)
<i>Apis spp.</i>	-	-	-	-	-	-	-	6.17	-	-	-	Joshi & Joshi (2010)
<i>A.m. monticollata</i>	-	-	-	-	-	-	-	5:25	7:04	-	-	Solomon and Likawent (2015)

PA: Pupae Area; **TB:** Total Brood; **TAB:** Total Un-sealed Brood; **NA:** Nectar Area; **PO:** Pollen Area; **HA:** Honey Area; **HY:** Honey Yield; **EFT:** Early Foraging Time; **LFT:** Late Foraging Time; **AB:** absconding rate; **QC:** Queen Cell

Table 3: Summary of defensive behavior of different races of local honeybees in Ethiopia

Races	TtFs_sec	Tt_sec _mag	TNo S__B	TNo S_G_	TDF_m_	References
<i>A.m. bandansii</i>	3.9	152	282	-	283.2 m	Mossie and Biratu (2019)
<i>A.m. wayi Gambela</i>	-	-	80	-	115	Aleme <i>et al.</i> (2017)
<i>A.m. scutellate</i>	40	-	29	281	400	Tarekegn <i>et al.</i> (2022)
<i>A.m. monticolla</i>	89.7	260	22.3	16.6	188.4	Merssa <i>et al.</i> (2016)

TtFs-sec: Total time for the first sting in seconds; **Tt-sec-mag:** Total time in seconds for mass aggressiveness; **TNoS-B:** Total number of stings on the leather ball; **TNoS-G:** Total number of stings on the glove; **TDF-m:** Total distance followed to the observer in meters.

2.3. Physicochemical Characteristics of Ethiopian Honey

A naturally sweet food with many uses, honey is well known for its versatility. Different bee species produce this tasty, sticky, and viscous liquid from flower nectar or the secretions of living plant parts (Gela *et al.*, 2023). Because of its unique composition, honey is used for industrial, nutritional, and medicinal purposes all over the world. Because honey is a primary energy source that prolongs the retention of nitrogen in the digestive system, it is especially advised for the elderly, pregnant women, and sick individuals (Kinat *et al.*, 2013). Honey is naturally a supersaturated solution of sugars, with fructose and glucose making up the majority of the sugar components.

Approximately 95% of the dry weight of honey is composed of carbohydrates (Gela *et al.*, 2023). Although honey contains a variety of other substances, its sugar content primarily determines its nutritional qualities (Alvarez-Suarez *et al.*, 2018). In addition to these, other components that enhance the quality and health advantages of honey include proteins, enzymes, amino acids, minerals, vitamins, organic acids, and phenolic compounds (Cianciosi *et al.*, 2018).

The majority of honey's volatile compounds, which also give it its distinct flavor and aroma, are alcohols, ketones, aldehydes, acids, and esters. Furthermore, flavonoids and phenolic acids, which have a variety of biological effects and function as natural antioxidants, are abundant in natural honey (Contreras-Martínez *et al.*, 2020). Ever since ancient times, products derived from honey bees, like propolis, pollen, and honey, have been touted as health benefits (Kamaruzzaman *et al.*, 2019; Singh and Takhellambam, 2021; Sanyal *et al.*, 2023). It also has distinct aromatic and organoleptic qualities that appeal to consumers in addition to carbohydrates, proteins, minerals, and vitamins (Solayman *et al.*, 2016; Pita-Calvo and Vázquez, 2018).

A range of beekeeping methods are employed in Africa, where the traditional method of producing honey is still widely used. Honey is traditionally harvested by hanging various types of hives on tree branches or by hunting from tree holes. In this instance, swarms of genetically identical wild bees live in hives constructed from locally accessible materials and positioned in forest trees (Gela *et al.*, 2023).

Conversely, the contemporary beekeeping system uses top bar hives and movable framed hives to produce honey. As a result, the honey production system and postharvest processing methods also affected the quality and composition of honey (Alvarez-Suarez *et al.*, 2018). The physical and chemical compositions of honey are generally influenced by a variety of factors, aside from production and processing methods, and honey derived from different sources exhibits distinct characteristics (Alvarez-Suarez *et al.*, 2018). The composition and quality of honey are affected by several variables, such as the sources of the flowers, their geographic and environmental origin, the time of year, how they are processed and stored, and other factors (Alvarez-Suarez *et al.*, 2018). Furthermore, the amount of enzymes that bees add during the honey-making process is crucial to the quality of the product, so the processes used by different bee species can also affect the composition of the honey. For example, collecting unripe honey while it is still in the nectar stage lowers the number of enzymes needed, which lowers the quality of the honey and lowers the demand for it on the market (Alaerjani *et al.*, 2022). The composition of honey collected from various locations and at different times of the year may vary based on the types of nectar and the bees' sources of forage. Furthermore, the qualities of honey harvested at different times of the year from the same region may differ. This implies that various seasonal and climatic factors, in

addition to pre- and post-harvest beekeeping techniques, may affect the quality of honey (Gela *et al.*, 2023).

Studies conducted in Ethiopia have determined the physicochemical characteristics and geographic origins of *Apis mellifera* honey that were collected from particular locations during different times of the year (Tesfaye *et al.*, 2016; Muluget *et al.*, 2017; Berhe *et al.*, 2018; (Yayinie *et al.*, 2021); Gebeyehu and Jalata, 2023). The current practice of adulterating honey with foreign substances is changing the natural composition of honey significantly and raising safety concerns for both marketing and consumption. Teferi (2023) conducted a review that examined the pervasive practices of adulterating honey in Ethiopia, which have resulted in a decline in quality and an impact on both the domestic and international honey markets. To preserve the natural honey quality and its provenance, it is essential to regularly monitor and measure the key characteristics of honey, such as moisture content, sucrose and reducing sugars, pH value, electrical conductivity, ash content, free acidity, diastase activity, and hydroxymethyl furfural (HMF), against the set standard quality parameters.

Higher levels of moisture, free acidity, and sucrose content were found in some honey samples that were purchased from neighborhood markets; these findings point to the adulteration of honey. The moisture content of honey samples collected from farm gates was significantly influenced by both hive type and agro ecology had a particular impact on the moisture, free acidity, and sucrose content of the samples collected from farm gates and local markets (Gela *et al.*, 2023)

2.3.1. Moisture content

The second most important component of honey is moisture (Belay *et al.*, 2017; Nega *et al.*, 2020; Gebeyehu and Jalata, 2023). The moisture content of honey is one of the key factors in the analysis of honey quality. This is because a honey's moisture content can affect how long it lasts on the shelf (Akalework *et al.*, 2020). A high moisture content (beyond 22%) causes unfavorable fermentation of honey by microorganisms and enzymatic factors during storage, which is detrimental to the shelf life of honey (Akharaiyi, 2016). The results for moisture indicate that beekeepers are using appropriate handling and storage conditions of honey, as the moisture content in honey is also related to environmental and geographic conditions as well as handling procedures at the apiary and during honey storage (Nascimento *et al.*, 2022).

Foods that contain more monosaccharide units also have higher water activity kinetics. Honey's higher fructose and glucose content prevents microorganisms from growing because the monosaccharide units bind water (Raweh *et al.*, 2023). The honey industries believed that moisture was the only factor that mattered in determining how honey would spoil, stabilize, and store (Gebremedhin *et al.*, 2013; Getu and Birhan, 2014; Additionally, standard authorities only took moisture into account when defining quality parameters. Microorganisms cannot be rationally controlled in their growth and multiplication by moisture content alone (Abselami *et al.*, 2018; Anza *et al.*, 2022). The quality increases with decreasing moisture and water content (Fiseha *et al.*, 2021; Nzeh *et al.*, 2022).

The amount of moisture in honey affects its flavor, viscosity, and fluidity (Gilbert *et al.*, 2021). Other characteristics of honey, such as density, specific gravity, refractive index, viscosity, and optical qualities, are influenced by its moisture content. The complex interplay of variables such as hygroscopic nature, extraction and handling techniques, season, climate, degree of maturation, initial moisture of the nectar, and its geographical origin determines the moisture content (Rana *et al.*, 2023; Sharma *et al.*, 2023a; Meena *et al.*, 2023). The water content of honey is what keeps it stable against granulation and fermentation. Typically, the moisture content of ripe honey is less than 18.6 % (Alqarni *et al.*, 2016).

Additionally, some research has shown that stingless bee honey has a high moisture content, often exceeding 40 % (Biluca *et al.*, 2016; Nordin *et al.*, 2018). These findings can be explained by a variety of factors, including environmental humidity, nectar collection from flowers growing beneath the ground, ripe fruits that are high in water content, or even various stingless bee species (Ávila *et al.*, 2018). It's also crucial to remember that stingless bee honey's high moisture content affects its sensory qualities because it has a lower viscosity and a shorter shelf life as a result of its faster fermentation (de Araújo *et al.*, 2023). Higher water content is associated with lower sugar content (Biluca *et al.*, 2016; Grando *et al.*, 2023).

2.3.2. Ash content

Ash is thought to be a quality criterion for honey, suggesting that the honey may have a botanical origin. Ash is also dependent on the soil and climate of the honey's origin (Kebede *et al.*, 2017; Berhe *et al.*, 2018; Gilbert *et al.*, 2021; Thakur *et al.*, 2022; Gebeyehu and Jalata, 2023; Sharma *et*

et al., 2023b). Because plants absorb nutrients from the soil, the ash content of honey is a representation of the total mineral residue found in the nectar. It is primarily determined by the composition of the nectar that is collected (Silva *et al.*, 2016; Nordin *et al.*, 2018). Certain nitrogen compounds, minerals, pigments, and aromatic substances contribute to honey's mineral content (Akalework *et al.*, 2020). Honey's ash or mineral content affects its color, flavor, medicinal value, quality, and a few physical attributes, among other qualities (Sharma *et al.*, 2021). While directly correlated with sugar levels and water levels in the samples, the soluble solids function as an indicator parameter of the rate in solution solids such as sugars, organic acids, and minerals (Biluca *et al.*, 2016; Grando *et al.*, 2023).

Despite the lack of a standard value for this parameter (for stingless bees) in the Codex Alimentarius (Codex Alimentarius Commission, 2001), studies using honey from *A. mellifera* have revealed that the average ash content of honey is 0.17 %, with a range of 0.02 % to 1.03 % (Chakir *et al.*, 2011). However, ash values for stingless-bee honey in the state of Pará ranged from 0.03 to 0.33 % and 0.09 to 1.11 %, respectively, according to (Gomes *et al.*, 2017) and (Silva *et al.*, 2013). The color and flavor of honey may be correlated with its mineral content; a higher mineral content results in a stronger, darker flavor that appeals to consumers (Silva *et al.*, 2016)

2.3.3. Free acidity

The acidity of honey arises from the combination of inorganic ions and organic acids, including gluconic acid, which is in balance with its lactones or esters, phosphate, and chloride. The assessment of honey fermentation, the verification of unifloral honey, and the separation of nectar from honeydew can all benefit from acid measurement. Honey with lower acid values has better antioxidant activity, prevents the growth of harmful microorganisms in honey, and indicates the absence of unwanted fermentations (Belay *et al.*, 2013; Kebede, *et al.*, 2017; Akalework *et al.*, 2020; Gilbert *et al.*, 2021; Tigistu *et al.*, 2021; Gebeyehu and Jalata, 2023). A significantly higher acid content in honey causes it to become sour and is a sign that sugars are fermenting into organic acids. On the other hand, if honey's acid content is at the necessary level, it helps preserve honey's flavor (Akalework *et al.*, 2020). The source of nectar, the type of bee, and the activity of bacteria or enzymes are all connected to free acidity (Machado De Melo *et al.*, 2018). It shows one of the honey's quality parameters and whether or not the honey has undergone fermentation (Sousa *et al.*,

2016). Furthermore, free acidity varies depending on the type of flower and how it is stored, which can affect how acidic honey is (Alves *et al.*, 2013; Tornuk *et al.*, 2013). Variations in the harvesting process, storage conditions, and geographic location (Berhe *et al.*, 2018), may be the cause in the instance of this study's findings.

Because of the low concentration of organic acids, which give honey its flavor and antimicrobial properties as well as stability in the food matrix, all types of honey have some acidity. Aspartic acid, citric, acetic, formic, fumaric, galacturonic, malonic, formic, acetoglutamic, gluconic, glutamic, butyric, glutaric, butyric, shikimic, propionic, pyruvic glyoxylic, 2-hydroxybutyric, -hydroxyglutaric, isocitric, lactic, malic, methylmalonic, quinic, succinic, tartaric, oxalic, and others are the most significant (Cianciosi *et al.*, 2018).

Honey has a slight acidity due to the production of the essential acid gluconic acid through the oxidation of glucose by the enzyme glucose oxidase (Raweh *et al.*, 2023). Therefore, the high concentration of gluconic acid in Acacia flowers, which are a rich source of nectar, may be the cause of the increase in FA (Awad *et al.*, 2017). Acidity is a crucial indicator of the start of the fermentation process, which is linked to several variables, including the amount of gluconic acid produced by the enzymatic breakdown of glucose, the source of the flowers, the mineral content, and the time of harvest (Karabagias *et al.*, 2014; Oroian and Ropciuc, 2017). Furthermore, free acidity is a quality parameter primarily associated with the presence of organic acids derived from the nectar, which are produced by the fermentation of sugars, which yields acetic acid, as well as by certain enzymatic mechanisms, such as glucose oxidase's conversion of glucose to gluconic acid (Ávila *et al.*, 2018). It's interesting to note that research using stingless bee honey has revealed higher free acidity contents than studies using *A. mellifera* honey. For instance, according to (Duarte *et al.*, 2018), the free acidity of honey from stingless bees ranged from 17 meq/ kg to 125 meq/ kg. On the other hand, (Chuttong *et al.*, 2016) found that the honey samples had high acidity, with values ranging from 440.0 to 592.0 meq/ kg.

2.3.4. HMF (*Hydroxyl Methyl Furfural*)

Honey's shelf life is determined globally based on HMF content standards that are established by national laws. When deciding which use-by date to print on their labels, packaging plants must adhere to the requirement. Adherence to HMF content regulations is crucial as it ensures that

buyers can purchase honey that has undergone minimal modifications (Belay *et al.*, 2013). Hexose sugars undergo acid-catalyzed dehydration to form HMF, with fructose being especially vulnerable. Furthermore, only trace amounts of HMF are found in fresh honey, and it has been observed that storage and prolonged heating of the honey increase its concentration (Tesfaye, 2016). Thus, HMF is a crucial metric for determining the purity of honey (Getu and Birhan, 2014; Abselami *et al.*, 2018). HMF (5-hydroxymethyl-2furaldehyde) is a naturally occurring compound that is produced when glucose or fructose are broken down at a pH of less than 5. One of the key markers of honey quality is its HMF content, which shows whether the honey is old or overheated (Akalework *et al.*, 2020).

HMF, which is commonly used as a gauge of honey freshness, is described as a breakdown product of fructose that forms gradually and naturally during honey storage and much more quickly when honey is heated (Berhe *et al.*, 2018; Mouhoubi-Tafinine *et al.*, 2018; Fiseha *et al.*, 2021). The honey's quality decreases with increasing HMF value. When honey is stored and heated for an extended period, its HMF concentration rises (Gomes *et al.*, 2010).

HMF is a sign of inadequate storage circumstances at elevated temperatures. Prior research revealed that aged honey has a high HMF content, even when it is in its natural nest (Gela *et al.*, 2021). Furthermore, there could be overheating while processing. Overheating honey and not storing it properly can result in unwanted compounds being produced and increase the formation of HMF (Belay *et al.*, 2017). Based on the quantity present, the quantification of HMF in honey samples is a reflection of either its freshness or a great indicator of quality degradation in honey samples (Sajid *et al.*, 2020; Khansaritoreh *et al.*, 2021). Honey of the highest quality typically has a lower HMF value (Tarapoulouzi *et al.*, 2023)

It is important to note that areas with hot, humid climates, like the Amazon region, encourage the development of 5-HMF (Bandeira *et al.*, 2018) even when kept in room temperature storage (Khalil *et al.*, 2010). Research using honey from Brazilian stingless bees in different climates found that fresh honey did not contain 5-HMF (Biluca *et al.*, 2016). Therefore, 5-HMF formation is generally favored by the climatic conditions used to store the *A. mellifera* and Meliponinae honey samples produced in the eastern Amazon region (Gomes *et al.*, 2022).

2.3.5. pH

Honey generally has an acidic nature, regardless of its varied geographic origin (Belay *et al.*, 2013). No matter where it comes from, honey has an inherent acidity that may be caused by the organic acids that give it flavor and keep it stable against microbial deterioration. During the extraction process, the pH of the honey samples is crucial because it influences the texture, stability, and shelf life of the honey. Since microorganisms affect the texture, maintain stability, and extend the shelf life of honey, it is important to prevent their growth and presence when storing honey. Low pH values support this idea (Akalework *et al.*, 2020; Gela *et al.*, 2021). Additionally, honey's low pH prevents the growth of microorganisms (Gebremariam and Brhane, 2014; Kebede *et al.*, 2017; Moloudian *et al.*, 2018; Tarapoulouzi *et al.*, 2023; Meena *et al.*, 2023). The quality improves with a decreasing pH (Nzeh *et al.*, 2022).

The amount of organic acids in the honey is correlated with its pH level. Other variables that can impact the structure, stability, and shelf life of honey, as well as the fermentation process, include the presence of inorganic ions and the conditions under which it is extracted and stored (Fuad *et al.*, 2018; Raweh *et al.*, 2023). Honey's acidic nature, which results from its low pH levels, helps it heal wounds and has broad-spectrum antimicrobial qualities by releasing oxygen from hemoglobin, preventing the growth of microorganisms, and extending its shelf life (Krishnasree and Ukkuru, 2017). The majority of honeys have low pH levels and are acidic. The extraction procedure and storage conditions may also increase acidity (Karabagias *et al.*, 2014). The pH values, which ranged from 3.10 to 5.04 mEq kg⁻¹, are consistent with earlier findings for honey samples from some species of the Meliponinae subfamily collected from the Amazon region (Silva *et al.*, 2013). Similar results were found in another study (Missio *et al.*, 2020) for *A. mellifera* honey samples, where acidity ranged from 3.86 to 4.41 meq /kg.

Table 4: Summary of physicochemical characteristics of recorded in Ethiopian honey.

Area	Moisture (%)	Total ash (%)	Reducing sugar	sucrose	acidity (meq kg ⁻¹)	HMF (Mg/kg)	pH	Water insoluble solid	Reference
Harena forest	17.89	0.19	69.48	2.43	34.57	0.84	3.87	0.12	Belay <i>et al.</i> (2013)
in and Around Gondar	18.52	0.23	67.83	7.55	28.24	6.32	3.81	3.2	Getu & Birhan (2014)
Southwestern forest of Ethiopia	18.3-21.2	-	74.26-75.86	4.8-5.2	7-10	1.2-2.56	4.05-5.97	-	Addi & Bareke (2021)
Gedebano Gutazer Wolene, Central Ethiopia	18.91	0.2	74.4	1.06	16.3	2.63	-	-	Akalework <i>et al.</i> (2020)
Jimma, Bale, Kefa and North Shewa Zone).	21.9	0.42	-	-	28.8	14.8	4.1	-	Gela <i>et al.</i> (2023)
Arba Minch Zuria district of Gamo zone, Southern Ethiopia	19.27	0.23	25	2.08	34.04	13.09	3.80	-	Anza <i>et al.</i> (2022)
Godere Woreda, Gambella, Ethiopia	18.76	0.34	74.19	2.66	24.48	9.91	3.9	0.06	Berhe <i>et al.</i> (2018)
East Gojjam Zone of Amhara Region, Ethiopia	17.5-18.19	0.09-0.26%	45.1-63.8	-	35.3-46.6	-	3.98-4.12,	-	Nega <i>et al.</i> (2020)
Masha, Gesha, and Sheko districts in southwestern Ethiopia	22.86	-	66.79	4.46	28.32	19.52	-	-	Getachew <i>et al.</i> (2014)

Table 4 Continued....

Area	Moisture (%)	Total ash (%)	Reducing sugar	sucrose	acidity (meq kg ⁻¹)	HMF (Mg/kg)	pH	Water insoluble solid	Reference
Selected districts of Bale Zone, Oromia Regional State, Ethiopia	20.34	0.29	73.12	0.1	57.66	9.45	4.2	-	Teshome <i>et al.</i> (2020)
Bale Natural Forest, Southeastern Ethiopia	18.80	0.21	66.41	4.48	32.43	36.35	3.75	-	Tesfaye (2016)
Guji Zone, Chena District, Southwestern Ethiopia	13.62	0.52	60.07	3.33	16.52		3.69	-	Tesema, (2015)
Selected districts of Bench Shecko zone, southwestern Ethiopia	16.5-20	0.08-0.3	67.5-70.1	2.57-5.17	21-41	1.8-17.1	3.6-4.2	-	Fiseha <i>et al.</i> (2021)
Northern, central, southern highland, and southwestern Ethiopia	19.33 - 22.33	0.17-0.21	67.11 - 75.75	1.00 - 3.73	38.33 - 50.00	46.53 - 67.68	3.40 - 4.10; 0.12 – 0.22	-	Getachew (2020)
Homesha district of western Ethiopia	-	0.16-0.48	-	0.12- 7.69	12.4-38.7		-	-	Adgaba <i>et al.</i> (2017)
Northeastern Ethiopia	16.4	0.17	65	6.1	23.9	1.8	4.02	0.09	Gobessa <i>et al.</i> (2012)
	11.87–16.70	0.12–0.49	39.95–59.04	0.98–4.11	19.55–35.57	23.31–59.03	2.93–4.53	-	Abera & Alemu (2023)

Table 4: continued...

Area	Moisture (%)	Total ash (%)	Reducing sugar	Sucrose	Acidity (meq kg ⁻¹)	HMF (Mg/kg)	pH	Reference
Doyogena and Kachabira Districts of Kembata Tambaro Zone, Southern Ethiopia	18.83	0.25	68.55	2.54	13.39	3.42	-	Tigistu <i>et al.</i> (2021)
Supermarkets for Addis Ababa and Adama	-	0.17-0.46	-	-	-	-	4.11-4.33	Yohannes <i>et al.</i> (2018)
Adigrat and the surrounding area	12.56-22.57	0.09-0.54	50.31-79.56	2.24-12.24	3.99-45.17	8.32-45.26	3.4-4.65	Gebremariam & Brhane (2014)
Tepi area	-	-	-	-	17-29	-	3.96-4.26	Yadeta & Kebede (2014)
Siltie Woreda, Siltie zone, SNNP	14.61-19.54	0.12-1.67	66.44-71.64	2.16-6.01	12.41-33.67	-	4.13-5.02	Kebede and Adgaba (2017)
West Shewa zone, Oromiya region	16.61-18.64	0.38-0.64	61.38-72.87	6.84-15.94	7.42-13.8	-	3.77-4.22	Eyobel <i>et al.</i> (2017)
Debre Nazret kebele of Tigray region, Ethiopia	17-23	0.06-0.38	-	-	17.33-32.7	4.22-9.76	2.99-4.45	Equar <i>et al.</i> (2015)
Tigray region Northern Ethiopia	17-21	0.02-0.44	67.7-80	1.27-4.24	18-43	-	-	Gebre-medhin <i>et al.</i> (2013)
Gomma Woreda of Southwestern Ethiopia	15.66-23.4	0.05-0.60	61.15-77.41	0.75-6.96	0.30-50.30	0.05-17-70	3.45-4.18	Kinati <i>et al.</i> (2011)
Selected Districts of Arsi zone	17.9-21	0.04-0.41	-	-	-	-	3.39-4.2	Mulualem Teklemedhin (2018)
Ethiopian monofloral honey	14.4-20.54	0.20-0.39	-	1.1-2.8	20-55	-	3.4-4.6	Degaga (2017)

2.4. Botanical Origin of Ethiopian Honey (Pollen analysis)

The fundamental factors that impact the commercial value of honey are its botanical and geographical origins, which vary primarily depending on the coverage of plant species (Gela *et al.*, 2023). Flowering plants provide honeybees (*Apis mellifera L.*) with nectar and pollen (Addi and Bareke, 2019). The most significant element that can affect honeybee activity and productivity is the availability of bee flora (Addi and Bareke, 2021). To increase honey production by forecasting the frequency of honey harvest, honey flow period, and seasonal colony management, it is crucial to understand the availability of major honeybee forage species and their flowering calendar (Bareke and Addi, 2018; Betelhem *et al.*, 2019; Bareke and Addi, 2019; Wolditsadik *et al.*, 2022; Ofjan and Etenesh, 2023). According to Bareke and Addi (2018), a flowering calendar is a timeline that shows the approximate dates and lengths of the major nectar and pollen source plants' blossoming periods.

The variety of nectar-producing plants in a given area determines the variety of honey produced there (Addi and Bareke, 2019). Honey pollen analysis (melissopalynology) is a method that can be used to identify different types of honey regarding purity, and geographical and botanical origin using microscopic examination of the sediments found in honey samples. It presents an accurate image of the local honeybee flora, which serves as a food source for honeybees and other pollinators in the ecosystem (Arege *et al.*, 2020; Amsalu and Tusa, 2023).

Differentiating pollen grains from one species to another is made possible by unique structural patterns and unique genetic inheritance codes found in each plant species (Addi and Bareke, 2019; Amsalu and Tusa, 2023). Practical beekeeping requires the documentation of bee plants and the creation of a flora calendar (Clara *et al.*, 2023), efficient maintenance of bee colonies throughout the year, including periods of abundance and scarcity (Amsalu and Tusa, 2023)). As a result, it is necessary to increase the beekeeping industry's efficiency and allow beekeepers to make the most of them to collect a healthy yield of honey (Amsalu and Tusa, 2023). Principal component analysis (PCA) has been used extensively in multivariate analysis of honey to classify honey types based on pollen analysis (Siddiqui *et al.*, 2017; Bareke and Addi, 2019; Akbari *et al.*, 2020), which is one of the honey quality parameters used to describe honey and determine its botanical origin. It is common practice to characterize honey-based primarily on the plant it comes from, and this has

been used to influence the type of honey that is sold (Addi and Bareke, 2021). Ethiopia is home to 6000–7000 flowering plants, the majority of which are bee plants, which include forest trees, bushes, herbs, weeds, and undergrowth. These plants are supported by a variety of climatic conditions, topography, and altitudes (Amsalu and Tusa, 2023).

There are three distinct climate zones in Ethiopia. Woina dega, Kolla, and Dega. The *Acacia*, *Albizzia*, *Combrétum*, *Citrus commiphora*, *Eucalyptus*, and *Croton* plants are found in the Kolla, or hot zone. *Acacia*, *Eucalyptus*, *Citrus*, *Coffee*, *Combertum*, *Croton*, *Guizotia*, *Trifolium*, *Olea*, and *Veronia* are found in the Woina dega or cool warm zone. The Dega, or cold zone, is home to *Gizotia*, *Olea*, *Rosa abyssinica*, and *Citrus albizzia*. Swarming occurs in April and September. Bees have fewer enemies and Dega flowers all year long. The flowering period of kola is brief, and the bees are aggressive and highly productive. Bees are highly active in Woina. Productive swarms were observed in the lowlands of Kolla, and ineffective swarms from Dega (Gangwar and Gebremariam, 2010).

Species of plants that attract honeybees in most sample plots of the highland representative area, the most common plants were *Guizotia abyssinica*, *Bidens spp.*, *Echinops Spp.*, *Vicia faba*, *Cynodon dactylon*, *Acacia seyal*, *Hypoestes trifolia*, *Becium grandiflorum*, *Acacia tortolis*, and *Ocimum bacilicum*. In contrast, the most common plants in the lowland area were *Acacia asak*, *Sorghum bicolor*, *Sesamum indicum*, *Acacia mellifera*, *Acacia tortolis*, *Acacia seyal*, *Acacia brevispica*, *Bidens spp.*, *grass spp.*, and *Grewia bicolor* whereas the most common species in the midland altitude representation of the northeastern dry land areas of Ethiopia's Amhara region were *Hypoestes trifolia*, *Ocimum bacilicum*, *Acacia tortolis*, *Becium grandiflorum*, *Bidens spp.*, *Acacia seyal*, *Sorghum bicolor*, *Echinops spp.*, *Vicia faba*, and *Guizotia abyssinica* (Abebe et al., 2014).

Eucalyptus camaldulensis (52.02%), which is regarded as monofloral honey, was the most common pollen type, according to mellissopalynological analysis. As secondary pollen types, *Terminalia spp.* (25.96%), *Guizotia spp.* (17.80%), and *Bidens spp.* (17.61%) were categorized as multifloral honey. Pollen types identified in honey samples from all agroecologies included *Terminalia spp.*, *Guizotia spp.*, *Vernonia spp.*, *Bidens spp.*, *Plantago spp.*, and *E. camaldulensis*. In the highland, midland, and lowland regions, beekeepers identified *Schefflera abyssinica*,

Vernonia amygdalina, and *Cordia africana* as the primary providers of pollen and nectar for honeybees, respectively. In all of southwest Ethiopia's agro ecologies, *V. amygdalina*, *Coffea arabica*, *Croton macrostachyus*, and *C. africana* were also frequently observed bee flora (Tulu *et al.*, 2023).

Honey pollen analysis is used to identify five dominant plant species. These include *Croton macronstachys*, *Acacia tortolis*, *Schefflera abyssinica*, *Guizotia scabra*, and *Eucalyptus camadulensis*. These plant species predominate in honey samples because of their abundance, and potential for producing nectar and pollen. In the Wando District of the West Arsi Zone, the primary source of monofloral honey is *Eucalyptus camaldulensis* (Wolditsadik *et al.*, 2022).

According to Gemechis (2013), the primary source of *Eucalyptus* mono-honey is *E.globulus*, although other significant species that are also honey producers include *E. citrodora* and *E. camaldulensis*. Gimbichu Dugda, Wando, and Ada'a Districts of East Shewa and West Arsi Zones are major honey-producing areas for *Guizotia scabra*, which produces mono-floral honey. (Gemechis *et al.*, 2013). It is also stated that the majority of *Guizotia* mono honey originates from *G. scabra*, as well as *G. abyssinica* and other weeds that flower at the same time and partially contribute to this honey (Admassu *et al.*, 2014b).

The honey samples belonged to 14 mono-floral kinds of honey that originated from 6 plant types that are produced in various zones, according to the honey pollen analysis. *Guizotia scabra* monofloral honey is produced from September to October in the districts of Dugda and Ada'a, and from September to November in Wando, Gimbichu, and Nagelle Arsi. In the Kofele districts, *Schefflera abyssinica* monofloral honey was also produced. *Guizotia scabra* and *Acacia tortolis* are the most common plant species in the mid-and lowlands of the Arsi zone because of their extensive distribution (Mekonen *et al.*, 2022).

There are 74 species of bee plants known to exist, divided into 41 families. *Acanthaceae* (12.2%), *Lamiaceae* (14.6%), *Fabaceae* (9.8%), and *Asteraceae* (29.3%) were the plant families most frequently represented by the highest species composition in the area Using the flowering calendar in Gera Forest, four main honey harvesting periods were determined (January, March, April, and early June for *Vernonia*, *Coffee*, *Schefflera*, and *Croton* honey, respectively). Four types of mono-

floral honey were identified in the Gera forest through pollen analysis: *Schefflera abyssinica*, *Vernonia amygdalina*, *Coffea arabica*, and *Croton macrostachyus* (Bareke & Addi, 2019). Fifteen honey samples were identified as multi-floral, and twenty-five honey samples were identified as mono-floral. In the North Wollo zone, 19 plant species from 12 different plant families were identified as the honeybee flora. *Asteraceae* (n = 9 samples), *Myrtaceae* (5), *Papilionaceae* (4), *Fabaceae* (2), *Hypericaceae* (2), *Acanthaceae* (1), *Brassicaceae* (1), *Ericaceae* (1), *Bidden spp.*, *Trifolium ruppelianum*, and *Eucalyptus globulus* are the top three honeybee floras in terms of overall relative percentage and frequency. The most dominant honeybee flora plant families accounted for > 45% of pollen grains in the studied samples. The high diversity index for eleven samples ranges from (H=1.56) in the districts of Lasta to (H=1.01) in Meket. In the North Wollo zone, the other thirty-four samples have low diversity indices between H=0.31 and 0.99 (Wubshet and Mebratu, 2022).

CHAPTER 3: METHODOLOGY

3.1. Description of the Study Area

The experimental part of the study was carried out in the South Wollo zone, Dessie Zuria district with an area of 937.32 km², located approximately 401 km north of Addis Ababa. Due to its high altitude, Dessie Zuria experiences the lowest temperatures in the region, ranging from 10°C to 16°C. Rain-fed agriculture is the predominant farming practice in this district, although rainfall is often inconsistent or insufficient. However, certain areas within Dessie Zuria receive substantial rainfall, averaging between 1200 and 1400 mm annually (Bihonegn, 2017). South Wollo (Eastern Amhara) is considered a highly promising zone in the Amhara region, known for its diverse honey bee flora (Alebachew *et al.*, 2022). According to the CSA (2013), the district produced approximately 1,137,859 kg of honey, with an average productivity of 7.10 kg per hive per year.

Monitoring (on farm performance evaluation), honey quality, and melissopaynology parameters were studied in South Wollo which is one of the 11 zones in the Amhara region and has a total size of 17,067.45 km² and is situated between 10.20⁰ and 11.71⁰ N and 38.41⁰ and 40.02⁰ E of Ethiopia (Fig. 1). Dessie town serves as the zone's administrative center. The zone has 20 woredas, of which Tehulederie (midland (in 01, 05, 08, 09, 12, 05 kebeles)), Kalu (lowland (in 01, 03, 05, 07, 09 and 31 kebeles)), and Dessie Zuria (highland (in Gelsha, Asegedo, Ayata, Saquaro, Adja and Kolawit kebeles)) were specifically chosen for this study based on their beekeeping potential, accessibility, and proximity to honey marketing and processing routes. The way of identifying of highland, midland and lowland woredas in the zone was according to information or data available in South Wollo Zone agricultural office. The zone experiences annual rainfall on average of 1162mm. The monthly minimum and maximum temperatures are 12.6⁰C and 26.4⁰C, respectively (Bihonegn *et al.*, 2017)

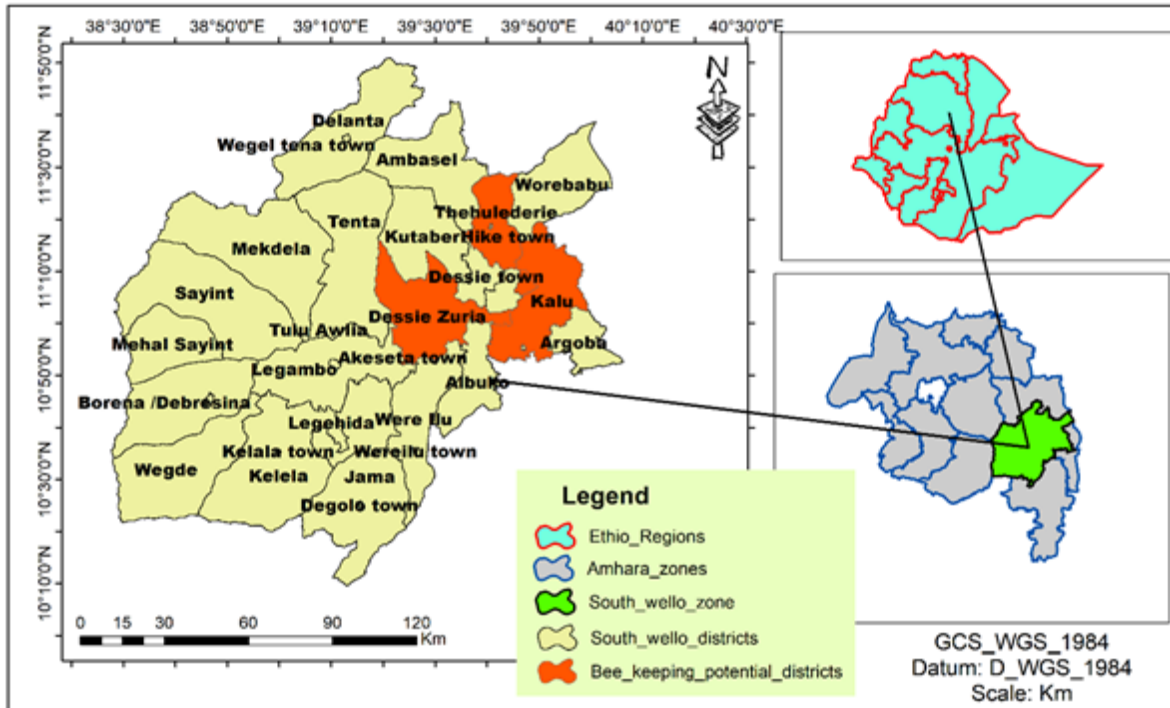


Fig. 1: Map of the study area

3.2 Experimental Design and Treatments

3.2.1 Apiary site preparation and colony management

To prevent robbery bees that share feeds, the trials were conducted at an optimum distance (5Km) away from the others. Thirty honey bee colonies (*A.mellifera. monticola*) were used in this experiment. Before the experiment, these colonies were divided into three groups weak, strong, and very strong based on their strength. Then these groups were randomly grouped into each treatment diet, by using a randomized complete block design (RCBD) with six replications (six colonies per treatment (each treatment group contains 2 weak, 2 strong, and 2 very strong colonies)). Before transferring, both hives and frames were washed, disinfected, and fumigated. Until transferring, traditional hive colonies were given sugar syrup and other locally available supplementation (*shiro* and *beso*).

3.2.2. Diet preparation and administration

Stinging nettle infusion was prepared from leaf flour of the stinging nettle plant (100 g nettle leaf meal in 1000 ml hot water). The infusion was used for preparing the syrup. Sorghum and Barly powders, *kerefa* (*Cinnamomum Zelanicum*), and sugar powder are prepared by grinding. *Shiro* is also prepared with spiced pea. Skimmed milk powder (NIDO) and bakery yeast were purchased from the market directly. The sugar syrup was prepared with a mixture of sugar and water at a 1:1 ratio. Syrup for T4 was made by infusion of Nettle. All feed treatments were administered as shown below. Feeds were rationed based on the energy and protein requirements of honeybees ($\leq 50\%$ carbohydrate and 4-60% protein related with the natural nectar and pollen composition).

1. **T1** = 50% sugar syrup + 14% roasted barley powder (*beso*) + 36% roasted spiced pea powder (*Shiro*).
2. **T2** = 50% powder sugar + 14% white sorghum powder + 36% bakery yeast
3. **T3** = 50% powder sugar + 14% white sorghum powder + 36% skimmed milk powder.
4. **T4**: 50% sugar syrup with an infusion of stinging nettle and 1% *kerefa* + 50% white sorghum powder.
5. **C**: Control groups were not given any supplementation.

To prevent other animals from consuming the feed, it was monitored throughout the day placed on the entrance at 5:00 in the morning and taken down at 7:20 at night. Depending on their consumption level, the feed was changed every week with freshly produced feed. Depending on their strength (weak, strong, and very strong), a weekly amount of 100, 150, or 200 g of feed was given respectively, and their feed intake was measured. To safeguard the freshness of the feeds, any color change of the water and feed in troughs were monitored carefully. The color of feed and water trough was considered the color preference of honey bees (Blue). Pure water was given in free access.

Table 5: Nutritional composition of experimental feeds

Nutritional composition	Treatments & <i>kerefa</i> nutrient compositions				
	T ₁	T ₂	T ₃	T ₄	<i>kerefa</i> (<i>Cinnamomum zelanicum</i>)
CP (%)	16.37	14.77	6.66	18.15	3.5
CF (%)	5.96	1.85	4.45	5.75	4.1
CH (%)	61.91	75.06	83.95	92.15	20
CFi (%)	6.76	6.29	4.65	6.65	23
Ash (%)	7.51	2.26	1.68	8.7	2.3
OM (%)	92.49	97.75	98.33	98.3	32
Moist. (%)	8.23	5.93	3.26	11.25	5.2
DM (%)	91.77	94.07	96.74	98.8	35

CP: Crude Protein; CF: Crude Fat; CH: Carbohydrate; CFi: Crude Fiber; OM: Organic Matter; Moist.: Moisture; DM: Dry Matter; T1: 50% sugar syrup + 14% roasted barley powder (*beso*) + 36% roasted spiced pea powder (*Shiro*); T2: 50% powder sugar + 14% white sorghum powder + 36% bakery yeast; T3: 50% powder sugar + 14% white sorghum powder + 36% skimmed milk powder T4: 50% sugar syrup with infusion of stinging nettle and 1% *kerefa* + 50% white sorghum powder; C: not given any supplementation.

3.3. Experimental Honeybee Performance Parameter Measurements

3.3.1. Productive and reproductive performance measurement

Space (cm²) covered by egg, larvae, pupae, nectar, pollen, and honey in the comb were measured using a frame-sized transparent grid meter (fig.2), which could fit well on the four wooden bars of the comb frames when placed on it. The grid consisted of squares each having a 1 cm² area (Sena *et al.*, 2012; Kumar *et al.*, 2013). The grid was placed on the comb for measuring various parameters and the number of squares of the grid covering the different parameters were counted on all the combs in a colony.

Total honeybee strength was measured in terms of frames covered by bees. The number of queen cells and the strength of all the experimental colonies were recorded. All parameters were measured at an interval of 21 days. Honey data was gathered when the honey was harvested. Weight differences between frames containing honey before and after honey extraction were recorded as the honey harvest/yield. These data were collected during the dearth period from 20

December to 22 March/2021 and the wet season from 27 June to 7 September /2022. To reduce errors, data was gathered from all experimental colony frames.

Initially, all treatments had equal mean amount of the following parameters:

Nectar area=30 cm ²	egg area=121.7 cm ²
Pollen area=45 cm ²	larvae area= 270 cm ²
Honey area=300 cm ²	pupa area=370 cm ²
Colony strength =4.2 frames covered by honeybees	queen cell= 0

Feed intake was measured according to the following formula:

Feed intake =Feed offered- feed refusal



Fig. 2: Method of productive and reproductive performance measurements

3.3.2. *Partial budget analysis*

Partial budget analysis was calculated based on the total cost of feed preparation which is the cost of feed and labor cost and on base of return, income generated from honey. The net profit was calculated by deducting the total cost of feeds and labor from the income generated.

3.3.3. *Feed laboratory analysis.*

At the Holeta Agricultural Research Center, a comprehensive analysis of various nutritional components was carried out, encompassing crude protein (CP), carbohydrate (CH), crude fat (CF),

crude fiber (CFi), organic matter (OM), moisture (moist.), dry matter (DM), and ash. To measure the dry matter content of a 1g feed sample, it was exposed to a 105°C oven for a duration of 24 hours. The Kjeldahl method of nitrogen analysis was employed to determine the crude protein content in the samples, while the protein content was computed using the formula $N \times 6.25$. To determine the dry matter and crude protein content, standard analysis procedures were adhered to.

To analyze the fiber fractions of the samples, a range of solutions was prepared. These included an acid detergent solution, used for acid detergent fiber analysis, potassium permanganate and demineralized solutions, used for lignin analysis, and a neutral detergent solution for neutral detergent fiber analysis. Both the neutral detergent fiber and acid detergent fiber were analyzed using a similar procedure, with the only difference being the solutions used. To begin, a solution was prepared. Next, a sample weighing 0.5g was combined with 50 ml of the respective solution and boiled for one hour. The resulting residues were then filtered and subjected to drying in an oven at 105°C for a period of 24 hours.

3.4. On-Farm Performance Evaluation of Local Honeybees

3.4.1. Data collection methods

A Purposive stratified random sampling method was used to select the sampling locations. After classifying the districts based on their agroecological location, a total of 90 colonies (30 colonies from each agroecology (highland, midland and lowland) under modern hives) were assigned to study the performance of honeybee colonies for foraging activity, defensive behavior, and absconding rate and assess those diseases, predators, and pests. The performance of the colonies was evaluated from 10 September to 30 December 2021 and 27 March to 23 June 2022 during the active season of the colony through the following parameters:

3.4.2. Evaluation of reproductive and productive performance

Space (cm²) covered by eggs, larvae, pupae, pollen, nectar, and honey in the comb was measured using a frame-sized transparent grid meter (fig.2), which could fit well on the four wooden bars of the comb frames when placed on it. The grid consisted of squares each having a 1 cm² area (Sena *et al.*, 2012; Kumar *et al.*, 2013). The grid was placed on the comb for measuring various

parameters and the number of squares of the grid covering the different parameters were counted on all the combs in a colony every 21 days. Comparisons of this study result with other findings was consider the consisted square of grids (conversion was made). Based on interviews and/or inspections for the development of queen cells, the reproductive swarming of the honeybee populations in the area was evaluated under the following circumstances.

The proportion of reproductively swarmed colonies to the total number of colonies owned by interview beekeepers was used to calculate the extent of reproductive swarming. According to the ratio of total colonies to newly built queen cells, the swarming tendency was estimated. To prevent double counting, these cells that had been counted were eliminated right away. During the active season, swarming tendency was assessed at intervals of 9 days. The honey yield was measured as each frame containing sealed honey was weighed to collect data on honey yield during the study period. The centrifugal honey extractor was then used to extract frames that contained honey. After the honey was extracted, empty frames were weighed. The weight after extraction was subtracted from the weight before the extraction to get the net honey yield (Mossie and Biratu, 2019).

3.4.3. Evaluation of foraging behavior, absconding rate and defensive behavior

Early foraging and late foraging times were also recorded 3 times per week. The time used to record was the East African time zone. The absconding rate was assessed by the ratio of colonies evacuating to the total number of colonies used for the experiment provided that all the colonies were kept under uniform environmental conditions (Alemu *et al.*, 2014). To measure the defensive behavior of the honeybee colony, a test of aggressiveness was used. A 2 cm diameter black leather ball was jerked for 60 seconds at a distance of 5 cm in front of the entrance of a colony. The gloved hands of the observer were situated 1 meter above the leather ball. The right gloved hand was moved during the test because it is used to jerk the thread that moves the leather ball. The gloved left hand was not moving. Then when the 1st sting was made in the leather ball, the time taken for the colony to become very aggressive, the number of stings in the gloves of the observer, the number of stings in the leather ball, and the distance that the bees followed the observer data were collected. The number of stings left in the gloves worn by the observer was counted afterward.

Each colony was tested five times with 10 minutes between tests at the active time of the day from 1:00 p.m. to 2:00 p.m. (Tarekegn *et al.*, 2022).

3.4.4. Disease, Predators, and Pests

During data collection diseases, predators, and pests were also recorded. Disease was identified by observing scientific symptoms in honeybees. Predators were known by observing them both outside and inside inspection. Pests are also identified by observing through internal inspection.

Diseases: are hazards of honeybees caused by microorganisms or parasites

Predators: are dangers of honeybees which attacks honeybees at some times

Pests: are risks of honeybees which live and reproduce in the hive of colonies permanently.

3.5. Honey Quality

3.5.1. Sampling techniques and sample size

A Purposive stratified random sampling method was used to select the sampling locations. After classifying the districts based on their agroecological locations, the sampling sites were selected using the random sampling technique. Fifteen representatives Kebeles were selected, five from the midlands (Tehuledere), five from the lowlands (Kalu), and five from the highlands (Dessie Zuria). For the study, a total of thirty samples were gathered, ten from each agroecology. All of the samples came from modern (frame) hives during two harvesting seasons (October-November (major harvesting season) and June –May (minor harvesting season)) from those hives in which on-farm performance evaluation was measured. Sampled honey was stored until analysis in hygienic, food-grade plastic containers (Fig.3).



Fig. 3: Sampled honey collected from the study area

3.5.2. Quality measurement procedures

The International Honey Commission's Harmonized Methods (HMIHC) were utilized to ascertain the quality compositions (moisture content, pH, acidity, HMF, mineral content, ash) of honey samples (Bogdanov *et al.*, 2009). At the Holeta Bee Research Center, an agricultural research institute in Oromiya, Ethiopia, the quality attributes of the collected honey samples were examined.

Moisture Content: By using a digital refractometer (Abe refractometer) that can be thermostated at 20°C and is routinely calibrated with distilled water from the refractive index of the honey by consulting a standard table, the moisture content of the honey sample was estimated. The sample was evenly smeared on the prism's surface, and two minutes later, the refractive index reading was recorded. Every sample was measured three times, with the averages of the three measurements being recorded along with the corresponding moisture content value.

pH: With a digital pH meter (Inolab, Germany), the pH of the honey was ascertained. In a 250 ml beaker, 10 grams of honey sample were dissolved in 75 ml of distilled water. After agitating the mixture and submerging the pH electrode in it, the pH was measured.

Free Acidity: Ten grams of honey were weighed using an electronic balance, and then added to a conical flask along with 75 ml of distilled water after the utensil had been rinsed. Phenolphthalein is used as an indicator when the solution is titrated in a burette against 0.1M NaOH solutions. The solution was titrated until it turned pink, turning it colorless. Free acidity is measured to one place

of decimals using the following formula: mill equivalents, or mill moles of acid/kg honey = ml of 0.1M NaOH x 10.

Mineral (ash): After burning the honey sample in an electric muffle furnace (Lenton Thermal Designs, England), the amount of ash was ascertained. The ash dish was first heated to an ashing temperature in an electrical furnace, then cooled to room temperature in a desiccator, and then weighed 0.001g (M_2). Subsequently, two drops of olive oil were added to the prepared ash dish to prevent frothing after 10 grams of honey sample had been weighed to the nearest 0.001g (M_0). After that, the water was drained and the ashing process was started without loss (by foaming and overflowing) at a low heat that was raised to 350–400° C with an electrical device. The sample was charred on a hot plate before being placed inside the furnace. Following the initial ashing with a hot plate, the dish was heated for an hour at 550° C in a muffle furnace that had been preheated. After being cooled in the desiccator, the ash dish was weighed. Until a constant weight was reached, the ashing process was carried out (M_1). The following formula is used to get the percent ash in grams per 100 grams of honey.

$$\text{Ash \%} = \frac{M_1 - M_2}{M_0} \times 100$$

Where M_0 = weight of honey taken, M_1 = weight of dish + ash, and M_2 = weight of dish.

Estimation of Hydroxyl Methyl Furfural (HMF): The reagents enlisted below, required to estimate the HMF content in honey samples were prepared as follows:

Carrez Solution I: 15 g of Potassium Hexacyanoferrate $K_2Fe(CN)_6 \cdot 3H_2O$ was dissolved in distilled water and volume was made to 100 ml.

Carrez Solution II: 30 g of Zinc Acetate, $Zn(CH_3COO)_2 \cdot 3H_2O$ was dissolved in distilled water and the volume was made to 100 ml.

Sodium Bisulphate solution 0.20 g /100 g (0.2 %):-0.20 g of solid Sodium Bisulphate ($NaHSO_4$) was dissolved in distilled water and the volume was made to 100 ml. Five grams of honey sample was taken approximately and diluted in 25 ml water and then poured into a volumetric flask. Then 0.5 ml of Carrez solution I was mixed with 0.5 ml of Carrez solution II and made up the volume. Then the solution was filtered through the filter paper and the first 10 ml of filtrate was rejected.

Five ml of sample was pipetted out in two test tubes and 5 ml of water was added to the first test tube and mixed well. Five ml of 0.2 % Sodium Bisulphate solution was added to the second test tube and mixed well for reference solution (Table 5)

The absorbance of the sample was determined against the reference solution with a UV Spectrophotometer at wavelength 284 and 336 nm by using 1cm-quartz cells within one hour. The sample and reference solution was diluted with water and sodium bisulphate if the absorbance 2 exceeds 0.6 at 284 nm following the equation provided by Bogdanov (2009) and Addis and Malede (2014).

$$\text{Dilution } D = \frac{\text{The final volume of Sample Solution}}{10}$$

$$\text{HMF expressed as mg/kg} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W.$$

A_{284} = Absorbance at 284 nm.

A_{336} = Absorbance at 336 nm

$$149.7 = 126 \times 1000 \times 1000 / 16830 \times 10 \times 5.$$

126 = Molecular weight of HMF.

16830 = Molar absorptive and HMF at 284 nm.

10 = Conversion of g into mg.

1000 = Conversion of g into kg.

5 = Theoretical nominal sample weight.

D = Dilution factor (in case dilution is required).

W = Weight in g of honey sample.

Table 6: Dilution of sample and reference solutions carried for estimation of HMF

Addition to test-tubes	Sample solution (in mL)	Reference solution (in mL)
Initial solution	5.0	5.0
Water solution	5.0	-
Sodium bisulphate (0.3%)	-	5.0

Source; (Bogdanov, 2009; Addis and Malede, 2014).

3.6. Melissopaynology (Pollen analysis)

3.6.1. Sampling techniques and sample size

This was done from honey samples collected for honey quality analysis. The honey samples were strained using double sieves and cheesecloth at the Holeta Bee Research Center (HBRC) laboratory. The honey samples were then stored at 4°C for further analysis (Bareke and Addi, 2019b).

3.6.2. Pollen identification

For honey pollen analysis, the method recommended by the International Commission for Bee Botany (Louveaux *et al.*, 1978) was adopted. Pollen types were identified by comparing them with reference slides of pollen collected directly from the plants in the study area. For quantification of the pollen types, at least 500 pollen grains were counted from each sample (Oliveira *et al.*, 2010). The percentage frequency of the pollen taxa in all the samples was calculated excluding polleniferous plant species which were observed during honey pollen analysis (Bareke & Addi, 2019b). Predominant pollen types (>45%) of the total pollen grains were counted; secondary pollen types (16%-45%), important minor pollen types (3%-15%), and minor pollen types (3%) were types of pollen allocated to one of the frequency classes for nectar source plants (Louveaux *et al.*, 1978). Honey with predominant pollen types is considered monofloral.

Plant types were categorized according to the following:

Herb: Are small sized plants with soft non-woody stems. They complete their life cycle with in one or two seasons.

Shrub: Are medium sized plants with woody stems. Have multiple branches arising from the base. There stems with flexibility but not fragility.

Tree: Large plants with a single main trunk that supports branches and leaves. Very thick, woody and hard stems.

Climber: They require support to grow upright. They have weak stems.

Grass: Are herbaceous plants with narrow leaves and jointed stems.

Crop: Plants that is grown in large amount by farmers. These plants can be grains, fruits, or vegetables cultivated for various purposes such as food, fiber, or fuel.

3.7. Statistical Analysis

The performance differences were tested using the analysis of Variance (ANOVA) procedure of the SAS general linear model (GLM). The least significant difference (LSD) at a 5% significant level was used for comparisons of means.

3.8. Statistical Models

Model 1: $Y = \mu + i_t^{\text{th}} + e_t^{\text{th}}$ (for experimental part)

Y= Responses of the treatment (productive and reproductive performances)

μ = overall mean

i_t^{th} = the i^{th} treatment factors (t_1, t_2, t_3, t_4)

e_t^{th} = the standard error of the mean ($e_{y\mu i t^{\text{th}}}$)

Model 2: $Y_{ij} = \mu + H_i + e_{ij}$ (for on-farm performance evaluation and honey quality)

Where: Y_{ij} = Observed dependent variables

μ = Overall mean

H_i = Fixed effect of agroecology ($i=3$; highland, midland and lowland)

e_{ij} = Residual error

CHAPTER 4: RESULTS

4.1. Effects of Different Feeds on the Performance of Honeybees

4.1.1. Effects of feeds on feed intake of honeybee colonies

Table 7 shows the feed intake performance of honeybees exposed to different supplementations. The intake of supplement feeds showed significant variation ($P < 0.05$). Experimental honeybee colonies consumed approximately 98% of the supplement feeds provided under T₄ and T₂, whereas only 54% of T₁ and 60% of T₃ supplements were consumed by experimental honeybee colonies. The experimental findings demonstrate that the intake of supplements was noticeably higher in treatments two (T₂) and four (T₄) compared to the other treatments ($P < 0.05$). Statistical analysis indicated no significant variation in consumption between T₄ and T₂, as well as T₁ and T₃ ($P > 0.05$). These results indicate that honeybee colonies find the feeds provided under treatments 4 (T₄) and 2 (T₂) more appealing than the other feeds.

Table 7: Effects of different supplemental diets on the feed intake of honeybee colonies

Parameters	Treatment groups					P- value
	T1	T2	T3	T4	C	
FO(g)	150.0±3.5a	150.0±3.5a	150.0±3.5a	150.0±3.5a	0±0b	<.0001
FR(g)	68.2±1.9 a	1.5±0.1b	59.5±1.7a	1.3 ±0.1b	0±0c	<.0001
FI(g)	81.8±1.9 b	148.5 ±3.5a	90.5 ±2.3b	148.7 ±3.5a	0±0c	<.0001
Perc.(%)	54.7±0.6 b	98.8±0.1a	60.4±0.6b	98.3±0.7a	0±0c	<.0001

FO: Feed Offered; FR: Feed Refusal; FI: Feed Intake; Perc: Percent; T1: 50% sugar syrup + 14% roasted barley powder (beso) + 36% roasted spiced pea powder (Shiro); T2: 50% powder sugar + 14% white sorghum powder + 36% bakery yeast; T3: 50% powder sugar + 14% white sorghum powder + 36% skimmed milk powder T4: 50% sugar syrup with infusion of stinging nettle and 1% kerefa.+ 50% white sorghum powder; C: not given any supplementation. ^{a-d} Means with different superscripts across a row are significantly different.



Bakery yeast content of feed (left) and syrup of nettle infusion (right).

Fig. 4: Provisioning of feeds

4.1.2. Effect of feeds on reproductive and productive performance of honeybees

Table 8 displays the effects of various supplementations on the reproductive and productive performance of honeybees after 2 phases. In terms of egged area (EA), T₄ exhibited the highest value at 464.9 cm², followed by T₂, T₃, and T₁. However, the difference between T₄ and T₂ was not statistically significant ($P > 0.05$). There was also no significant difference ($P > 0.05$) between T₁ and T₃. The control colonies had the lowest value (83.4 cm²), which differed significantly ($P < 0.05$) from all other experimental colonies. Similarly, when considering the larval area (LA), T₄ had the highest value (553.9 cm²), while the control group had the lowest value (163.1 cm²) compared to the other treated colonies. There were no significant differences ($P > 0.05$) between T₂) and T₄, as well as T₁ and T₃.

The pupae area (PA) measurement showed that T₄ had the highest value (627.1cm²), but the difference between T₄ and T₂ was not significant ($P > 0.05$). Colonies that did not receive any supplementations had the lowest value and had a significant difference ($P < 0.05$) compared to all other experimental colonies. There were no significant differences ($P > 0.05$) between T₁ and T₃, as well as between T₂ and T₃. In terms of the number of queen cells, T₄ had the highest value (0.2), followed by T₂ (0.1), but there was no statistical difference ($P > 0.05$) between them. T₁, T₃, and the control groups did not have any queen cell construction (QC) throughout the experiment and

did not show any significant differences compared to T₂. Regarding colony strength (CS), T₄ had the highest number of frames covered by bees (6.4 frames), but the statistical difference with T₂ (5.5) was not significant ($P > 0.05$). Colonies that did not receive any supplementations had the lowest value (2.8 frames) and had a significant difference ($P < 0.05$) compared to all other experimental colonies. There were no significant differences ($P > 0.05$) between T₁ and T₃, as well as between T₂ and T₃.

In terms of the nectar area, T₄ (54.3 cm²) gathered a greater amount of nectar compared to T₂, T₃, or T₁. However, the statistical analysis revealed that the difference between T₄ and T₂ was not significant ($p > 0.05$). On the other hand, there were no significant differences detected between T₁ and T₃. The control colonies registered the smallest value of 16.4 cm², which was significantly lower ($p < 0.05$) compared to all other experimental colonies. While there was a minimal difference in pollen area measurement between T₄ (68.7 cm²) and T₂ (64.6 cm²), T₄ consistently maintained the highest value. The control colonies exhibited the lowest values of 21.6 cm² and displayed significant differences ($p < 0.05$) compared to the other experimental colonies. There was no noticeable difference observed between T₁ and T₃, as well as between T₂ and T₃.

The experimental diets had an impact on the honey area as well. Among the colonies, those that received T₄ (311.2 cm²) showed the highest value, while T₂ did not significantly differ ($p > 0.05$) from T₄. While there was no statistically significant difference between T₃, T₁, and T₂ ($p > 0.05$). In the comparison of control colonies (103.9 cm²), it was observed that their honey area was smaller than all experimental colonies, and this difference was found to be statistically significant. Among the experimental colonies, T₄ had the highest honey yield of 7 kg, followed by T₂. However, there was no statistically significant difference between T₄ and T₂. T₃ and T₁ displayed higher honey yields compared to the control groups (0.7 kg). Moreover, a statistically insignificant difference ($p > 0.05$) was observed between the honey yields of T₁ and T₃.

Table 8: Effects of experimental feeds on reproductive and productive performance of honeybees

Reproductive Parameters	Treatment groups					P value
	T ₁	T ₂	T ₃	T ₄	C	
EA (cm ²)	251.2±17.8 ^b	433.8±35.3 ^a	279.4±19.7 ^b	464.9±37.9 ^a	83.4±4.3 ^c	<.0001
LA (cm ²)	408.0±15.8 ^b	542.5±31.3 ^a	434.9±18.9 ^b	553.9±33.6 ^a	163.1±11.1 ^c	<.0001
PA (cm ²)	520.5±18.0 ^c	607.7±27.8 ^{ba}	534.5±19.1 ^{bc}	627.1±28.4 ^a	213.5±15.2 ^d	<.0001
QC (No)	0±0 ^b	0.1±0.1 ^{ba}	0.0±0.0 ^b	0.2±0.1 ^a	0.0±0.0 ^b	<.0001
CS (frame)	4.4±0.2 ^c	5.5±0.3 ^{ba}	4.6±0.2 ^{bc}	6.4±0.4 ^a	2.8±0.1 ^d	<.0001
Productive Parameters						
NA (cm ²)	37.6±1.3 ^b	50.1±2.6 ^a	40.7±1.8 ^b	54.3±3.2 ^a	16.4±1.3 ^c	<.0001
POA (cm ²)	48.8±2.5 ^c	64.6±4.3 ^{ba}	53.3±2.8 ^{bc}	68.7±4.5 ^a	21.6±1.9 ^d	<.0001
HA (cm ²)	254.3±10.5 ^b	295.6±10.9 ^{ba}	262.3±10.2 ^b	311.2±12.8 ^a	103.9±12.8 ^c	<.0001
HY (kg)	2.8±0.2 ^b	6.0±0.7 ^a	3.8±0.6 ^b	7.0±0.7 ^a	0.7±0.1 ^c	<.0001

EA: Egg Area; LA: Larvae Area; PA: Pupae Area; QC: Queen cells CS: Colony Strength; NA: Nectar Area; POA: Pollen Area; HA: Honey Area; HY: Honey Yield; T₁: 50% sugar syrup + 14% roasted barley powder (beso) + 36% roasted spiced pea powder (Shiro); T₂: 50% powder sugar + 14% white sorghum powder + 36% bakery yeast; T₃: 50% powder sugar + 14% white sorghum powder + 36% skimmed milk powder T₄: 50% sugar syrup with infusion of stinging nettle and 1% kerefa. + 50% white sorghum powder. C: not given any supplementation. ^{a-d} Means with different superscripts across a row are significantly different.

4.1.3. Profitability of experimental feeds

Table 9 presents the profitability of experimental diets. Among them, T₄ showed the lowest cost of feed, with a value of 73.6 ETB, whereas T₃ had the highest cost at 473.6 ETB. There was no significant difference ($p > 0.05$) between T₁ and T₂ or T₄ in terms of feed cost. It is worth noting that the labor cost remained unchanged across all treatments. Statistical analysis indicated no significant difference ($p > 0.05$) between T₁ and T₃. In terms of gross expenditure, T₃ had the highest value of 474.9 ETB, followed by T₂, T₁, and T₄. However, there was no statistically significant difference ($p > 0.05$) between T₁ and T₂ or T₁ and T₄.

The honey price of all treatment colonies was charged an equal amount of 425.0 ETB. Among the treatments, T₄ (2975 ETB) had the highest impact on gross return, followed by T₂ (2550 ETB). However, there was no statistically significant difference between T₄ and T₂ ($p > 0.05$). The control colonies, on the other hand, had a lower value of 297.5 ETB compared to the other colonies. The difference between the control colonies and T₁ was statistically insignificant ($p > 0.05$). There

were no significant differences ($p > 0.05$) observed among T₁, T₂, and T₃. However, the colonies treated with T₄ (2900 ETB) showed the highest net profit compared to those treated with T₂, T₃, or T₁, as well as the control colonies which had the lowest net profit (297.5 ETB). Statistical analysis revealed no significant difference ($p > 0.05$) between T₁ and T₃, as well as between T₂ and T₄.

Table 9: Profitability of experimental diets

Parameters	Treatment groups					P value
	T ₁	T ₂	T ₃	T ₄	C	
CoF (ETB)	122.5±10.0 ^{cb}	178.7±12.6 ^b	473.6±30.9 ^a	73.6±6.2 ^c	0±0 ^d	<.0001
CoL(ETB)	1.3±0.8	1.3±0.8	1.3±0.8	1.3±0.8	0±0	0.702
HY (Kg)	2.8±0.2 ^b	6.0±0.7 ^a	3.8±0.6 ^b	7.0±0.7 ^a	0.7±0.1 ^c	<.0001
GE (ETB)	123.8±9.6 ^{cb}	180±12.2 ^b	474.9±30.5 ^a	74.9±5.8 ^c	0±0 ^d	<.0001
P_of_h_/kg	425.0±7.5	425.0±7.5	425.0±7.5	425.0±7.5	425.0±7.5	1.000
GR(ETB)	1190±87.8 ^{dc}	2550±307.7 ^{ba}	1615±256.4 ^{bc}	2975±325.2 ^a	297.5±52.5 ^d	<.0001
NP (ETB)	1066.2±81.2 ^b	2370±301.0 ^a	1140.1±237.6 ^b	2900.1±321.3 ^a	297.5±52.5 ^c	<.0001

COF: Cost of Feed; COL: Cost of Labor; HY: Honey Yield; GE: Gross Expenditure; P of h kg: price of honey per kg; GR: Gross Return; NP: Net Profit; ETB: Ethiopian Birr; Kg: Killo gram; T1: 50% sugar syrup + 14% roasted barley powder (beso) + 36% roasted spiced pea powder (Shiro); T2: 50% powder sugar + 14% white sorghum powder + 36% bakery yeast; T3: 50% powder sugar + 14% white sorghum powder + 36% skimmed milk powder T4: 50% sugar syrup with infusion of stinging nettle and 1% kerefa. + 50% white sorghum powder; C: not given any supplementation. ^{a-d} means with different superscripts across a row are significantly different.

4.2. On-farm performance evaluation of local honeybees in different agro-ecologies of South Wollo Zone

4.2.1. Reproductive and productive performance of local honeybees on their agroecology

Table 10 indicates the reproductive and productive performance of honeybees in different agro ecologies. Highland showed the highest Egg area (EA), Larvae area (LA), and Pupae area (PA) (1343.8, 1407.05, and 1471.83 cm²) respectively than lowland and midland which had the lowest brood area. There was a statistical difference ($P < 0.05$) between agro-ecologies for measurements of all brood parameters. Extent of swarming (exrsw) were showed a significance difference ($P <$

0.05) with higher value in lowland (2.57 and 8.57) and lower in midland (0.71 and 2.38) but highland was not statistically differed ($P > 0.05$) with both lowland and midland. Swarmed tendency (swate) was not affected by agro- ecology ($P > 0.05$). It was 14.76 for lowland, 14.29 for highland and 6.67 for midland. All above parameters Pollen area (POA), Nectar area (NA), Honey area (HA), and Honey yield (HY) were maximum in highland (15.28, 343.17, 386.13, and 22.13) followed by lowland (8.97, 201.15, 236.02 and 18.88) and midland (6.01 142.54, 150.39 and 12.83). There was a significant difference between agro- ecologies ($P < 0.05$) in all these parameters.

Table 10: Performance evaluation of the reproductive and productive performance of local honeybees in different agro-ecologies

Reproductive parameters	Agro- ecologies			p-value
	Highland	Midland	Lowland	
EA (cm ²)	1343.80±47.49a	519.25±18.61c	824.78±29.80b	<.0001
LA (cm ²)	1343.80±47.49a	1407.05±47.89a	874.57±30.60b	<.0001
PA (cm ²)	1471.83±48.71a	618.76±22.97c	926.10±31.45b	<.0001
Exrsw (%)	7.14±1.77a	2.38±0.86b	8.57±2.03a	0.025
Swate (%)	14.29±3.07	6.67±2.08	14.76±3.17	0.083
Productive parameters				
NA (cm ²)	343.17±15.17a	142.54±6.83c	201.15±8.40b	<.0001
POA (cm ²)	15.28±0.44a	6.01±0.22c	8.97±0.25b	<.0001
HA (cm ²)	386.13±17.98a	150.39±8.53c	236.02±9.50b	<.0001
HY (kg)	22.13±0.88a	12.83±0.62c	18.88±0.70b	<.0001

EA: egg area; LA: larvae area; PA: pupae area; Exrsw: extent of swarming; Swate: swarmed tendency. POA: pollen area; NA: nectar area; HA: honey area; HY: honey yield. ^{a-d} Means with different superscripts across a row are statistically significant.

4.2.2. Foraging ability, absconding rate, defensiveness, and disease assessment of local honeybees

The study area's Foraging ability, absconding rate, defensiveness and disease assessment of local honeybees is shown in Table 11. Despite the numerical differences between the agro-ecologies and had no impact ($P > 0.05$) on absconding rate. In the lowland, highland, and midland, the values were 1.90 %, 2.38 %, and 4.28 % respectively. The foraging time showed a significant difference ($P < 0.05$) both early and late. Colonies found in Highland were wake up a later than the others (5:55 am). still, the lowland colonies were back to their hive later than others (7:04 p.m). In the

early foraging period, there was no discernible difference ($P > 0.05$) between midland (5:23) and lowland (5:13). Additionally, there was no statistically significant difference ($P > 0.05$) between highland and midland at the late foraging time (6:55 and 6:59 p.m. respectively). Colonies in the lowland agro-ecology have been shown to have good foraging ability, which causes them to awaken earlier and return to their hives later than other colonies displayed in rest agro ecologies.

Except for TNoS-B, which was 21.28 in the lowland, 21.71 in the highland, and 21.71 in the midland, it showed significance ($P < 0.05$). Highland (89.43) and 258.86) and midland (87.85 and 259.28), respectively, recorded higher values in TtfFs_sec and Tt_sec_mag without a statistically significant difference ($P > 0.05$) between them. While lowland had significantly lower results than the other two agro ecologies (53.29 sec. and 142.28 sec.). With a statistical difference between them, the highland recorded the highest TNoS_G_ and TDF_m (15.00 and 192.57 m), followed by the lowland and midland.

Table 11: Evaluation of foraging, absconding, and defensiveness of local honeybees in different agro ecologies.

Foraging behavior	Agro- ecologies			p-value
	Highland	Midland	lowland	
EFT (am)	5:55±0.07a	5:23±0.01b	5:13±0.01b	<.0001
LFT(pm)	6:55±0.05b	6:59±0.05b	7:04±0.01a	<.0001
Absconding rate	2.38±0.92	4.28±1.70	1.90±0.84	0.353
Defensiveness				
TtfFs_sec	89.43±0.63a	87.85±1.04a	53.29±0.70b	<.0001
Tt_sec_mag	258.86±0.62a	259.28±0.81a	142.28±0.66b	<.0001
TNoS__B	21.71±0.52	21.71±0.66	21.28±0.70	0.860
TNoS_G_	15.00±0.65a	7.28±0.62c	12.28±0.66b	<.0001
TDF_m_	192.57±1.00a	182.42±0.51c	187.57±0.89b	<.0001

EFT: Early foraging time; **LFT:** Late foraging time **TtfFs-sec:** Total time for the first sting in seconds; **Tt-sec-mag:** Total time in seconds for mass aggressiveness; **TNoS-B:** Total number of stings on the leather ball; **TNoS-G:** Total number of stings on the glove; **TDF-m:** Total distance followed to the observer in meters. ^{a-d} means with different superscripts across a row are statistically significant.

The study area experienced disease, pests, and predators during the data collection period, as shown in Table 12. There were several honeybee diseases found in colonies, including chalkbrood and nosema. ants, termites, spiders, birds, Varroa mite and wasps were seen among the predators. Among the pests observed were wax moths and bee lice.

Table 12: Disease, predators, and pests observed in the study area.

Disease	Predators	Pests
Chalkbrood	Ants	Wax moth
Nosema	Termites	Bee lice
Varroa mite	Spiders	
	Birds	
	Wasps	

4.3. Evaluation of Honey Quality

Table 13 displays the Evaluation of honey quality harvested in the major and minor phases in highland, midland, and lowland areas of South Wollo zone. In the major harvesting season (October), Except for the pH ($P > 0.05$), all parameters were significantly differed ($P < 0.05$). The lowest FA value (15.00) was found in honey collected from lowland, whereas the highest FA value (21.55) was found in honey collected from midland. Between midland and lowland, there was a statistically significant difference ($P < 0.05$), but not between the others. Honey collected from lowland had a minimum MC of 16.38, which was significantly different from the other honey areas ($P < 0.05$). However, there was no statistically significant difference ($P > 0.05$) found in the honey obtained from midland and highland (17.64). The honey gathered from lowland also had the lowest AC (0.04), and there was a significant difference ($P < 0.05$) between it and the other honey areas. Midland honey had the highest AC (0.2) than highland honey (0.15), but there was no statistical difference between the two ($P > 0.05$). The highest HMF value was found in honey that was collected from lowland (4.48), and it differed significantly ($P < 0.05$) from midland and highland, but not significantly ($P > 0.05$) from either of them.

In the minor harvesting season (June), every parameter in the agro-ecologies showed a significant difference ($P < 0.05$), except for pH. midland honey had the highest recorded FA (29.67), which differed significantly ($P < 0.05$) from the other honey. The honey from lowland had the lowest FA (15.97), but there was no statistically significant difference ($P > 0.05$) between it and the honey from highland (16.60). The least amount of honey (15.67) was collected from lowland, and when compared to the other honey, MC showed a significant difference ($P < 0.05$). Despite midland honey having the highest MC content (18.28), there was no significant difference ($P > 0.05$) between it and highland (17.82). Midland honey had the highest AC (0.13), and it differed significantly ($P < 0.05$) from the other honey. While the AC content of the honey from lowland (0.04) was higher than that of the honey from highland (0.03), there was no statistically significant

difference ($P > 0.05$) between the two. The lowest HMF was found in midland honey (3.53) and it differed significantly ($P < 0.05$) with others. The highest recorded result for honey collected from the lowland was 13.00, but there was no statistical difference ($P > 0.05$) between it and honey from the highland (11.21).

Table 13: Evaluation of honey quality harvested in the major and minor harvesting season in highland, midland, and lowland areas of South Wollo zone.

Quality parameters (major harvesting season)	Highland (mean and range)	Midland (mean and range)	Lowland (mean and range)	p-value
FA (meq kg-1)	16.05±1.5ab (10-29)	21.55±2.1a (10-38)	15.00±1.5b (10-31)	0.019
MC (%)	17.64±0.4a (13.3-20.3)	18.76±0.2a (17.3-21)	16.38±0.4b (13-18.5)	<.0001
AC (%)	0.15±0.0a (0.02-0.49)	0.2±0.0a (0.02-0.55)	0.04±0.0b (0.01-0.1)	0.004
pH	3.19±0.1 (2.5-3.6)	3.09±0.1 (2.5-3.6)	3.06±0.1 (2.5-3.5)	0.355
HMF(mg/kg)	0.20±0.1b (0-0.9)	0.52±0.3b (0-1.9)	4.48±1.2a (0-15.6)	<.0001
Quality Parameters (minor harvesting season)				
FA (meq kg-1)	16.60±1.5b (12-35.5)	29.67±2.4 a (12-45)	15.97±1.2b (12-38.5)	<.0001
MC (%)	17.82±0.1a (17-18.6)	18.28±0.8a(17-19.2)	15.67±0.5b (12-20)	<.0001
AC (%)	0.03±0.0b (0.01-0.06)	0.13±0.0a (0.02-0.4)	0.04±0.0b (0.01-0.08)	0.003
pH	3.06±0.0 (2.8-3.4)	3.13±0.1 (2.7-3.7)	3.24±0.1 (2.7-3.7)	0.077
HMF(mg/kg)	11.21±2.1a (0-30.5)	3.53±1.2b (0-23.7)	13.00±2.4a (0-37-8)	0.003

FA: Free Acidity; MC: Moisture Content; AC: Ash Content; HMF: hydroxyl methyl furfural; meq = milli equivalent. Values in parentheses are ranges. ^{a-b} Means with different superscript letters across a row are significantly different.

Table 14 presents national and international quality standards over all means of honey quality parameters in the study area. As it shows, every parameter satisfies both international and national requirements except the pH. These honeys have the potential to penetrate the worldwide honey market as a result.

Table 14: Overall honey quality in the study area compared with export marketing standards.

Parameters	Mean ± SE	CAC	EU	QSAE
FA (meq kg-1)	19.14±0.9	≤ 50	≤ 40	40 max.
MC (%)	17.43±0.2	≤ 21	≤ 21	21 max.
AC (%)	0.09±0.0	≤ 0.6	≤ 0.6	0.60 max.
pH	3.13±0.0	3.4-6.1	NA	NA
HMF(mg/kg)	5.49±0.7	≤ 60	≤40	40 max.

FA: Free Acidity; MC: Moisture Content; AC: Ash Content; HMF: hydroxyl methyl furfural; CAC = Codex Alimentarius Commission; EU = European Union; meq = mill equivalent; QSAE = Quality and Standards Authority of Ethiopia, NA, not available.

The effect of season on the quality of honey harvested in the highland, midland, and lowland of south Wollo is displayed in Table 15. The analysis's findings indicated that honey harvested from highland, only AC and HMF were significantly impacted by the season ($P < 0.05$), while the other variables did not differ significantly ($P > 0.05$). While, as the data suggests, honey harvested from midland, only FA and HMF demonstrated significance ($P < 0.05$), while the others showed no statistically significant effects from the season ($P > 0.05$). According to the analysis in lowland, only HMF was impacted by the season ($P < 0.05$), while the other variables did not exhibit a statistically significant difference ($P > 0.05$).

Table 15: Effect of season on quality of honey harvested in highland, midland, and lowland of south Wollo.

Agroecology	Parameters	Season 1(major)	Season 2 (minor)	P-value
Highland	FA (meq kg-1)	16.05±1.5 ^{ab}	16.60±1.5 ^b	0.797
	MC (%)	17.64±0.4	17.82±0.1	0.634
	AC (%)	0.15±0.0 ^a	0.03±0.0 ^b	0.003
	pH	3.19±0.1	3.06±0.0	0.113
	HMF(mg/kg)	0.20±0.1 ^b	11.21±2.1 ^a	<.0001
Midland	FA (meq kg-1)	21.55±2.1 ^b	29.67±2.4 ^a	0.015
	MC (%)	18.76±0.2	18.28±0.8	0.084
	AC (%)	0.2±0.0	0.13±0.0	0.697
	pH	3.09±0.1	3.13±0.1	0.237
	HMF (mg/kg)	0.52±0.3 ^b	3.53±1.2 ^a	0.015
Lowland	FA (meq kg-1)	15.00±1.5	15.97±1.2	0.675
	MC (%)	16.38±0.4	15.67±0.5	0.294
	AC (%)	0.04±0.0	0.04±0.0	0.527
	pH	3.06±0.1	3.24±0.1	0.055
	HMF (mg/kg)	4.48±1.2 ^b	13.00±2.4 ^a	0.003

FA: Free Acidity; MC: Moisture Content; AC: Ash Content; HMF: hydroxyl methyl furfural; meq = mill equivalent. ^{a-b} Means with different superscript letters across a row are significantly different.

The effects of season on the quality of honey in the study area are presented in Table 16. As it shows, Most quality parameters did not show a significant difference ($P > 0.05$) in terms of harvesting seasons except AC and HMF content of honey ($P < 0.05$). The highest AC was found

from honey harvested during the first season (0.13) but HMF was higher for honey harvested during the second season (9.25).

Table 16: Seasonal effect on overall mean honey quality in the study area

Parameters	Season 1	Season 2	P-value
FA (meq kg ⁻¹)	17.53±1.04	20.75±1.4	0.064
MC (%)	17.59±0.2	17.27±0.2	0.336
AC (%)	0.13±0.0 ^a	0.07±0.0 ^b	0.004
pH	3.12±0.0	3.14±0.0	0.596
HMF(mg/kg)	1.74±0.5 ^b	9.25±1.2 ^a	<.0001

FA: Free Acidity; MC: Moisture Content; AC: Ash Content; HMF: hydroxyl methyl furfural; meq = mill equivalent. ^{a-b} Means with different superscript letters across a row are significantly different.

4.4. Pollen Analysis of Sampled Honey

Table 17 presents identified bee plant species from sampled honey in the highland in the major harvesting season. Except for samples of D5, which were *Guizotia scabra*, *Eucalyptus Globulus* covers the majority of the highland (>45%). *Rumex spp.* was a secondary pollen source in D2 and D3. Some of the plants that served as secondary sources for samples D4 & D5, and D8 & D9, respectively, were *Guizotia scabra* and *Maesa lanceolata*. Among the plants that were identified, *Plantago lanceolata*, *Dracaena Schizantho*, *Leonatis Africana*, and *Belpharis limasirfolia* were grouped as important pollen sources for D7 and D8, respectively. A few other significant pollen sources as minor included *Hypoestes trifolia* in D1, *Acacia spp.*, *Ocimum spp.*, *Bersama abyssinica* in D3, *Maytenat obscura* in D4, *Vicia faba* and *Gossypiam hirsutam* in D5, *Polyscias ferruginla* in D6, and *Bidens spp.* in D10.

Table 17: Identified bee plant species from sampled honey in highland in the major harvesting season

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
D1	<i>Eucalyptus globulus</i> = 85.3%	-	<i>Guizotia scabra</i> = 8.8%	<i>Trifolium spp</i> 2.9% <i>Plantago spp</i> =1.5% <i>Hypoestes trifolia</i> - 1.5%
D2	<i>Eucalyptus globulus</i> = 71.4%	<i>Rumex spp</i> = 20.4%	-	<i>Trifolium spp</i> = 2 % <i>Guizotia spp</i> =4% <i>Dracaena Schizantho</i> = 2%
D3	<i>Eucalyptus globulus</i> = 49.1%	<i>Rumex spp</i> =23%	<i>Guizotia scabra</i> =15.3% <i>Trifolium spp</i> =9.2%	<i>Acacia spp</i> =1.5% <i>Btepharis linaricfoliya</i> =0.5% <i>Ocimum spp</i> =0.8% <i>Bersama abyssinica</i> =0.8% <i>Maytenat obscura</i> =0.7%
D4	<i>Eucalyptus gloubulus</i> =71.9%	<i>Guizotia scarba</i> =24%	<i>Rumex spp</i> =3.5%	
D5	<i>Guizotia scabra</i> =70.6%	<i>Maesa lanceolata</i> =16.3%	<i>Eucalyptus globulus</i> = 6.5% <i>Trifolium spp</i> =3%	<i>Hypoestes spp</i> =0.3% <i>Rumex spp</i> =1.6% <i>Vicia faba</i> =0.9% <i>Gossypiam hirsutam</i> = 0.8%
D6	<i>Eucalyptus globulus</i> =66.6%	<i>Rumex spp</i> =31.3%	-	<i>Polyscias ferruginla</i> =0.8% <i>Guizotia spp</i> =1.3%
D7	<i>Eucalyptus globulus</i> =48.1%	<i>Maesa lanceolata</i> = 38.5	<i>Plantago spp.</i> =9.6% <i>Dracaena schizontha</i> =3.7%	-

D8	<i>Eucalyptus globules</i> =46.3%	<i>Guizotia spp</i> =37%	<i>Leonatis Africana</i> = 7.4% <i>Schinus molle</i> =3.7% <i>Belpharis limasirfolia</i> = 5.6%	-
D9	<i>Eucalyptus globulus</i> =58%	<i>Maesa lanceolata</i> = 22.4%	<i>Guizotia scabra</i> =6% <i>Dracaena schizontha</i> =8% <i>Biden spp</i> =4%	<i>Trifolium spp</i> = 1.6%
D10	<i>Eucalyptus globulus</i> = 91.1%		<i>Trifolium spp</i> = 8.4%	<i>Bidens spp</i> = 0.4%

D: Sample names collected from Dessie zuria

Identified bee plant species from sampled honey in the midland in major harvesting season displayed in table 18. *Eucalyptus globules* was found predominantly except in T1, T7 and T9 (*Schefflera abyssinica*), T4 (*Acacia spp*) and T10 (*Trifolium spp*). Among secondary plant source, grass in T1 and T2, *Guizotia scabra* in T3, *Syzgium guineans* in T6, *Rumex spp.* in T7 and T10 and *Maesa lanceolata* in T9 were listed. Other plant types found as important were, *Acanthus sennii* in T1, *Hypoestes trifolia* in T3, *Apodytes dimidiato* in T7 and *Ocimum spp* in T8. *Coffea arabica*, *Cesalpinia decapetola*, *Erica arborea*, *Phoenix abyssinica*, *Maize*, *Brassica carinata* and *Justicia schimperiana* in T3, *Bidens spp* and *Vernonia schimperi* in T4, *Impatiens rothii* and *Dracaena schizanthae* in T5, *Dombiyya toirida* in T7, *Vicia faba* in T9, and *Polyscias ferruginla* in T10 were identified plant types as minor pollen and nectar source for these samples.

Table 18: Identified bee plant species from sampled honey in the midland in major harvesting season

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
T1	<i>Eucalyptus globulus</i> = 62.5%	<i>Grass spp</i> = 25%	<i>Acanthus sennii</i> = 12.5%	-
T2	<i>Eucalyptus globulus</i> =75%	<i>Grass spp</i> = 16.7%	<i>Guizotia scabra</i> = 8.3%	
T3	<i>Eucalyptus globulus</i> 56.8%	<i>Guizotia scabra</i> = 22.7%	<i>Hypoestes spp</i> = 4.5% <i>Maesa lanceolata</i> = 11.4%	<i>Coffee arabica</i> = 0.1% <i>Cesalpinia decapetola</i> = 0.1% <i>rumex spp</i> =1.6% <i>Erica arborea</i> = 0.1% <i>Apodytes dimidiato</i> = 0.2% <i>Phoenix abyssinica</i> = 0.1% <i>Zea mays</i> = 0.1% <i>Brassica carinata</i> =0.2% <i>Ocimum spp</i> =0.6% <i>Justicia spp.</i> = 0.9%
T4	<i>Acacia spp</i> = 78%	-	<i>Eucalyptus globulus</i> =6% <i>Rumex spp</i> =8% <i>Guizotia scarab</i> =5.1%	<i>Bidens spp</i> = 0.2% <i>Brassica carinata</i> = 0.2% <i>Vernonia spp</i> =0.6% <i>Trifolium spp</i> = 1.4%
T5	<i>Schefflera abyssinica</i> = 61.6%	<i>Eucalyptus globulus</i> = 26.7%	<i>Maesa lanceolata</i> = 7.7% <i>Guizotia scabra</i> =3.6%	<i>Impatiens rothii</i> =0.3% <i>Dracaena schizontha</i> = 0.1%
T6	<i>Eucalyptus globules</i> =61.2%	<i>Syzgium guineans</i> = 20.4%	<i>Rumex spp</i> = 6.1% <i>Hypoestes spp</i> =10.2%	<i>Ocimum spp</i> = 0.2% <i>Guizotia scabra</i> = 0.6% <i>Justicia spp</i> = 1.2% <i>Grass spp</i> = 0.1%
T7	<i>Schefflera abyssinica</i> = 66.7%	<i>Rumex spp</i> = 20%	<i>Apodytes dimidiate</i> = 3.5% <i>Eucalyptus</i> = 4%	<i>Guizotia scarab</i> = 1.3% <i>Dombiya toirda</i> = 0.5% <i>Hypoestes spp</i> = 1.3% <i>Maesa lanceolata</i> = 2% <i>Dracaena schizontha</i> = 0.7%

T8	<i>Eucalyptus globulus</i> =60.9%	-	<i>Ocimum</i> = 3.8% <i>Maesa lanceolata</i> =11.3% <i>Syzgium guineans</i> =9.4% <i>Schiffera abyssinica</i> =4.7%	<i>Hypoestes</i> =1.9% <i>Guizotia scabra</i> = 2.6 <i>Rumex spp</i> = 0.6% <i>Trifolium</i> =2.8% <i>Caesalpinia decapetala</i> = 2%
T9	<i>Schefflera abyssinica</i> =60.5%	<i>Maesa lanceolata</i> = 16.8%	<i>Rumex spp</i> = 15.1% <i>Guizotia scarab</i> = 6.7%	<i>Impatient votail</i> = 0.6% <i>Vicia faba</i> = 0.2%
T10	<i>Trifolium spp</i> =55.1%	<i>Rumex spp</i> = 31.2%	<i>Guizotia scabra</i> = 3.7% <i>Eucalyptus globulus</i> = 5.5%	<i>Polyscias ferruiginea</i> = 2.9% <i>Ocimum spp</i> = 0.1% <i>Caesalpinia decapetala</i> =0.1% <i>Zea mays</i> =0.04% <i>Eucea kenau</i> = 0.4% <i>Crotonma crostaechus</i> = 0.1% <i>Hypoestes spp</i> = 0.2% <i>Coffea arabica</i> = 0.7%

T: Sample name collected from Tehuledere

Table 19 shows identified bee plant species from sampled honey in the lowland in major harvesting season. *Eucalyptus globules* cover a predominance percentage in all honey samples except in K3 which was *Schefflera abyssinica*. *Guizotia scabra* in K4 and k5 serve as secondary pollen source. *Coffea arabica* and *Rumex spp* in K1, *Maesa lanceolata* in K2, *Dracaena schizantho* in K4 were among the identified plants that serve as important pollen and nectar sources for honeybees produce these sample honey. *Trifolium spp* in K2, *Plantago lanceolata* in K4, *Prunes persica* in K5, *Brucea antidysentefica*, *Cleame usambarica* and *Schinus molle* in K6 and *Satureja paradox* and *Phytolacea dodecandra* in K9 were other plants and recorded as minor source for these samples.

Table 19: Identified bee plant species in the lowland in major harvesting season

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
K1	<i>Eucalyptus globulus</i> = 82%	-	<i>Guizotia scabra</i> = 4.1% <i>Coffee arabica</i> = 4.9% <i>Rumex spp</i> = 9%	-
K2	<i>Eucalyptus globulus</i> =90.9%	-	<i>Maesa lanceolata</i> = 5.3% <i>Guizotia scabra</i> =3%	<i>Trifolium spp.</i> =0.8%
K3	<i>Schefflera abyssinica</i> = 74%	-	<i>Eucalyptus</i> =13.3% <i>Maesa lanceolata</i> =7.2% <i>Rumex spp</i> = 4.8%	<i>Guizotia Scarba</i> =0.5% <i>Dracaera schizantha</i> =0.2%
K4	<i>Eucalyptus globulus</i> =59.2%	<i>Guizotia scabra</i> .= 29.2%	<i>Dracaera schizantha</i> = 7.7%	<i>Trifolium spp</i> =0.6% <i>Plantago lanceolata</i> =2.9%
K5	<i>Eucalyptus globulus</i> =64.8%	<i>Guizotia spp.</i> = 32.4%		<i>Plantago lanceolata</i> =2.7% <i>Prunes persica</i> =0.1%
K6	<i>Eucalyptus globulus</i> = 92.1%		<i>Guizotia scabra</i> = 3.2%	<i>Coffea arabica</i> =1.3% <i>Brucea antidysentefica</i> = 0.5% <i>Bidens spp</i> = 0.5% <i>Cleame usambarica</i> = 0.5% <i>Schinus molle</i> = 1.3%
K7	<i>Eucalyptus globulus</i> =83.3%	-	<i>Guizotia scabra</i> = 7.5% <i>Rumex spp</i> = 9.2%	-
K8	<i>Eucalyptus globulus</i> = 94.2%	-	-	<i>Guizotia scarba</i> = 2.3% <i>Trifolium spp</i> = 1.5% <i>Satureja paradox</i> = 1.9%
K9	<i>Eucalyptus globulus</i> =94.3%	-	-	<i>Plantago lanceolata</i> = 0.7% <i>Trifolium spp</i> = 1.4% <i>Satureja paradox</i> = 2.9% <i>Phytolacea dodecandra</i> =0.7%

K10	<i>Eucalyptus globulus</i> = 96.8%	-	-	<i>Guizotia scabra</i> = 2.9% <i>Phytolacea dodecandra</i> = 0.3%
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K: *Sample name collected from Kalu*

Plant types identified in the first season in all agro-ecologies are present in table 20. From 30 honey samples, 41 plant types were identified which were combinations of trees, herbs, shrubs as well as crops. All these plants had a reward of both pollen and nectar sources except *Rumex spp*, *Maytenat obscura*, *Plantago lanceolata*, *Hypoestes trifolia*, and *Phytolacca dodecandra* which provided only pollen, and *Schefflera abyssinica* which only proved nectar for honeybee. Most plants had flowering calendars of expected months which were found in this harvesting season.

Table 20: Plant types identified in the major season in all agro-ecologies

No.	Scientific name	Local name	Life Form	Flowering time	Reward
1	<i>Guizotia scabra</i>	Mech	herb	Sep-Nov	Nectar and pollen
2	<i>Maesa lanceolata</i>	Abaliyeh	tree	Sep-Oct	Pollen
3	<i>Dracaena schizanthae</i>	Chowyeh, tabatos	tree	Sep -Nov	pollen and nectar
4	<i>Bidens spp</i>	Adey Abeba	Herb	Sep-Oct	Pollen and nectar
5	<i>Eucalyptus globulus</i>	bahirzaf)	Tree	All over the year	nectar and pollen
6	<i>Trifolium spp</i>	Maget	Herb	Aug-Dec	nectar and pollen
7	<i>Rumex spp.</i>	Embacho	Herb	Sep-Nov	Pollen
8	<i>Maytenat obscura</i>	Atat	shrub,	July-Oct	Pollen
9	<i>Apodytes dimidiato</i>	Donga, Celeqleqqa	tree,	Sep - Nov	pollen and nectar
10	<i>Brassica carinata</i>	Gomenzer	Crop	Sep-Oct	pollen and nectar
11	<i>Leonatis africana</i>				
12	<i>Schinus molle</i>	Kundo Berbere	Tree	March	pollen and nectar
13	<i>Belpharis limasirfolia</i>				
14	<i>Plantago lanceolata</i>	Gorteb	Herb	May-June	Pollen
15	<i>Hypoestes trifolia-</i>	Tqur Telenj	Herb	Sep-Nove	Pollen
16	<i>Acacia spp</i>	Girar	Tree	Sep-Nov	pollen and nectar
17	<i>Ocimum spp</i>	Besobila	Herb	Sep-Nov	nectar and pollen
18	<i>Bersama abyssinica</i>	Affajeshn, Azamr	Tree	Sep-Nov	pollen and nectar
19	<i>Phoenix abyssinica</i>	Hosa'na, Sellen Hosana, Zembaba	Tree	Sep-Nov	pollen and nectar
20	<i>Justicia schimperiana</i>	Sensel	shrub,	Sep-Nov	pollen and nectar
21	<i>Vicia faba</i>	Bakela	Crop	August	Nectar and pollen
22	<i>Gossypiam hirsutam</i>	Tit	shrub	Oct-.Dec	pollen and nectar
23	<i>Polyscias ferruginla</i>	Yeznjerowenber	Tree	Sep-Dec	pollen and nectar
24	<i>Grass spp</i>	Sar	grass	July-Dec	pollen and nectar
25	<i>Acanthus sennii</i>	Koshashile	shrub	July -Sept	pollen and nectar
26	<i>Coffea Arabica</i>	Bunna	Shrub	March,	pollen and nectar
27	<i>Cesalpinia decapetola</i>	Yeferenj-Kitkitta, Qontir	herb/shrub	Sep-Nov	pollen and nectar
28	<i>Erica arborea</i>	Adal	shrub	Oct- Dec	pollen and nectar
29	<i>Maize</i>	Bekolo	Crop	Aug-Sept,	pollen and nectar
30	<i>Vernonia schimperi</i>	Grawa	Herb	Sep-May	pollen and nectar
31	<i>Schefflera abyssinica</i>	Geteme	Tree	March-April	Nectar

No.	Scientific name	Local name	Life form	Flowering time	Reward
32	<i>Dombiya toirda</i>	Wulkefa	Tree	Sep-Nov	pollen and nectar
33	<i>Prunes persica</i>	Kok	Tree	Sep-Nov.	Pollen and nectar
34	<i>Satureja paradox</i>	Tenadm	Herb	Sep-Oct	pollen and nectar
35	<i>Impatiens rothii</i>	Gursht	Shrub	Sep-Nov	pollen and nectar
36	<i>Euclea racemosa</i>	Dedehe	Shrub	Aug-Dec	pollen and nectar
37	<i>Brucea antidysentefica</i>	Abalo, Wagnos	herb/shrub	Sep-Oct	pollen and nectar
38	<i>Phytolacca dodecandra</i>	Endod	shrub	Sep-Nov	Pollen
39	<i>Syzgium guineans</i>	Doqma	Tree	March	nectar and pollen
40	<i>Croton macrostachyus</i>	Bsana	Tree	Apri-June,	nectar and pollen
41	<i>Cleame usambarica</i>		Shrub		

Table 21 displays unique plants in each agro ecology. Some plants were unique for each agro ecology. From sampled honey midland honey contains a large no of unique plants (15 plants) that were not found in other sampled honey (highland and lowland). 4 plants and 5 plants were unique in highland and lowland respectively.

Table 21: Unique plants in each agro ecology of the study area in major harvesting season

Highland	Midland	Lowland
<i>Maytenat obscura</i>	<i>Grass spp</i>	<i>Prunes persica</i>
<i>Leonatis africana</i>	<i>Acanthus sennii</i>	<i>Brucea antidysentefica</i>
<i>Belpharis limasirfolia</i>	<i>Cesalpinia decapetola</i>	<i>Cleame usambarica</i>
<i>Bersama abyssinica</i>	<i>Erica arborea</i>	<i>Satureja paradox</i>
<i>Gossypiam hirsutam</i>	<i>Apodytes dimidiato</i>	<i>Phytolacea dodecandra</i>
	<i>Phoenix abyssinica</i>	
	<i>Zea mays</i>	
	<i>Brassica carinata</i>	
	<i>Justicia</i>	
	<i>Vernonia spp</i>	
	<i>Impatiens rothii</i>	
	<i>Syzgium guineans</i>	
	<i>Dombiya toirda</i>	
	<i>Eucea kenau</i>	
	<i>Crotonma crostaechus</i>	

Table 22 indicates the Frequency, and percent of the total of identified plants in the major harvesting season. During this season, *Eucalyptus globules* take the first rank with 16.8% from total, and *guizotia* spp is found in the second rank with 13.9%. *Rumex spp*, *trifolium spp*, and *maestant* spp were found in the third, fourth, and fifth with 8.7%, 6.9%, and 6.4% in total respectively. Some plants had similar ranks with equal percentages. Among these *Dracaena schizanthae* and *Hypoestes -trifolia-*, *Plantago -lanceolata*, *Ocimum spp*, *Bidens spp*, and *Schefflera abyssinica* stands 6th, 7th, and 8th rank with the percentage of 4.0%, 2.9% and 2.3% from total plants respectively. *Prunes persica*, *Leonatis africana*, *Phoenix abyssinica*, *Acanthus sennii* and *Vernonia schimperi* were among identified plants had the smallest percentage (0.6%) and stands at the last rank.

Table 22: Frequency, and percent of total of identified plants in the major harvesting season

Plants	Highland	Midland	Lowland	Total	Rank
<i>Guizotia scabra</i>	8 (4.6%)	8 (4.6%)	8 (4.6%)	24 (13.9%)	2
<i>Maesa lanceolata</i>	3 (1.7%)	6 (3.5%)	2 (1.2%)	11 (6.4%)	5
<i>Dracaena schizanthae</i>	3 (1.7%)	2 (1.2%)	2 (1.2%)	7 (4.0%)	6
<i>Bidens spp</i>	2 (1.2%)	1 (0.6%)	1 (0.6%)	4 (2.3%)	8
<i>Eucalyptus Globulus</i>	10 (5.8%)	9 (5.2%)	10 (5.8%)	29 (16.8%)	1
<i>Trifolium spp</i>	6 (3.5%)	2 (1.2%)	4 (2.3%)	12 (6.9%)	4
<i>Rumex spp.</i>	5 (2.9%)	7 (4.0%)	3 (1.7%)	15 (8.7%)	3
<i>Maytenat obscura</i>	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	11
<i>Apodytes dimidiato</i>	0 (0.0%)	2 (1.2%)	0 (0.0%)	2 (1.2%)	10
<i>Brassica carinata</i>	0 (0.0%)	2 (1.2%)	0 (0.0%)	2 (1.2%)	10
<i>Schefflera abyssinica</i>	0 (0.0%)	4 (2.3%)	0 (0.0%)	4 (2.3%)	8
<i>Dombiya toirda</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Prunes persica</i>	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.6%)	11
<i>Satureja paradox</i>	0 (0.0%)	0 (0.0%)	2 (1.2%)	2 (1.2%)	10
<i>Leonatis africana</i>	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	11
<i>Schinus molle</i>	1 (0.6%)	0 (0.0%)	1 (0.6%)	2 (1.2%)	10
<i>Belpharis limasirfolia</i>	2 (1.2%)	0 (0.0%)	0 (0.0%)	2 (1.2%)	10
<i>Plantago –lanceolata</i>	2 (1.2%)	0 (0.0%)	3 (1.7%)	5 (2.9%)	7
<i>Hypoestes –trifolia-</i>	2 (1.2%)	5 (2.9%)	0 (0.0%)	7 (4.0%)	6
<i>Acacia spp</i>	1 (0.6%)	1 (0.6%)	0 (0.0%)	2 (1.2%)	10
<i>Ocimum spp</i>	1 (0.6%)	4 (2.3%)	0 (0.0%)	5 (2.9%)	7
<i>Bersama abyssinica</i>	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	11
<i>Phoenix abyssinica</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Justicia schimperiana</i>	0 (0.0%)	2 (1.2%)	0 (0.0%)	2 (1.2%)	10
<i>Impatiens rothii</i>	0 (0.0%)	3 (1.7%)	1 (0.6%)	4 (2.3%)	8
<i>Euclea racemosa</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Brucea antidysentefica</i>	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.6%)	11
<i>Phytolacea dodecandra,</i>	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.6%)	11
<i>Vicia faba</i>	1 (0.6%)	1 (0.6%)	0 (0.0%)	2 (1.2%)	10
<i>Gossypiam hirsutam</i>	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	11
<i>Polyscias ferruginla</i>	1 (0.6%)	1 (0.6%)	0 (0.0%)	2 (1.2%)	10
<i>Grass spp</i>	0 (0.0%)	3 (1.7%)	0 (0.0%)	3 (1.7%)	9
<i>Acanthus sennii</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Coffee Arabica</i>	0 (0.0%)	2 (1.2%)	2 (1.2%)	4 (2.3%)	8
<i>Cesalpinia decapetola</i>	0 (0.0%)	3 (1.7%)	0 (0.0%)	3 (1.7%)	9
<i>Erica arborea</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Zea mays</i>	0 (0.0%)	2 (1.2%)	0 (0.0%)	2 (1.2%)	10
<i>Vernonia schimperi</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Syzgium guineans</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Croton macrostachyus</i>	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	11
<i>Cleame usambarica</i>	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.6%)	11
Total	52 (30.1%)	78 (45.1%)	43 (24.9%)	173 (100.0%)	

Table 23 shows identified bee plant species from sampled honey in the highland minor harvesting season (June). In this agroecology, *Eucalyptus globulus* was the dominant plant except for D6 and D10 which was *Terminalia spp.* Among plants that served as secondary important pollen sources

were *Hypoestes Trifolia* and *Cordia africana* in D2, *Croton macrostachys* in D7, and *Erica spp* in D8. *Veronia spp* in D2, *Rumex spp* in D3, *Ehertia cymosa* in D5 *Olea europea* in D7 *Satureja spp* in D8, and *Schefflera abyssinica* in D10 were as important pollen sources for these honey. Other identified plant types in sampled honey served as minor pollen source which was *Dombeya spp* and *Acica spp* in D1, *Hagenia abyssinica* in D4, *Plantago spp* in D6, and *Plectrntus spp* in D10.

Table 23: Identified bee plant species from sampled honey in the highland minor harvesting season.

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
D1	<i>Eucalyptus globulus</i> =98%			Not identified= 2%
D2		<i>Hypoestes trifolia</i> =28% <i>Eucalyptus globulus</i> =40% <i>Cordia-africana</i> =20%	<i>Croton macrostachys</i> =3.2% <i>Veronia spp</i> = 4%	<i>Dombeya Spp</i> =1.6% <i>Acacia Spp</i> = 1.2% <i>Satureja Spp</i> =2%
D3	<i>Eucalyptus globulus</i> = 95.2 %		<i>Rumex spp</i> = 4.8%	
D4	<i>Eucalyptus globulus</i> 60.4%	<i>Terminalia spp</i> =32.2%	<i>Cordia africana</i> =4%	<i>Rumex spp</i> = 0.8% <i>Hypoestes trifolia</i> = 2% <i>Veronia spp</i> = 0.4% <i>Hagenia abyssinica</i> = 0.2%
D5	<i>Eucalyptus globulus</i> = 80.9%		<i>Rumex spp</i> = 4.8% <i>Ehertia cymosa</i> = 14.3%	
D6	<i>Terminalia spp</i> =70.4%	<i>Eucalyptus globulus</i> = 24.6 %		<i>Dombeya Spp</i> = 2.8% <i>Rumex spp</i> = 0.4% <i>Plantago lanceolata</i> = 0.7% <i>Olea europea</i> = 1.4%
D7	<i>Eucalyptus globulus</i> = 55.5%	<i>Croton Macrostachys</i> =37%	<i>Olea europea</i> = 5.5%	<i>Plantago lanceolata</i> =1.8%
D8	<i>Eucalyptus globulus</i> = 57.1%	<i>Erica spp</i> =34.3%	<i>Satureja spp</i> =5.7%	<i>Rumex spp</i> = 1.1% <i>Veronia spp</i> = 1.7%
D9	<i>Eucalyptus globulus</i> = 77.4%	<i>Croton Macrostachys</i> =19.3%		<i>Acica spp</i> = 0.7% <i>Rumex spp</i> = 2.6%
D10	<i>Terminalia spp</i> = 86%		<i>Schefflera abyssinica</i> = 11.5%	<i>Hypoestes Trifolia spp</i> = 1.7% <i>Plectrthus spp</i> = 0.8%

D: sample name collected from Dessie zuria

Identified bee plant species from sampled honey in the midland minor harvesting season (June) presented in table 24. In this agro ecology (midland), *Eucalyptus globulus* is also dominantly found in 4 samples of honey (T5, T7, T9, and T10). While *Schefflera abyssinica* and *Terminalia spp* was found dominantly in T6 and T2 respectively. *Hypoestes trifolia*, *Acica spp*, *Rumex spp*, and *Veronia spp* were plants found in T1, T2, T3, and T9 and served as a secondary sources respectively. *Trifolium spp* in T1, *Echinops macrochaetus* in T3, *Croton macrostachys* in T4, *Guizotia scarba*, *Coffea arabica*, and *Cordia africana* in T8, were among plants that served as important sources of honey bees which produce these honey. *Brassica carinata* and *Dombeya spp* were other identified plant sources used as minor sources for honey represented by T1 and T8 respectively.

Table 24: Identified bee plant species from sampled honey in the midland minor harvesting season.

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
T1		<i>Eucalyptus globulus</i> = 39.6% <i>Hypoestes trifolia</i> = 26.4% <i>Schefflera- abyssinica</i> = 19.8%	<i>Rumex spp</i> =5.9% <i>Trifolium spp</i> = 4%	<i>Veronia spp</i> =1.3% <i>Coffea arabica</i> = 2% <i>Brassica Carirata</i> = 1%
T2	<i>Terminalia spp</i> = 55.2%	<i>Acica spp</i> = 31%	<i>Hypoestes trifolia</i> = 13.8%	-
T3		<i>Eucalyptus globulus</i> = 43.9% <i>Hypoestes Trifolia</i> = 29.3% <i>Rumex spp</i> 19.5%	<i>Echinops macrochaetus</i> = 7.3%	-
T4		<i>Eucalyptus globulus</i> = 44.9% <i>Rumex spp</i> =25.6 <i>Acica spp</i> 16% <i>Guizotia spp</i> =16%	<i>Croton macrostachys</i> = 6.4%	<i>Veronia spp</i> = 1% <i>Hypoestes trifolia</i> = 2.2% <i>Olea europea</i> = 0.6%
T5	<i>Eucalyptus globulus</i> =83.3%	-	<i>Croton macrostachys</i> =11.1% <i>Rumex spp</i> =3.9%	<i>Trifolium spp</i> =1.7%
T6	<i>Schefflera- abyssinica</i> = 60%	<i>Eucalyptus globulus</i> = 35%	<i>Rumex spp</i> = 5%	-
T7	<i>Eucalyptus globulus</i> = 90%		<i>Veronia spp</i> = 10%	-
T8		<i>Eucalyptus globulus</i> =43.2%	<i>Veronia spp</i> =7.2% <i>Guizotia scabra</i> =10.1% <i>Coffea arabica</i> =5.7% <i>Hypoestes trifolia</i> =12.4% <i>Cordia africana</i> = 14.4%	<i>Rumex spp</i> =2.9% <i>Croton Macrostachys</i> =2.2% <i>Dombeya Spp</i> =1.4%
T9	<i>Eucalyptus globulus</i> =48.4%	<i>Rumex spp</i> =28.2% <i>Veronia spp</i> =20.2%	<i>Terminalia spp</i> = 3.2%	-
T10	<i>Eucalyptus globulus</i> = 85%		<i>Rumex spp</i> =15%	-

T: Sampled name collected from Tehuledere

Identified bee plant species from sampled honey in the lowland second harvesting season (June) are displayed in Table 25. In this area, *Eucalyptus globulus* also served as a predominant plant in all sampled honey except K4 which was *Terminalia spp.* *Rumex spp.*, *Croton macrostachys*, *Satureja spp.*, and *Plantago spp.* were identified plants that serve as secondary sources for K1, K2, K8, and K10 respectively. *Acacia spp.* and *Veronia spp.* serve as an important source for k4 & k8 and k6 & K7 respectively. While, *Trifolium spp.*, *Plectrnthus spp.*, and *Echinops macrochaetus* were among the plants under minor sources for K1, K4, and K8 respectively.

Table 25: Identified bee plant species from sampled honey in the lowland in minor harvesting season.

Samples	Predominant pollen (>45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)
K1	<i>Eucalyptus globulus</i> = 74.3%	<i>Rumex spp</i> = 22.9%	-	<i>Trifolium spp</i> = 2.8%
K2	<i>Eucalyptus globulus</i> = 83.3%	<i>Croton macrostachys</i> = 16.7%	-	-
K3	<i>Eucalyptus globulus</i> = 92.2%	-	<i>Croton macrostachys</i> 5.7%	<i>Veronia spp</i> = 2.1%
K4	<i>Terminalia spp</i> = 82.2%	-	<i>Acica spp</i> = 13.7%	<i>Plectrnthus punctatus</i> = 4.1%
K5	<i>Eucalyptus globulus</i> = 87.7%	-	<i>Croton macrostachys</i> = 3.5% <i>Rumex spp</i> = 8.8%	-
K6	<i>Eucalyptus globulus</i> = 66%	<i>Croton macrostachys</i> = 30.4%	<i>Veronia spp</i> = 3.6%	-
K7	<i>Eucalyptus globulus</i> = 88.1%	-	<i>Veronia spp</i> = 10.4%	<i>Rumex spp</i> = 1.5%
K8	<i>Eucalyptus globulus</i> = 58.6%	<i>Satureja spp</i> = 27%	<i>Acica spp</i> = 3.6% <i>Rumex spp</i> = 9%	<i>Echinops macrochaetus</i> = 1.8%
K9	<i>Eucalyptus globulus</i> = 99.2%	-	-	<i>Rumex spp</i> = 0.8%
K10	<i>Eucalyptus globulus</i> = 58.8%	<i>Plantago lanceolata</i> = 29.4 %	<i>Croton macrostachys</i> = 11.8%	-

Table 26 presents the identified plants in the study area identified plants were a combination of trees, shrubs, and herbs. Their flowering season was under expected seasons for this harvesting period. All 23 identified plants served as both pollen and nectar sources except *Rumex spp*, *Hypoestes trifolia*, *Olea africana*, and *Hagenia abyssinica* for only pollen and *Schefflera abyssinica* for only nectar source.

Table 26: Identified plants in the study area in the minor harvesting season.

No.	Scientific name	Local Name	Life form	Flowering season	Reward
1	<i>Eucalyptus globulus</i>	Bahi zaf	tree	All over the year	Pollen and nectar
2	<i>Plantago spp</i>	Gurteb, Gortem, Yebeg Lat, Womebert	Herb/shrub	Sep-Nov	Pollen and nectar
3	<i>Croton macrostachys</i>	Bisana	tree	Feb-Aug	Pollen and nectar
4	<i>Rumex spp</i>	Embach, Embacho	Herb/shrub	Sep-Nov	pollen
5	<i>Satureja spp</i>	Tosgn, Yeloskit	herb	Sep-Nov	Pollen and nectar
6	<i>Acica spp</i>	Girar	tree	Dec-May	Pollen and nectar
7	<i>Echinops macrochaetus</i>	Kosheshila	herb	March- April	Pollen and nectar
8	<i>Hypoestes trifolia</i>	Tej matebiya	Herb	Sep-Oct	pollen
9	<i>Dombeya spp</i>		herb		
10	<i>Terminalia spp</i>	Abalo, Tagyie	tree	March-June	Pollen and nectar
11	<i>Cordia Africana</i>	Wanza	tree	Sep-Nov	Pollen and nectar
12	<i>Hagenia abyssinica</i>	Koso	tree	March-April	pollen
13	<i>Veronia spp</i>	Grawa	herb	Dec—Jan	Pollen and nectar
14	<i>Ehertia cymosa</i>	Game	tree	Sep-Nov	Nectar and pollen
15	<i>Olea europea</i>	Weira	tree	March-Jun	Pollen and nectar
16	<i>Erica spp</i>	Asta	shrub	Oct- Dec	Pollen and nectar
17	<i>Schefflera abyssinica</i>	Geteme	tree	March- April.	nectar
18	<i>Plectrnthus spp</i>	Agwashir, Yefiyel Doqa, Yefiyel Duba, Yemariyam-Weha-Qeji	shrub	March-april	Pollen and nectar
19	<i>Trifolium spp</i>	Maget	herb	Oct-Feb	Pollen and nectar
20	<i>Brassica carirata</i>	Gomen	crop	Sep-Oct	Pollen and nectar
21	<i>Coffea Arabica</i>	Bunna	shrub	March	Pollen and nectar
22	<i>Olea Africana</i>	Woyira	tree	Oct-Feb	Pollen
23	<i>Guizotia scabra</i>	Mech	shrub	Sep-Nove	Pollen and nectar

Unique plants for each agro ecology in the second harvesting season shows in Table 27. Some plants were unique for specific agro ecology from identified plants. Among these *Ehertia cymosa*,

Erica spp, and *Hagenia abyssinica* were only found from honey harvested from Dessie zuriya (highland). *Brassica carirata*, *Guizotia scabra*, *Coffea arabica*, and *Olea africana* were plantes found only from honey harvested in tehuledere (midland). In this season there was no plant identified as unique for Kalu (lowland). These indicated that all identified plants from the honey of this area were found either or both in highland and midland areas.

Table 27: Unique plants for each agro ecology in the minor harvesting season

Highland	Midland	Lowland
<i>Ehertia cymose</i>	<i>Brassica carirata</i>	-
<i>Erica spp</i>	<i>Guizotia scabra</i>	-
<i>Hagenia abyssinica</i>	<i>Coffea arabica</i>	-
	<i>Olea africana</i>	-

Tabel 28 showed frequency, percent within plants, and percent of the total of identified plants during pollen analysis during the second harvesting season. *Eucalyptus globulus* had the highest percentage (22.5%) of all the plants during this season and was ranked first. Among the identified plants, *Rumex spp*, *Croton macrostachys*, and *Hypoestes trifolia* had percentages of 15.8%, 9.2%, and 6.7%, respectively, placing them in second, third, and fourth place. In this season, some plants had equal percentages and ranks like *Plantago spp*, *Satureja spp*, *Dombeya spp*, *Cordia africana*, *Olea europea*, *Schefflera abyssinica* and *Trifolium spp* had 2.5% of the total and found at the rank of 6th. *Hagenia Abyssinia*, *Ehertia cymosa*, *Erica spp*, *Brassica carirata*, and *Olea africana* were plants that recorded the lowest percentage in total (0.8%) and found the last rank.

Table 28: Frequency and percent of the total of identified plants during pollen analysis during minor harvesting season.

Plant	Dessie zuriya	Tehuledere	Kalu	Total	Rank
<i>Eucalyptus globulus</i>	9 (7.5%)	9 (7.5%)	9 (7.5%)	27 (22.5%)	1
<i>Plantago spp</i>	2 (1.7%)	0 (0.0%)	1 (0.8%)	3 (2.5%)	6
<i>Croton macrostachys</i>	3 (2.5%)	3 (2.5%)	5 (4.2%)	11 (9.2%)	3
<i>Rumex spp</i>	6 (5.0%)	8 (6.7%)	5 (4.2%)	19 (15.8%)	2
<i>Satureja spp</i>	2 (1.7%)	0 (0.0%)	1 (0.8%)	3 (2.5%)	6
<i>Acica spp</i>	2 (1.7%)	1 (0.8%)	2 (1.7%)	5 (4.2%)	5
<i>Echinops macrochaetus</i>	0 (0.0%)	1 (0.8%)	1 (0.8%)	2 (1.7%)	7
<i>HypoestestTrifolia</i>	3 (2.5%)	5 (4.2%)	0 (0.0%)	8 (6.7%)	4
<i>Dombeya spp</i>	2 (1.7%)	1 (0.8%)	0 (0.0%)	3 (2.5%)	6
<i>Terminalia spp</i>	2 (1.7%)	2 (1.7%)	1 (0.8%)	5 (4.2%)	5
<i>Cordia Africana</i>	2 (1.7%)	1 (0.8%)	0 (0.0%)	3 (2.5%)	6
<i>Hagenia abyssinca</i>	1 (0.8%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	8
<i>Veronia spp</i>	3 (2.5%)	5 (4.2%)	3 (2.5%)	11 (9.2%)	3
<i>Ehertia cymosa</i>	1 (0.8%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	8
<i>Olea europea</i>	2 (1.7%)	1 (0.8%)	0 (0.0%)	3 (2.5%)	6
<i>Erica spp</i>	1 (0.8%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	8
<i>Schefflera abyssinica</i>	1 (0.8%)	2 (1.7%)	0 (0.0%)	3 (2.5%)	6
<i>Plectrntus spp</i>	1 (0.8%)	0 (0.0%)	1 (0.8%)	2 (1.7%)	7
<i>Trifolium spp</i>	0 (0.0%)	2 (1.7%)	1 (0.8%)	3 (2.5%)	6
<i>Brassica carirata</i>	0 (0.0%)	1 (0.8%)	0 (0.0%)	1 (0.8%)	8
<i>Coffea Arabica</i>	0 (0.0%)	2 (1.7%)	0 (0.0%)	2 (1.7%)	7
<i>Olea Africana</i>	0 (0.0%)	1 (0.8%)	0 (0.0%)	1 (0.8%)	8
<i>Guizotia scarab</i>	0 (0.0%)	2 (1.7%)	0 (0.0%)	2 (1.7%)	7
Total	43 (35.8%)	47 (39.2%)	30 (25.0%)	120 (100.0%)	

CHAPTER 5: DISCUSSION

5.1. Effects of Different Feeds on Performances of Honeybees

5.1.1 Effects of feeds on feed intake honeybee colonies

Results from Ullah *et al.* (2021) study showed that the maximum amount ingested per week was (74.34 g) soybean fortified meal was highly consumed which was lower than the result of the present study (148.7g) (Table 7). On the other hand, the outcomes of Mahesh *et al.* (2021), who offer Brewer's yeast (42g) + Gram (4g) + Skimmed Milk Power (4g) + Sugar (50g) + Pollen (10g) diet got a maximum net consumption of (67.85%) which was less than the feed intake tallied in the current study. In addition, the maximum amount of food (71.90 g) consumed by honey bees each week, was obtained by Islam *et al.* (2020) for 100 g (30 g soybean flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup., which was also lower than the result in the present study.

In contrast, the maximum total feed intake (100%) was recorded by El-Wahab *et al.*(2016) for 10g brewery yeast + 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A, D, and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup), which was a higher result than the current finding. Confirming this (Dogra *et al.* 2023) fed Defatted soya flour = 150g; Wheat flour = 150g; Deactivated yeast = 100g; Sugar = 266g; Water = 134ml (Total = 800 g + 40 ml Rum) recorded 100% fed consumption. For honeybee colonies to survive when there is a lack of available natural nectar, sugar feeding is essential. The sugar-feeding type affects the growth and health of bee colonies to increase their resistance (Frizzera *et al.*, 2020; Ahmad *et al.*, 2021). According to Mohammed *et al.* (2019), Agarwal *et al.* (2023), and Al Mārghitaş *et al.* (2010), white sorghum powder and syrup of nettle infusion have higher attractiveness, respectively. Findings of Haleem *et al.* (2015), El-Wahab *et al.* (2016) and Agarwal *et al.* (2023) support the attraction of yeast and skimmed milk powder to honey bees. The result of Ricigliano

et al. (2022) revealed that sugar-negative feed is less acceptable by honey bees than sugar-positive ones.

5.1.2. Effects of feeds on reproductive and productive performance of honeybees

Regarding unsealed brood (egg and larva) , results found in the current study (464.9 cm² 553.9 cm² which is a total of 1,018.8 cm²) (Table 8) which were lower than the findings of Sihag and Gupta (2013) who fed protein+ carbohydrate +vitamin and mineral and found a maximum of 7515 cm² of unsealed brood area. Irandoust and Ebadi (2013) reported an egg area of 18235 cm² during the supplementation of wheat gluten. The result of Gameda (2014) is also higher than the current findings. He stated that the mean amount of brood produced in the dearth period was 1274.3 cm² for colonies fed on pea flour. Hunde (2022) found a maximum area of 300 cm² of brood area which fed soya bean diets. On the opposite, Kumar and Agrawal (2014) composed of soy flour-1part, brewer's yeast-1part, soy protein hydrolysate 1part, sugar-1part, glucose-1part reported 76.2 cm² of unsealed brood area which was lower than the current finding. Kumari and Kumar (2020) reported a total area of unsealed brood (egg and larvae) which was 1121 cm² during offering of Bee-sup. Hunde (2022) found a maximum area of 300 cm² of brood area which fed soya bean diets.

The best result in T₄ in this study may be due to the presence of nettle infusion having the ability to increase brood surface area (Al Mārghitaş *et al.*, 2010). Yeast in T₂ also has high amount of protein levels and generally provides a more balanced set of amino acids and is also more attractive than soya bean flour which is confirmed by (Jach *et al.*, 2022). Proteineous supplemental diets have been shown to have a positive effect on the efficiency of queen egg laying (Szawarski *et al.*, 2019; Palmer-Young *et al.*, 2021). On the opposite, omega-3 polyunsaturated fatty acids (PUFAs) and the presence of leucine, isoleucine, and valine in white sorghum (Mohammed *et al.*, 2019; Pezzali *et al.*, 2020; Masresha and Belay, 2020; Pontieri *et al.*, 2022) and high amount of protein level in yeast (Jach *et al.*, 2022) caused to record higher larvae area in T₄ and T₂. This is consistent with Zheng *et al.*, (2014), who claimed that ten of the 21 amino acids the proteins' building blocks are necessary for the development of worker bees and the rapid growth of larvae.

According to Brodschneider and Crailsheim (2010), colony development is restricted by a lack of at least one of the essential amino acids in the food protein and the highest requirements are l-leucine, l-isoleucine, and l-valine. The importance of supplementing honey bees with essential amino acids was also noted by (Hendriksma *et al.* 2019). According to Shumkova and Balkanska, (2020), it is anticipated that foods like Apimix and Apipasta, which are high in vitamins and amino acids, will encourage bee colonies to produce more bee brood. For honey bees to lay eggs, improve brood rearing, increase yield, and avoid diseases and stress, food quality and availability are essential (Branchiccela *et al.*, 2019; Topal *et al.*, 2022; Timuroğlu *et al.*, 2022).

The pupae area found in the current study (627.1 cm²) (Table 8) was also smaller than that of Sihag and Gupta, (2013) who supplemented Protein +carbohydrate +vitamin and mineral and found a maximum pupae area of 7495 cm². Ahmad *et al.* (2021) who fed 45 g soybean flour + 15 g Brewer's yeast + 75 g powdered sugar + 7.5 g skimmed milk + 7.5 g date palm pollen + 200 mL sugar syrup supplement with Vitamin C) informed a maximum worker-sealed brood area of 2277.3 cm², and Khan *et al.* (2022) who offered sucrose solution indicated a maximum worker-sealed brood area of 2153 cm². Ullah *et al.* (2021) who provided the main component of soybean flour (traditional pollen substitute), sugar cane syrup resulted in the highest worker brood area (1489.27 cm²/colony) which is greater than the current study. Islam *et al.* (2020) offered 30 g soybean flour + 15 g Brewer's yeast+ 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D, and E vitamins + 150 ml sugar syrup discovered that the colony's maximum worker brood area of (1562.0 cm²), which is higher than the findings of the present study.

Kumari and Kumar (2020) also reported a maximum sealed brood area of 1938.3 cm² during supplementation of Bee-sup. However, the outcome of the current study was superior to Sena *et al.* (2012) who fed 3 kg of Feedbee/colony, reported 773.5 cm², and Kumar and Agrawal, (2014) who supplemented 16.7% defatted soya flour+16.7% brewery yeast+ 8.3% Parched Gram+8.3% Protein Hydrolysate+33.3% sugar+16.7% glucose resulted in 66 cm². Kumar *et al.* (2021) Soybean flour (60g) + Honey (35g) + Yeast (5g) + Vitamins (1 g/kg) provided diet stated an overall maximum brood development of (790.70 cm²) which was lower than the result found in the current study. Additionally, El-Wahab *et al.* (2016) supplemented 10 brewery yeast+ 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A, D and E vitamins + 45g powdered sugar + 20ml

orange juice + 10ml mint oil + 30ml sugar syrup) reported that the maximum sealed worker brood area of 285.8 cm². This is supported by research from Lamontagne-Drolet *et al.*, (2019), Islam *et al.* (2020) and Hossaini *et al.* (2020) who stated that honey bees increased the surface of sealed brood areas after consuming various supplements and pollen substitutes. According to Tawfik *et al.* (2020), colonies that received a supplemental diet had significantly more sealed brood area (258.33 inch²/colony) additionally, this outcome was higher than the current finding.

Supplemental diets increased the population density in comparison to the non-supplemented control group (Islam *et al.*, 2020; Moumeh *et al.*, 2020; Kumari and Kumar, 2020; Ahmad *et al.*, 2021; Sarioğlu-Bozkurt *et al.*, 2022; Topal *et al.*, 2022). Mahmood *et al.* (2013) fed Pollen a supplemental gram diet of 250 g per colony + brewer's yeast + sugar supplemental feeding and Irandoust and Ebadi (2013) through feeding of soya bean flour and wheat gluten and Dogra *et al.* (2023) who supplement Defatted soya flour = 150g; Wheat flour = 150g; Deactivated yeast = 100g; Sugar = 266g; Water = 134ml (Total = 800 g + 40 ml Rum) recorded a maximum total brood (sealed and unsealed) area of 4945 cm², 22680 cm² and 4945 cm² respectively which was highest result than the present study. Ahmad *et al.* (2021) through a supplement of 150 g (45 g soybean flour + 15 g Brewer's yeast + 75 g powdered sugar + 7.5 g skimmed milk + 7.5 g date palm pollen + 200 ml sugar syrup supplement with Vitamin C) got worker sealed brood area of 2277.3 cm². The variation of the current finding with these findings may be due to differences in feed type (nutritional composition of feed), feed intake, queen performance as well as initial colony strength.

Colony strength in the present study (6.4 frames/colony) showed a significant effect with experimental diets when compared to control colonies. Results in this study were lower than the findings of Sihag and Gupta (2013) who offered Protein +carbohydrate +vitamin and mineral and reported a maximum of 18 frames covered by honey bees per colony, Gameda (2014) who fed pea flour recorded a maximum result of 9.7 frames covered by bees per colony. Ahmad *et al.*, (2021) who supplement sucrose solution stated a maximum of 14.5 frames covered by bees and Hunde (2022) who provided soya bean diets a recorded of 9.4 frames covered by bees. Islam *et al.* (2020) fed 30 g soybean flour + 15 g Brewer's yeast+ 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup found the highest (10 bee frames/colony),

Similarly, Ullah *et al.* (2021) who offered main component of soybean flour (traditional pollen substitute), sugar cane syrup, reported a maximum of (8 bee frames/colony) which was also greater than the value recorded by the current study. Kumar and Agrawal (2014) who supplemented 16.7 % defatted soya flour+16.7% brewery yeast+ 8.3% rParched Gram+8.3% eProtein Hydroly-sate+33.3% sugar+16.7% glucose also reported a higher value than the present study which found a maximum of 13.7 frames covered by bees. Kumari and Kumar (2020) who offered Bee-sup reported 8.3 frames covered by bees. Nevertheless, El-Wahab *et al.* (2016) reported a maximum of 5.3 frames, Kumar *et al.* (2021) who fed Soybean flour (60g) + Honey (35g) + Yeast (5g) + Vitamins (1 g/kg) had a maximum strength of 5.8 frames of bees which were lower results when compared to the present study. Dogra *et al.* (2023) who provided various grain flour (3%) =150g; Skimmed milk (3%) =150g; Dried yeast (2% =100g; Honey (8%) = 400g reported a maximum frame of bees (6) which was almost confirm the present study.

The difference may be due to initial brood area, feed quality and quantity. El-Wahab *et al.* (2016), Branchiccela *et al.* (2019), Ahamad *et al.* (2020), and Eshbah *et al.* (2018) also stated giving a mixture of food with sugar syrup improves brood and adult colonies of honey bees and colony strength has correlation with brood amount in the hive. Similarly, Topal *et al.* (2022) indicated that most protein supplementations affect the health and longevity of honey bee colonies. Hoover *et al.* (2022) Islam *et al.* (2020), Ahamad *et al.* (2020), and Ahmad, *et al.* (2021) revealed that supplemental diets increased the population density in comparison to the non-supplemented control group. The existence of a complex network between nutrition, gut microbiota, immunity, and pathogen infection was stated by (Castelli *et al.*, 2020; Hesham *et al.*, 2022; Mortazavi *et al.*, 2020). Hristov *et al.* (2020) stated adult worker bees' metabolic health is influenced by the nutritional quality of their larval diets which are better for survival and have higher metabolic rates per unit of body mass.

Supplemental feeds had no statistically significant impact on the number of queen cells constructed in the current study. After the experiment, colonies that had received T₄ and T₂ recorded a maximum number of queen cells of 0.1 and 0.2 respectively which was a lower value than (El-Wahab *et al.*, 2016) who reported a maximum mean number of 0.83. This different findings may be due to feed quality and colony strength differences. This supported research from (Boes, 2010 and Stürup *et al.*, 2013) that suggested a colony might modify queen production depending on the

season (day length and temperature), as well as other environmental cues like colony size and food availability. The nutrition of honey bee larvae influences the development of new queens favorably (Czekońska *et al.*, 2015). Protein-rich supplemental diets have an advantageous effect on the production cells of queens (Haleem *et al.*, 2015; Szawarski *et al.*, 2019; Estegamat and Gholami, 2010; Cengiz *et al.*, 2019). The quality of royal jelly, which is secreted by the hypo pharyngeal glands, depends on the source of honey bees' food and the time of year (Sherif *et al.*, 2018; Hu *et al.*, 2019; Shakeel *et al.*, 2020). For instance, the amount and structure of essential Royal Jelly components like amino acids, carbohydrates, and vitamins are significantly altered when bees are fed sugar syrup (Shi *et al.*, 2018; Khan and Ghramh, 2022).

Nectar area was maximal in colonies fed T₄ (54.3 cm²) (Table 8). Results in this study were lower than the findings of Hunde (2022) who supplemented diet soya beans reported a nectar area of 258.9 cm². Kumar *et al.* (2017) who indicated nutrition is one factor that affects the amount of nectar present in the hive in honey bee colonies. Chaand *et al.* (2017) stated colony strength affects Pollen, nectar, and honey area. Sihag and Kaur (2018) also identified nectar stores are positive with brood and colony strength. The nectar area has decreased to some extent from the initial present study. This is confirmed by Huang (2012) and Amro *et al.* (2016) documented that honey bee usually prefers more natural pollen and nectar as compared to supplementations and substitute diets.

The honey area in the present study was also higher in colonies that received T₄ (311.2 cm²) (Table 8) which was lower than the following findings. Sihag and Gupta (2013) who supplement Protein+carbohydrate+vitamin and minerals reported a honey area of 9800 cm², Kumari and Kumar (2020) who supplement Bee-sup also documented a 1963.5 cm² honey area. Mahmood *et al.* (2013) who fed the Pollen supplemental gram diet 250 g per colony + brewers yeast + sugar supplemental also reported a maximum honey area of 3820 cm². Dogra *et al.*, (2023) who supplemented Defatted soya flour = 150g; Wheat flour = 150g; Deactivated yeast = 100g; Sugar = 266g; Water = 134ml (Total = 800 g + 40 ml Rum) documented 602.2 cm² of honey area.

But Kumar and Agrawal (2014) who fed 16.7% defatted soya flour+16.7% brewery yeast+ 8.3% Parched Gram+8.3% Protein Hydrolysate+33.3% sugar+16.7% glucose reported 59.7cm² which was lower than the result recorded in the current study. The difference may be due to differences in

brood area, colony strength, feed quality and intake. These highly performed colonies also recorded higher feed intake with higher nutritional value. Also, maximal brood area, colony strength, and higher nectar were observed in these colonies. These parameters have positively correlated with the honey area. This is similar to the findings of Chaand *et al.* (2017) and Sihag and Kaur (2018) who stated colony strength affects Pollen, nectar, and honey area. Pollen supplements positively affect brood area, colony strength, and honey store (Kumsa and Takele, 2014).

The pollen area was affected by experimental diets in the current study. The finding in the current study was (68.7 cm²) which was lower than the findings of Sihag and Gupta (2013), who supplemented Protein+carbohydrate+vitamin and minerals and recorded a maximum area of 252 cm², Mahmood *et al.* (2013) who supplemented Pollen supplemental gram diet 250 g per colony + brewer's yeast + sugar found 127.9 cm² area of pollen. Irandoust and Ebadi (2013) who offered wheat gluten, reported 143.5 cm², and Hunde (2022), which fed soya bean diets recorded a pollen area of 219.9 cm². Colonies that received T₄ and T₂ in the present study collected more pollen compared with others. Control colonies preserved lower pollen than other treatment colonies. This result was related to their feed intake and brood area amount. Sihag and Gupta (2013) and El-Wahab *et al.* (2016) stated that there are very high and significant correlations between unsealed brood/sealed brood and pollen in all the colonies receiving pollen substitute diets. Colony strength affects pollen, nectar, and honey area (Divya *et al.*, 2017).

Pollen stores have a positive correlation with brood and colony strength (Sihag and Kaur, 2018), 2018). Mahfouz (2016) and Khan *et al.* (2022) stated that pollen supplementation improves the storing of foods in the hive during a dearth period. Pollen area has decreased to some extent in present studies from initial. This confirms the finding of Huang (2012) and Amro *et al.* (2016) who stated that honey bee usually prefers more natural pollen and nectar as compared to supplementations and substitute diets. The nutritional quality of larval diets impacts the metabolic functioning of adult worker bees, with diets more optimal for survival resulting in a higher metabolic rate per unit of body mass. As foraging bees already experience extremely high metabolic demands, differences in the quality of larval nutrition could impact the metabolic function which may negatively influence the foraging efficiency of workers. Subsequently, this could impact the build-up of pollen and nectar stores available for brood rearing and overwintering,

with consequences for overall colony success (Nicholls *et al.*, 2021; Kumar *et al.*, 2021; Hunde, 2022).

Oskay (2021) stated that supplemental feeding is generally used by beekeepers to sustain colony maintenance strategy to promote their honey bee colonies when nectar and pollen sources are scarce in nature or when agricultural plants are cultivated. Tesfaye (2019) indicated that giving pollen supplementary feeding at the time of the dearth period maintains the strength of the bee colony within the hive and attains to the next natural pollen and nectar flow season. Also, Chaabane (2019) revealed that the key factor in ensuring bee health is monitoring the nutritional status of the colony and saving bee population declines from nutritional shortages caused by several factors.

Honey yield was maximal colonies received T₄ (7 kg) which was higher than the finding of El-Wahab *et al.* (2016), who supplemented 10 brewery yeast+ 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A, D, and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup) reported 3.7kg. On the other hand, Irandoust and Ebadi (2013) reported 8.9 Kg during the offering of soya bean flour. Sihag and Gupta (2013), 11.3 kg, Tolera (2014) reported 26.8 kg for colonies fed on pea flour, Islam *et al.* (2020) offered 30 g soybean flour + 15 g Brewer's yeast+ 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup reported honey yield of 7.5 kg.

Ahmad *et al.* (2021) who fed sucrose solution documented a maximum honey yield of 13 kg, Ullah *et al.*, (2021), who provided the main component of soybean flour (traditional pollen substitute), sugar cane syrup recorded 8.7 kg, Hunde (2022) reported 11.5 kg of honey which fed soya bean diets and those the above-mentioned results were higher than the current study. This result was positively related to their brood area, nectar and honey stored, and colony strength. This was similar to the findings of (Sihag and Gupta, 2013 and El-Wahab *et al.*, 2016). Similarly, very high and significant correlations existed between colony strength and unsealed brood/sealed brood and pollen and honey stores in all the colonies receiving pollen substitute diets stated (El-Wahab *et al.*, 2016; Shehata, 2016; Saini *et al.*, 2018; Tawfik *et al.*, 2020; Islam *et al.*, 2020).

Additionally, honey yield also increased significantly in those bee colonies that fed on supplemental diets than the control colonies that fed only sugar syrup. Strong bee colonies rear

more brood and produce more honey than weak colonies (Mladenović and Radoš, 2010; AL-Kahtani, 2013; Gabka, 2014). The higher nutritional composition and intake in these colonies in the present study results in higher honey yield recorded. This was in line with (Ricigliano *et al.*, 2022) documented that the sum of dietary essential amino acid deficiencies relative to leucine content negatively affected honey bee colony performance and as a result their honey yield performance. (Crone and Grozinger, 2021) stated that in honeybees (*Apis mellifera*) there is growing evidence that the impact of multiple stressors can be mitigated by quality nutrition. Pollen which is the primary source of protein and lipids in bee diets is particularly critical for generating more resilient phenotypes. Nichols and Ricigliano (2022) stated incorporating algae feeds and supplements into existing beekeeping practices has the potential to sustainably improve honey bee colony productivity and health. Hashm *et al.* (2021) presented that honey yield variation is caused by a variety of stressors that affect the immune system of bees, such as pathogens, insecticides, and inadequate diets as well as the bee itself.

5.1.3. Profitability of experimental feeds

In the present study, higher net profit and colony strength were recorded in T₄ and T₂ (Table 9). Numerically T₄ was higher than T₂ but had no statistical difference. This was due to the higher attraction, nutritional value, and palatability of feed presented in these treatments. Saffari *et al.* (2010) reported that the pollen substitute artificial diet must contain the required ingredients, texture, and consistency that are attractive and palatable to honeybees. It must have nutritional values and be free from anti-nutritional factors. Sihag and Gupta (2011) also documented that it must be easy to prepare and economically viable for beekeepers. Therefore, a cheap/low-cost and acceptable pollen substitute diet is one of the prime needs of beekeepers to sustain beekeeping.

5.2. On-Farm Performance Evaluation of Local Honeybees in Different Agro-Ecologies of South Wollo Zone

5.2.1. Reproductive and productive performance evaluation of local honeybees on their agroecology

The current study's maximum brood area was 1343.8 cm², 1407.1 cm², and 1471.8 cm² for eggs, larvae, and pupae, respectively (Table 10). When compared with the results of Alemu *et al.* (2014)

findings, which evaluated colonies of *A.m. Scutellata* and reported a total brood area of 216 cm², Mossie and Biratu (2019) findings for *A.m. bandansii* reported a total brood area of 252.5 cm². As well as this, Negash and Argaw (2022) reported 148 cm² for *A.m. scutellata*, Aleme *et al.* (2017) reported an unsealed brood area of 127cm² and a sealed brood area of 94 cm² for *A.m. wayi Gambela*. Tarekegn *et al.* (2022) reported unsealed (54 cm²) and sealed (127cm²) for *A.m. scutellata*. All of the aforementioned findings were lower than the outcome of the current study.

However findings of Hunde and Hora (2022) on *A.m. bandansii* found a brood area of 6114.1cm² and Merssa *et al.* (2016) on *A.m. monticola* found an unsealed brood area of 20.4 cm² and sealed 2546.1cm² were higher than the result of the current study even though, the unsealed brood area lower than the current. The reasons for differences for these findings may be due to difference in races, temperature and flora availability. The maximum brood raising was seen during the active season, which could be due to the region's abundance of honeybee flora, which ranges from weeds to forest trees and provides enough nutrition for bees during this month. *Apis mellifera* bees in tropical areas raise brood all year but lower the number reared during the rainy season (Tarekegn *et al.*, 2022).

Weather parameters play a major role in influencing brood rearing, pollen area, nectar area, bee strength, and honey production as well as the activity of pollen foragers (Manoj *et al.*, 2017). The bee's activity and production rate are determined by the temperature conditions in its surroundings (Nazlı *et al.*, 2023). One of the most significant elements influencing beekeeping and honeybees is temperature. Determining the ideal temperature is one of the most crucial steps toward making beekeeping and its products economically viable (Filmon, 2023; Krystyna *et al.*, 2023; Rafael *et al.*, 2023). Colony performance was impacted by the beehive type and honey bee race (Abou-Shaara *et al.*, 2013).

According to Alqarni *et al.* (2014), pollen harvesting was impacted by solar radiation, but brood-rearing activities were connected with monthly pollen storage. Based on climatic conditions, vegetation cover, altitude, and other abiotic and biotic factors, honey bee races are dispersed throughout the nation for each region's agro-ecologies (Aleme and Nwankwo, 2021). According to Shumkova and Balkanska (2020), it is anticipated that foods like Apimix and Apipasta, which are high in vitamins and amino acids, will encourage bee colonies to produce more bee larvae. The amount of harvested and transmitted bee pollen in the hives increases as a result, strengthening the

bee colonies. Pollen contains nutrients such as proteins, minerals, vitamins, and lipids that are crucial for adult longevity, brood development, and colony growth (Abou-Shaara, 2014; Arien *et al.*, 2015; Aleme *et al.*, 2017; Topal *et al.*, 2022; Negash and Argaw, 2022).

The current study's measurements of pollen, nectar, and honey area were strongly influenced by the agro-ecology of the study's area. The current study measured amounts of pollen, nectar, and honey of 15.3 cm², 343.2 cm², and 386.1 cm² respectively. It showed a lower value of pollen and higher value of nectar and honey than results reported by Alemu *et al.* (2014) showed 1.2 cm² and 2.6 cm² of pollen and nectar respectively for *A.m.scutellata* and Aleme *et al.* (2017) reported 2.5cm², 1.3 cm² and 5.8 cm² of pollen nectar and honey area for *A.m. wayi gambela* respectively as well as Negash and Argaw (2022) who found 3.9 cm² and 5.3 cm² of pollen and nectar for *A.m.scutellata* respectively. In addition, the result of Merssa *et al.* (2016) for *A.m. monticola* was 1 cm² and 10.6 cm² of pollen and nectar respectively which was also lower than the the current study. In comparison to the present study, Tarekegn *et al.* (2022) reported that the pollen area (2 cm²) and nectar area (1.4) of *A.m. scutellata* were lower than the present finding. However, Hunde and Hora (2022) results from which reported 19.48 cm² and 136 cm² of pollen and nectar for *A.m. bandansii*, respectively, were higher in pollen but not nectar.

Foraging activity by honey bee colonies depends on gathering pollen, nectar, honey, and its yield (Hristov *et al.* 2020). The main tendency of nectar and pollen storage activity does coincide with the pattern of brood rearing of the investigated colonies in both agro-ecologies, it was further validated. The distance of honeybee forages, colony size, food availability, month, and time of day may all play a role in the difference between the active and dearth seasons of foraging activity. According to Topal *et al.* (2022), weather factors play a significant impact in regulating pollen foraging activity, pollen area, nectar area, bee strength, and honey output. Adult worker bees' metabolic processes are influenced by the nutritional quality of their larval meals, with diets that are better suited for survival having a greater metabolic rate per unit of body mass (Crone and Grozinger, 2021).

Like all living things, honeybees require a balanced and sufficient diet. Reduced immunity, more stress, a shorter life span, and colony loss can all result from malnutrition. For brooding behaviors to continue and for the colony's young population to grow, pollen is required (Divyaet *et al.*, 2017; Hoover *et al.*, 2022). According to Sihag and Kaur (2018), there is mounting proof that high-

quality nutrition can reduce the negative effects of several stresses on honey bees (*Apis mellifera*). For the development of more resilient phenotypes, pollen, the main source of protein and fats in bee diets, is particularly important. Beehive type and honey bee race have an impact on colony performance (Abou-Shaara *et al.*, 2013).

According to Manoj *et al.* (2017), diet is one element that influences how much nectar is present in honey bee colonies' hives. According to El-Wahab (2016) and Gabka (2014), colony strength has an impact on the pollen, nectar, and honey areas. Pollen and nectar stores were also identified by Shehata (2016), Saini *et al.* (2018), and Tawfik *et al.* (2020) as being positively correlated with brood and colony strength. The principal honey harvesting season of the year, which runs from October to November, saw larger honey yields than usual.

This could be attributed to the abundance of food supplies, which may have a significant impact on the health and productivity of honeybee colonies. The mean honey output (kg) per harvest per hive for this race (*A. m. monticola*) was higher than the race found in the highland area. The higher honey yield was recorded during the primary honey harvesting season of the year from October to December and this might be due to the abundant availability of forage sources that could profoundly impact honeybee colony strength and productivity. The maximum honey yield of the present study was 22.2 kg which was higher than the range of the national level (19.1kg). For the *A.m bandansii*, Mossie and Biratu (2019), reported a maximum honey yield of 12.33 kg.

In addition, Zewudu *et al.* (2012) for *A.m. Bandansii* found 12.5 kg, Hunde and Hora (2022) found 11.8 kg for *A.m. scutellata*, and Merssa *et al.* (2016) reported 18.2 kg for *A.m. monticola*, all of which were lower results than the current finding. The honey productivity of bee colonies depends on the type of honeybee race, agro-ecology, availability of pollen and nectar, health status of the colonies, and strength of the colonies (Mossie and Biratu, 2019; Islam *et al.*, 2020; Tawfik *et al.*, 2020). According to Büchler *et al.* (2013) and Delaplane *et al.* (2013), all of the colonies' unsealed/sealed brood, pollen, and honey supplies, as well as colony strength, environmental conditions, and beekeeping management practices were highly and significantly correlated.

The greatest reproductive swarming season for *A.m. monticola* occurred between October-December. This is likely because these months coincided with periods of peak plant blossoming, which provided a surplus of bee forages. These months represent the honeybees' active season in

the research area. Due to repeated queen cell growth and advanced markers of swarm preparation, the race produced more queen caps per hive in October, demonstrating a strong desire to swarm. These findings are consistent with earlier studies that found tropical honeybees typically have a high tendency to reproduce by swarming and a propensity to rapidly increase their population, which leads to the rapid multiplication of colonies (Hunde and Hora, 2022; Tarekegn *et al.*, 2022). A study based on months also indicates that 60% of *A. m. monticola* colonies' peak swarming period is in October-December (Merssa *et al.*, 2016). The variation among these reports might be due to the agro-ecological difference in the study areas. The expression of these behavioral traits can be strongly influenced by environmental factors and beekeeping management techniques (Büchler *et al.*, 2013; Delaplane *et al.*, 2013).

The swarming tendency in the current study was not affected by agro-ecology. And recorded a maximum of (4.4) queen cells. This result was higher than the findings of Tarekegn *et al.* (2022) who reported 3.42 for *A.m. scutellata*, Hunde and Hora (2022) who reported 2.2 for *A.m. bandansii*, Alemu *et al.* (2014) who reported 2 for *A.m. scutellata* and Aleksander *et al.* (2015) who found 2.44. However, it was lower than the findings reported by Merssa *et al.* (2016) which was (30) on *A. m. monticola*, and by Negash and Argaw (2022) who found (5.2) for *A. m. Scutllata*.

5.2.2. Foraging, defensiveness, absconding rate, and disease assessment of local honeybees

The honey bees found in our study area were early foragers in both seasons (EFT 5:13 am and LFT 7:04 pm) (Table 11). Joshi and Joshi (2010) discovered that honey bee workers started foraging activity at 6.17 am. Additionally, compared to *A.m. scutellata*, *A.m. monticola* exhibits a longer cessation time. According to Solomon and Likawent (2015) research report, honey bee races (*A. m. monticola*) assigned to different agro ecologies foraged as early as 5:25 am and returned as late as 7:04 pm. This behavior showed high competence in natural resources. For EFT and LFT, Tarekegn *et al.* (2022) recorded for *A.m.scutellata* at 6:10 a.m. and 6:29 p.m. Aleme *et al.* (2017) reported EFT and LFT for *A.m. woyi gambela* at 6:25 am and 6:25 pm. Additionally, according to Mossie and Biratu (2019) and Merssa *et al.* (2016) for *A.m. bandansii* and *A.m. monticola* reported times of 6:25 am, 6:40 pm, and 5:55 am, 7:04 pm, respectively. The LFT of

colonies discovered in the current study was 7:04 pm, which was also the LFT of colonies found in Merssa *et al.* (2016) and Solomon and Likawent, (2015).

Numerous researchers have noted variations in foraging timing in response to various geographic and environmental conditions. According to Alqarni *et al.* (2014), temperature has the greatest impact on colony activity and bee vitality. The kind and number of floral resources in the area affect foraging behavior. According to the flowering times of the available resources, honeybees adjust their foraging behavior (Mossie and Biratu, 2019). Adult worker bees' metabolic processes are influenced by the nutritional quality of their larval diets; diets that are better for survival result in higher metabolic rates per unit of body mass. Differences in the quality of larval nutrition could have an impact on metabolic function, which could have a negative impact on the foraging efficiency of workers as foraging bees already experience extremely high metabolic demands (Nichols and Ricigliano, 2022).

The honey bee (*Apis mellifera*) colony adapts its foraging behavior right away to meet its sudden need, and a negative or positive feedback mechanism regulates the necessary parameter when the colony's internal parameters are changed (Sihag and Kaur, 2018). It is useful to be aware of when the bees are most aggressive and when they are least aggressive. The findings suggest that the *A.m.monticola* race was less aggressive, which suggests that the race was unable to establish robust colonies throughout the active season, leading to good hygienic behavior and high-quality honey production. Another benefit of the race's extremely aggressive behavior is that it makes for a strong defense against honeybee pests. In fact, rather than being a genetic trait of the race, the aggressiveness of the race may be attributed to better apiary management, improved bee forage, and the carrying capacity being optimized in a study area.

The temperament (defensiveness) of colonies found in the current study was affected by the agro ecology of the study area. The maximum time (second) for the first sting was 89.4, the time taken for mass aggressiveness was 259.3 sec., the total number of stings on the ball was 21.7, the total number of stings on the glove was 17, and the total distance followed was 192.5 meters. Compared to the current study Valter *et al.* (2014) found that the average observer's following distance ranged from 23.33 meters to 216.6 meters. Merssa *et al.* (2016) reported the total time for the first sting, 89.7 sec., total time for mass aggressiveness, 260 sec., the number of stings on the glove, 16.6, the

number of stings on the leather ball, 22.3 and total distance followed by 188.4 m for *A.m. monticola* which confirmed the result of the present finding.

A.m. bandansii, which was more aggressive than colonies found in the current study area, was reported by Mossie and Biratu (2019) to take 3.9 seconds for the first sting, 152 seconds for mass aggressiveness, 282 stings on the ball, and 283.2 meters to follow. In addition, Tarekegn *et al.*, (2022) reported that *A. m. scutellata*, which is also very aggressive, stung 400 meters away and took 40 seconds to do so, as well as 281 stings on the glove and 29 stings on the leather ball. For *A.m. Wayi Gambela*, Aleme *et al.* (2017) also noted a total of 80 stings on the ball and the total distance followed was 115 m. According to Alemu *et al.* (2014), environmental factors, genetics, colony strength, large honey and pollen stores, and the volume of alarm signaling all contribute to the variation in honeybee aggression. Based on climatic conditions, vegetation cover, altitude, and other abiotic and biotic factors, honey bee races are dispersed throughout the nation for each region's agroecologies (Aleme and Nwankwo, 2021).

The highest absconding rate ever found in this study was 4.3%. The outcome was less favorable than those reported by Negash and Argaw (2022), who reported 63.3% for *A.m. scutellata*, Hunde and Hora (2022), 54% for *A.m. bandansii*, Alemu *et al.* (2014), 27% for *A.m. scutellata*, Aleme *et al.* (2017)), 26% for *A.m. scutellata*, Tarekegn *et al.* (2022), 30% for *A.m. scutellata*, Merssa *et al.* (2016), 37.5% for *A.m. monticola*. Absconding is the act of a colony leaving its nest and dispersing into a swarm to establish itself somewhere else. According to the study, fewer people fled the tested colonies. This might be brought on by less frequent disturbances, pest attacks, a lack of bee forage during the rainy season that might be brought on by scarcity, or pesticide use in the area for crop production (Dubale, 2017). High absconding was also seen during the transfer of the colony from the traditional hive, which may have been caused by inadequate internal feeding and the lack of a queen cage (Tesfaye, 2019). The main predators and pests that started bee colony absconding were various. The main causes were the disease, the seasonality of bee flora, and environmental changes (Shitaneh *et al.*, 2022).

Similar diseases, predators, and pests were found in east and west Gojjam according to (Ayele *et al.*, 2020). Additionally, Tsegaye (2015) and Jatema and Abebe (2015) reported on it from the

eastern regions of the Amhara Region, Gomma Woreda, and the Walmara district, respectively. The top three honeybee pests, according to studies by (Adeday *et al.*, 2012; Godifey, 2015; Haftu *et al.*, 2015; Tsegaye, 2015), are ants, wax moths, and bee-eater birds. Many factors have been identified as contributing to the decline of honeybee colonies, such as pesticides, diseases, pathogens, commercial pollination methods, and environmental change (Asrat *et al.*, 2023; Khushdeep *et al.*, 2023; Nicole *et al.*, 2023). A dangerous external parasite called Varroa is responsible for a number of problems for beekeepers, including declining adult numbers, stunted growth in young bees, erratic brood patterns, growth of disease-causing pathogens, adult bee departure from the hive, low honey productivity, and colony losses at an advanced stage (Yashdeep *et al.*, 2023).

5.3. Evaluation of Honey Quality

In general, the chemical composition of honey depends on several factors such as plant source (botanical origin of the nectar) visited by bees, season, environmental and climatic conditions, production methods, storage conditions, etc (Yang *et al.*, 2017; Weldegebriel *et al.*, 2018; Lazareva *et al.*, 2023; Nouri, 2023; Pop *et al.*, 2023) but the main constituents are more or less identical in all honey types.

The moisture content of sampled honey in the present study was 17.59 %, 17.27 %, and 17.43 %, and ranges of 12-20 % for season 1, and season 2, over all mean and ranges respectively (Table 16 and 14). This indicates it was under the national and international standards (≤ 21 % for CAC (Codex Alimentaris Commission, 2001), EU (European Union, 2002), and QSAE (Quality Standard Authority of Ethiopia, 2005). Honey collected in Ethiopia's southwest forest had a moisture content of 18.3-21.2 % (Addi and Bareke, 2021). Akalework *et al.* (2020) reported that honey produced in Gedebano Gutazer Wolene, Central Ethiopia, had a mean moisture content of 18.98 %.

According to research by (Gela *et al.*, 2023), the moisture content of the honey from the Jimma, Bale, Kefa, and North Shewa zones was 21.9 %. According to Alemayehu *et al.* (2021), honey produced in the Arba Minch Zuria district of the Gamo zone in Southern Ethiopia has a mean moisture content of 19.27 %. According to Berhe *et al.* (2018) and Teshome *et al.* (2020), the mean moisture content of honey collected from Godere Woreda, Gambella, Ethiopia, as well as

selected districts of Bale Zone, Oromia Regional State, Ethiopia, was 18.76 %, and 20.34 %, respectively. According to Tesfaye (2016), honey produced in Southeast Ethiopia's Bale Natural Forest had 18.80 % moisture content.

A range of 19.33-22.03 % was reported by Getachew (2020) for honey that was collected from specific districts in the Bench Shecko zone, located in southwest Ethiopia. According to (Tigistu *et al.*, 2021), honey from the Doyogena and Kachabira Districts in the Kembata Tambaro zone of Southern Ethiopia had a mean moisture content of 18.83 %. The average moisture content determined by the current study was lower than all of the previously mentioned findings.

Conversely, comparable findings to the present study were reported by Kebede *et al.* (2017), Equar *et al.* (2015), and Degaga (2017) for honey produced in the Tigray region of Ethiopia, the Siltie Woreda, the Siltie zone, SNNP, Debre Nazret Kebele, and Ethiopian monofloral honey, respectively, with moisture contents of 14.61-19.54 %, 17.23, and 14.4-20.54 %. Fiseha *et al.* (2021) reported that the honey collected from the Chena District in Southwest Ethiopia had moisture content ranges of 16.5-20 %. Gobessa *et al.* (2012) reported that honey produced in the Homesha district of western Ethiopia had a mean moisture content of 16.4 %. According to Abera and Alemu (2023) and Teklit and Freihwot (2016), the moisture content of honey produced in northeastern Ethiopia, and honey collected from the wild in the eastern zone areas of Tigray Ethiopia was found to be 11.87-16.7 %, and 16.00 %, respectively.

Also, the results of the current study were superior to all of the previously mentioned results. According to Nega *et al.* (2020), the honey produced in Ethiopia's Amhara Region's East Gojjam Zone had a moisture content ranging from 17.5 to 18.19 %. The lower limit of this range was maximum, and the upper limit was minimum when compared to the current study. Furthermore, Mulugeta *et al.* (2017) found that when compared to the current study, the range of honey produced in the West Shewa zone, Oromia region, was 16.61–18.54 %, with a maximum by its lower limit and a minimum by its upper limit.

The kinds of nectar that the honeybees consumed, the soil and climate of the area, and the methods used to handle honey after harvest all affect the physicochemical characteristics of a particular honey. The degree of honey maturity, the honey sample's botanical origin, the methods used for harvesting, and the extraction of honey from the comb to the bees' ripening process are all directly

related to the water content (Berhe *et al.*, 2018; Nega *et al.*, 2020; Nascimento *et al.*, 2022; Gebeyehu and Jalata, 2023) .

Ash content obtained in the current study was 0.13 %, 0.07 %, and 0.09 %, and 0.01-0.55 % for season 1 season 2, over all mean and ranges and which fulfills the national and international quality standards (≤ 0.6 for CAC, EU and QSAE). Akalework *et al.* (2020) reported that sampled honey from Gedebano Gutazer Wolene, Central Ethiopia, contained 0.2 % ash. Gela *et al.* (2023) stated that honey produced in the Jimma, Bale, Kefa, and North Shewa zones had a mean ash content of 0.42%. Alemayehu *et al.* (2021) found that the honey from the Arba Minch Zuria district in the Gamo zone of Southern Ethiopia had an ash content of 0.23 %. According to Berhe *et al.* (2018), honey produced in Godere Woreda, Gambella, Ethiopia, has an average ash content of 0.34 %. Teshome *et al.* (2020), reported mean ash content of 0.29 %, for honey harvested from selected Districts of Bale Zone, Oromia Regional State, Ethiopia.

According to Tigistu *et al.* (2021), the honey from the Doyogena and Kachabira Districts in the Kembata Tambaro zone of Southern Ethiopia has an ash content of 0.25 %. Kebede *et al.* (2017) and Mulugeta *et al.* (2017) reported that the ash content ranged from 0.12-1.67 % and 0.38-0.64 % for honey produced in Siltie Woreda, siltie zone, SNNP, Ethiopia and West Shewa zone, Oromia region Ethiopia. According to Teklit and Firehiwet (2016), the ash content of sampled honey that was collected from the wild in Tigray, Ethiopia's eastern zone was 0.47 %. And all of the aforementioned results exceeded the current study's findings.

Conversely, Nega *et al.* (2020) reported on the range of 0.09-0.26 % ash content of sampled honey collected from the East Gojjam Zone of Amhara Region, Ethiopia which is higher by its lower limit but lower by its upper limit than the current study. Fiseha *et al.* (2021), Getachew (2020), Adgaba *et al.* (2020), and Abera and Alemu (2023) reported arrange of ash contents 0.08-0.3 %, 0.17-0.2 % 0.1, 6-0.48 and 0.12- 0.49 % for Chena District, Southwestern Ethiopia, Selected districts of Bench Shecko zone, southwestern Ethiopia, Northern, central, southern highland and southwestern Ethiopia and northeastern Ethiopia respectively and which were higher by their lower limit but lower by their upper limit than the current study.

Weldegebriel *et al.* (2018) found that the ash contents of honey found in supermarkets in Addis Ababa, ranged from 0.17 to 0.46 %. These values were higher by their lower limit but lower by their upper limit. Furthermore, ash content ranges 0.04-0.41 %, and 0.20-0.39 % were reported by Mulualem and Teklemedhin (2018), and Degaga (2017) for honey collected from Selected Districts of Arsi zone, Oromia, Ethiopia, and Ethiopian monofloral honey, respectively. It was anticipated that the varying soil types, the concentration of minerals in the nectar of various apiaries, and the diverse botanical origins of the honey samples would result in variations in ash content across locations (Pauliuc *et al.*, 2020). Research showing that factors such as harvesting, beekeeping practices, processing, handling, and the material bees gather while foraging for flowers could all affect how much ash is in honey Akinwande and Oladapo, 2022; Thakur *et al.*, 2022). However, nowadays, the measurement of electrical conductivity is often used instead of ash content. High ash content can make sugars more difficult for honeybees to process, potentially leading to dysentery and even death for the bees (Anna *et al.*, 2020). If you're interested in honey quality, you might want to explore the European Honey Directive and the Codex Alimentarius Standard for Honey, both of which are currently under revision (Vincent *et al.*, 2016)

According to the present study, the free acidity content of sampled honey was 17.53 meq/kg, 20.75 meq/kg and 19.14 meq/kg, and 10-45 meq/kg for season 1 season 2, range and overall mean and it also under the national and international quality standards (≤ 50 for CAC and ≤ 40 for EU and QSAE) even though only 2 samples resulted above 40 (44 and 45). According to studies by Gela *et al.* (2023), Alemayehu *et al.* (2021), and Berhe *et al.* (2018) honey collected from Jimma, Bale, Kefa, and North Shewa zone, Ethiopia, Arba Minch Zuria district of Gamo zone, Southern Ethiopia, and Godere Woreda, Gambella, Ethiopia, yielded results of 26.8 meq/kg, 34.04 meq/kg, and 24.48 meq/kg, respectively.

Nega *et al.* (2020) found FA of a range between 35.3–46.6 meq/kg for honey produced in the East Gojjam Zone in the Amhara Region of Ethiopia. The means of FA were also reported by Teshome *et al.* (2020), Tesfaye (2016), Fiseha *et al.* (2021), and Getachew (2020) which were 57.66 meq/kg, 32.43 meq/kg, 21.41 meq/kg, and 58.33 meq/kg for honey produced in selected Districts of Bale Zone, Oromia Regional State, Ethiopia, Bale Natural Forest, Southeastern Ethiopia, Chena District, Southwestern Ethiopia and Selected districts of Bench Shecko zone, southwestern

Ethiopia respectively. Degaga (2017) reported a higher 20–55 meq/kg of FA in Ethiopian monofloral honey. Furthermore, every result listed above was greater than the study's findings.

Conversely, Akalework *et al.*, (2020) reported a mean FA of 16.3 meq/kg for honey produced in Gedebano Gutazer Wolene, while Addi and Bareke (2021) documented a range between 7-10 meq/kg for honey from Ethiopia's southwest forest. Tesema Areda (2015) reported 16.52 meq/kg of honey that was gathered in Ethiopia's Guji Zone. According to (Tigistu *et al.*, 2021), honey produced in the Doyogena and Kachabira Districts of the Kembata Tambaro zone in Southern Ethiopia had a mean FA of 13.39 meq/kg. Mulugeta *et al.* (2017) reported that honey produced in Ethiopia's West Shewa zone and Oromiya region had an FA arrangement ranging from 7.42 to 13.8 meq/kg. Furthermore, all of the results previously mentioned were lower than the current study's outcome.

Furthermore, for honey produced in Northern, Central, Southern Highland, Southwest Ethiopia, and Northeastern Ethiopia, by Adgaba *et al.* (2017) and Abera and Alemu (2023) found a range of FA 12.4-38.7 meq/kg and 10.55-35.57 meq/kg, respectively and which were higher by their lower limit but lower by their higher limit. For honey produced in Adigrat and the surrounding area. Kebede *et al.* (2017) discovered that the honey sampled in Siltie Woreda, Siltie zone, SNNP, had an FA range of 12.41-33.67 meq/kg, which was higher by its lower limit but lower by its upper limit. Equar *et al.* (2015) discovered that the honey sampled from Debre Nazret kebele in the Tigray region of Ethiopia had an FA between 17.33 and 32.7 meq/kg, which is higher than the honey's lower limit but lower by its upper limit when compared to the current study. These difference may due to soil type, nectar source and bee type according to (Machado De Melo *et al.*, 2018 ; Akalework *et al.*, 2020; Gilbert *et al.*, 2021; Tigistu *et al.*, 2021; Gebeyehu and Jalata, 2023)

Free acidity is an essential parameter related to honey composition. It reflects the presence of organic acids (such as gluconic acid) and inorganic ions (like phosphates, sulfates, and chlorides). Higher values may suggest fermentation of sugars into organic acids. If you encounter honey with elevated free acidity, it's essential to assess its quality and consider potential impacts on taste and safety. For instance, higher acidity may contribute to the production of hydroxymethylfurfural (HMF), a compound that forms during honey storage (Srinivasan and Vasudeva, 2014)

The present study resulted in HMF of 1.74 mg/kg, 9.25 mg/kg, 0-37.8 mg/kg, and 5.49 mg/kg for season 1 and season 2, range and overall mean respectively which falls under the national and international level (≤ 60 for CAC and ≤ 40 for EU and QSAE). According to Getu and Birhan (2014), honey produced in and around Gondar, Amhara, Ethiopia, and contained 6.32 mg/kg of HMF. Gela *et al.* (2023), Alemayehu *et al.* (2021) and Berhe *et al.* (2018), reported mean HMF of 14.8 mg/kg, 13.09 mg/kg and 9.91 mg/kg for honey produced in Jimma, Bale, Kefa and North Shewa zone, Ethiopia, Arba Minch Zuria district of Gamo zone, Southern Ethiopia and Godere Woreda, Gambella, Ethiopia respectively. Additionally, mean HMF was reported by Teshome *et al.* (2020) and Tesfaye (2016) for selected districts in the Bale Zone, Oromia Regional State, Ethiopia, and the Bale Natural Forest, Southeastern Ethiopia, respectively, which were 9.45 mg/kg and 36.35 mg/kg. Getachew (2020) and Abera and Alemu (2023) reported that honey collected from selected districts in the Bench Sheko zone of southwestern Ethiopia and northeastern Ethiopia had a range of HMF between 46.53-67.68 mg/kg and 23.31-59.03 mg/kg respectively. Furthermore, the results mentioned above were superior to the findings of the current investigation.

However, a mean HMF of 2.63 mg/kg was reported by Akalework *et al.* (2020) for honey produced in Gedebano Gutazer Wolene, Central Ethiopia. According to Tigistu *et al.* (2021), the HMF content of honey produced in Doyogena and Kachabira Districts of Kembata Tambaro zone in Southern Ethiopia was found to be 3.42 mg/kg. All of the aforementioned outcomes were lower than the current study's outcome. Conversely, Fiseha *et al.* (2021) found that the honey from Chena District in Southwest Ethiopia had an HMF range of 1.8–17 mg/kg, which was higher by its lower limit but lower by its upper limit when compared to the range found in the current study. A range of HMF 1.2-2.56 mg/kg for honey collected from Ethiopia's southwest forest was reported by Addi and Bareke (2021) and this range was higher by its lower limit but lower by its upper limit. The different value of the current finding in respecting of those other mentioned findings may be due to, climatic condition, moisture content, condition and time of storing according to (Akalework *et al.*, 2020; Sajid *et al.*, 2020; Gela *et al.*, 2021; Fiseha *et al.*, 2021; Khansaritoreh *et al.*, 2021; Gomes *et al.*, 2022; Tarapoulouzi *et al.*, 2023).

The present study recorded pH of 3.12, 3.14, 2.5-3.8, and 3.13 for seasons one, 2, range, and overall mean respectively additionally it is also under international quality standards (3.4-6.1 for CAC).

The honey produced in the Haremma forest had a mean pH of 3.87, according to Belay *et al.*, (2013). Honey produced in and around Gondar has a mean pH of 3.81 (Getu and Birhan, 2014). Addi and Bareke, (2021) found the pH range of Ethiopia's Southwestern Forest honey is 4.05–5.97. Gela *et al.*, (2023), Alemayehu *et al.* (2021) and Berhe *et al.* (2018) reported a mean pH of 4.2, 3.8, and 3.9 for honey produced in Jimma, Bale, Kefa, and North Shewa zone, Ethiopia, Arba Minch Zuria district of Gamo zone, Southern Ethiopia and Godere Woreda, Gambella, Ethiopia respectively. Nega *et al.* (2020) documented that honey collected from the East Gojjam Zone of the Amhara Region, Ethiopia, had a pH range of 3.98–4.12.

A mean pH of, 4.2, 3.75, and 3.69 was reported by Teshome *et al.* (2020), Tesfaye, (2016), and Tesema, (2015) for honey produced in selected Districts of Bale Zone, Oromia Regional State, Ethiopia, Bale Natural Forest, Southeastern Ethiopia and Guji Zone, Ethiopia respectively. According to studies conducted by Fiseha *et al.* (2021) and Getachew (2020), the pH range of honey obtained from Chena District in Southwest Ethiopia and selected districts of Bench Shecko zone in Southwest Ethiopia was found to be between 3.6-4.2 and 3.4-4.1 respectively. Abera and Alemu (2023) and Weldegebriel *et al.* (2018) reported that the pH range of sampled honey varied between 2.93-4.53 and 4.11-4.33 for the northeastern Ethiopia and supermarkets in Addis Ababa and Adama respectively.

Furthermore, PH ranges of 4.13-5.02 were recorded by Mulugeta *et al.* (2017) for honey produced in Adigrat surrounding and Tepi areas in Ethiopia respectively. Mulualem and Teklemedhin (2018) and Degaga (2017) also found pH of honey ranged between 3.39-4.2 and 3.4-4.6 in Selected Districts of Arsi zone and Ethiopian monofloral honey respectively. All of these results were higher than the results found in the current study. These may be due to differences in geographic origin, shelf life, growth of microorganisms and fermentation processes (Fuad *et al.*, 2018; Moloudian *et al.*, 2018; Tarapoulouzi *et al.*, 2023; Meena *et al.*, 2023; Raweh *et al.*, 2023)

5.4. Pollen Analysis of sampled honey

Pollen analysis is must to identify that honey botanical and geographic origins (Belay *et al.*, 2015). This approach provides more accurate results than visual surveys, which makes it a vital tool for researching the forage used by honeybees and the growth of local apiculture (Begum *et al.*, 2021). For the major and minor harvesting seasons in the current study, 41 and 23 plants, respectively,

were identified. The plants were composed of trees, herbs/shrubs, and crops. The most common plant in all agro-ecologies was *Eucalyptus*. Chala (2012) disclosed Gomma district and midland honeybee flora compositions include natural trees, herbs, and perennial crops like coffee. In the lowland agro-ecology of the Shebe-Sombo district, honeybee plants or flora are those that, when in bloom, provide an abundance of both nectar and pollen.

The present study identified several major plants that function as both predominant and secondary sources, including *eucalyptus*, *shefflera*, *guizotiya*, *hypoestus*, *rumex*, *croton*, *acacia*, *cordiya*, and *plantago*. This was consistent with Betelhem *et al.* (2019) report, which stated that *accacia*, *coffee arabica*, *vernonia*, and *eucalyptus* were the main pollen-bearing plants in the Gedoe Zone. In the current study, some of these plants have been identified as being particular to a particular agro-ecology. Due to variations in topography, climate, and farming practices, honeybee plant distribution and type, as well as the length of their flowering period, differ from location to location (Azmir *et al.*, 2013; Laha *et al.*, 2017; Mewcha and Yemane, 2016; Betelhem *et al.*, 2019; Homrani *et al.*, 2020; Balkanska *et al.*, 2020; Ofjan and Etenesh, 2023). Tesfu and Dawit (2021) also identified these plants as major plants in the Southwestern Parts of Southern Nations, Nationalities, and Peoples Region, Ethiopia. These plants were found in higher densities in the sample plots' highland, midland, and lowland regions.

However, because of deforestation, there were fewer trees and shrubs in the study area (Abebe *et al.*, 2014). The study area's flowering phenology, which is typical of the herbaceous flora across the nation, is greatly enhanced by the summer's "Kiremt" rains, which culminate in autumnal flowering intensity (Abebe *et al.*, 2014). Although *shefflera* and *croton* were the most common plants in the jimma zone, (Belay *et al.*, 2017) noted that this was because Ethiopia generally had a diverse flora and habitat for honey bees. Along the agro-ecology (climate, altitude, latitude, topography, and soil type), there are variations in the distribution of natural vegetation, fruits, and cultivated crops (Abera and Minyahel, 2021). The plants identified in this study were also listed by (Belay *et al.*, 2013) in his findings and by (Addi & Bareke, 2021) in Ethiopia's southwest forest.

These plants were also identified by (Belay *et al.*, 2015) for the honey from the Haremma forest in Bale, Ethiopia. Nearly every plant found in this study was also found in Ethiopia's Western Amhara Region (Keralem *et al.*, 2017). The variety of honey types produced in a particular area is

determined by the diversity of plants that provide nectar. Additionally, different locations have different topographies, climates, farming techniques, cultural practices, plant species composition, and flowering times (Clara *et al.*, 2023). Utilizing pollen analysis, honey's purported botanical and geographic origins are confirmed (Adekanmbi *et al.*, 2019). *Eucalyptus globulus*, *Acacia tortolis*, *Guizotia scabra*, *Eucalyptus camaldulensis*, and *Hypoestes forskaolii* were the species with the highest percentage of pollen types (> 45%) (Wolditsadik *et al.*, 2022).

Compared to dominant species, secondary and significant minor honey source plant species had a greater diversity (Wolditsadik *et al.*, 2022). By resolving the issue of colony overstocking, the productivity of honeybee colonies is increased by adjusting the number of colonies with available resources (Admassu, 2015). All agro ecology honey was found to contain *Terminalia* species (*Combretaceae*) and *Guizotia* species (*Asteraceae*), whereas highland and midland agro ecology honey contained *Eucalyptus camaldulensis* (*Myrtaceae*) as secondary pollen. In lowland honey, *bidens* species were also secondary pollen types (Admassu, 2015).

Research by Gok *et al.* (2015) and Aina *et al.* (2015), found that honeybees were observed to forage on a variety of plant species from both natural and agricultural ecosystems because honey has a distinct pollen spectrum. Pollen types found in various agro ecologies included *Terminalia spp.*, *Guizotia spp.*, *Vernonia spp.*, *Bidens spp.*, *Plantago spp.*, and *E. camaldulensis*. In honey samples collected from particular regions, pollen types such as *Acacia spp.*, *Alea africana*, *Prunes persica*, *Brucea antidysentefica*, *Cleame usambarica*, *Zea mays*, and *belpharias* were noted. Depending on the agro ecology of the area, the distribution and diversity of plant life in a given area can be linked to the presence of common and specific pollen types in honey samples (Aina *et al.*, 2020). In the current study, every honey sample from both seasons had multiple flowers in it. According to a study by Bareke and Addi, (2020), the predominant pollen type in lowland honey was found to be *E. camaldulensis* (*Myrtaceae*), which was referred to as monofloral honey. Typically, only one plant species is used to produce monofloral honey (Bareke and Addi, 2020) and over 45 percent of the total pollen content is composed of pollen. This might be because honeybees have a floral constancy behavior that helps them stick to a single species of plant reward food and continue until the flower stops producing pollen or nectar (Belay *et al.*, 2015; Bareke and Addi, 2020).

According to reviews by Tulu *et al.* (2023), *E. camaldulensis* is one of the most significant plants used by bees as a forage source for monofloral honey in Ethiopia. In the Wando district, *E. camaldulensis* was a significant source of honey plants that produced monofloral honey, according to research similar to this one (Wolditsadik *et al.*, 2022). Since the honey samples from the three agro ecology areas contained a variety of low-density pollen types, they were identified as multiflora honey (Aina *et al.*, 2015; Aina *et al.*, 2020). This could be because bees visit flowering plant species in a given area less frequently and honeybee preferences are based on the availability of flora in a given region (Aina *et al.*, 2015; Aina *et al.*, 2020). In line with the conclusions of (Aina *et al.*, 2020), the current study found that several honey samples could be classified as multiflora honey because they contained a combination of secondary pollen, significant minor pollen, and minor pollen. The presence of cultivated plant pollen types, such as *Zea mays* (*Mytaceae*), in honey samples, suggests that bees obtain their floral supplies from agro-ecosystems. For honeybees to survive and reproduce, especially in the absence of natural flowering plants, this plant is essential.

Similar results were noted by (Aina *et al.*, 2020) in Kenya. Because honey from cultivated crops is distinct, managing the local agro-ecosystem can support honeybee survival and boost honey production. The majority of the honeybee plants found through pollen analysis in honey samples matched the responses of beekeepers to the honeybee flora in the research regions. To evaluate the quality, provenance, and possible therapeutic benefits of honey samples, melissopalynology the study of pollen grains found in honey to identify plant species visited by bees while foraging for nectar is crucial (Tulu *et al.*, 2023). Pollen grains can be used to determine which floral sources contribute to the distinct flavor and aroma of African honey. A wide variety of pollen types, including some unusual African pollen that is unique to Africa and cannot be found anywhere else in the world, are known to be present in African honey (Kek *et al.*, 2018).

Numerous investigations have recorded the pollen spectra of various African countries, including Ethiopia. In line with these results, a study on the pollen spectra of honey from different parts of Ethiopia was carried out by (Hailu and Belay, 2020). The study identified several pollen species, including Brassica, Acacia, and Eucalyptus that are frequently found in African regions. Additionally, a variety of pollen image atlases are available to compare and identify different

pollen types, such as the "Pollen Atlas of the Tropics of Africa" by (Gosling *et al.*, 2013) in West Africa (Manuel *et al.*, 2021) in South Africa, and (Schüler and Hemp, 2016) in East Africa.

Images of pollen grains were used to identify the plant types in a sampled honey (Warui *et al.*, 2019; Ikegbunam and Okwu, 2021) on the melissopalynological characterization of Nigerian and Kenyan honey, respectively. This study lends support to the current investigation. These pictures offered visual assistance in distinguishing between the various kinds of pollen present in samples of honey. Given that honey adulteration is a prevalent problem in the industry, it is imperative to authenticate honey associated with Ethiopia. Melissopalynology has been used in several studies to authenticate honey samples from various parts of the world (Moumeh *et al.*, 2020; Zhang and Abdulla, 2022). Therefore, talking about the findings regarding Ethiopian honey's authenticity can provide important information about the caliber and purity of honey samples.

The beekeeper can learn the dates and length of the blooming season of significant nectar and pollen plants, which can provide insight into the area's honey flow period, by creating a floral calendar (Amsalu and Tusa, 2023). Plants bloomed in September, November, and March for the most part, but some also flowered in August, September, October, and February for March. The eucalyptus blossomed throughout the year. The current study's flowering calendar was consistent with Mulualem *et al.*'s 2021 and (Ofjan and Etenesh, 2023) and the plant types were comparable to those of (Addi and Bareke, 2019; Tulu *et al.*, 2023).

Tesfu and Dawit (2021) confirmed this and reported regarding the number of plant species that bloom throughout the year, data from June to August's heavy rains showed that a greater proportion of plants were seen to be in bloom from September through the end of December. A surprisingly small number of plant species were seen to bloom from January to May (Abebe *et al.*, 2014) in Southwestern Parts of Southern Nations, Nationalities and Peoples Region, Ethiopia. Each location has a different flowering season due to the variety of plant habitats, environmental factors, and plant distributions (Abera *et al.*, 2021). In line with (Addi and Bareke, 2021) in the Southwestern forest of Ethiopia and (Amsalu and Tusa, 2023) in the East Wollega Zone, Western Oromia, Ethiopia. Also similar to a report of (Keralem *et al.*, 2017) in the Weastern Amhara region of Ethiopia. The tree species such as *Schefflera abyssinica*, *Acacia tortolis*, *Croton macrostachyus*, and *Eucalyptus spp* are flowered in March-May (Wolditsadik *et al.*, 2022). Admassu *et al.* (2015)

also stated that *Schefflera abyssinica*, *Acacia spp*, and *Croton macrostachyus* are the most important honey-producing trees and flowered from April to May.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

Seasonal honeybee colony management is very essential for maintaining healthy and strong honeybee colonies to produce high volume and quality bee products. The findings of the present study show that dearth period feeding enhanced both productive and reproductive performance of honeybee colonies during the following honey flow seasons. Particularly, T4 (50% sugar syrup with an infusion of stinging nettle and 1% *kerefa*, along with 50% white sorghum powder) and T2 (50% powdered sugar, 14% white sorghum powder, and 36% bakery yeast) significantly improved honeybees' performance, which led the beekeeping business profitable. The higher performance of both feed supplements may be attributed to their high acceptability by honeybees, high proteins level in nettle infusion and omega-3 polyunsaturated fatty acids (PUFAs) and the presence of leucine, isoleucine, and valine in white sorghum as well as high amount of protein level in yeast. In addition, the availability and low production costs of T4 and T2 feed supplements in the study area led to the highest net return to the beekeepers than other feeding treatments.

On farm performance evaluation of honeybee colonies in highland agro-ecology performed better in terms of productive and reproductive performance compared to midland and lowland agro-ecological regions of the study areas. This could be explained by the abundance of surplus nectar and pollen source bee plants, population strength, and genetic factors contributing to the superior performance of highland honeybee colonies. Moreover, highland honeybee colonies were found to be more docile than colonies kept in midland and lowland areas. In relation to foraging, honeybee colonies in lowland were found to be active foragers and defensive compared with highland and midland colonies, which may be explained by environmental conditions and genetic factors contributing to the superior foraging abilities of honeybee colonies.

All honey samples from the three agro-ecologies comply with both national and international honey quality standards. Primarily, honey produced in lowland areas was found to be of high quality compared to honey produced in highland and midland areas in both harvesting seasons. This variation may be attributed to factors related to the botanical origin from which bees collect nectar, including mineral content, enzymes, water content, soil, and the environment. In addition, honey pollen analysis reveals that midland areas possess unique plant species in addition to those found in both lowland and highland areas, indicating its potential to produce monoflora honey of

its kind. In both harvesting seasons, *Eucalyptus globulus* ranks first followed by *Guizotia scabra*, *Rumex* species, *Trifolium* species, and *Maestant* species, as the most abundant plants found in honey samples.

Based on the findings of the current study, the following recommendations are forwarded:

- The effects of T4 and T2 feed supplementation during dearth periods shall be evaluated for honey quality.
- To boost the production and productivity of bee products, maintaining healthy and strong honeybee colonies is indispensable. It is, therefore, highly recommended to create awareness among beekeepers about the importance of feed supplementation during dearth periods to ensure the well-being of their colonies.
- Stinging nettle and white sorghum are plants that grow in specific regions. To enhance their cultivation, the beekeeping extension system can play a greater role in expanding their distribution. This can be achieved by providing beekeepers with the necessary knowledge and resources to grow these plants in new areas, thereby increasing their availability.
- Bee pollen collection potential and its contribution for productive and reproductive performance during dearth period feeding shall be investigated. The productivity of a honeybee colony is directly linked to the availability and quality of pollen and nectar resources from the surrounding plants. Consequently, to maintain productive honeybee populations, it is crucial to investigate the honeybee colony carrying capacity across different agro-ecological regions.
- Due to budget and laboratory facility limitations, some important honey quality parameters were not addressed in the present study. Therefore, additional honey quality analysis including sugar profile, diastase test, adulteration, chemical residues, and other required commercial honey quality analysis parameters shall be conducted to meet international market demands.

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APENDEIXES

Appendix 1 LIST OF PUBLICATIONS

Published articles



RESEARCH ARTICLE

AGROBIOLOGICAL RECORDS

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)

Open Access Journal

EVALUATION OF PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF HONEYBEE COLONIES (*Apis mellifera monticola*) IN SOUTH WOLLO, AMHARA, ETHIOPIA

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ABSTRACT

Despite the country's potential for apicultural resources, native races, productive and reproductive performances are at relatively low levels. Numerous factors, including environmental factors and honeybee races, may be to blame for this. As a result, this study was carried out to assess local honeybee race performance and choose the best colonies in the various agro ecologies of the study area. For this purpose, 90 colonies of *Apis mellifera monticola* (30 colonies per agro ecology under on farm) were kept in improved box hives and assessed for egg area, larvae area, pupae area, nectar and honey area, as well as honey yield and swarming tendency. Egg sizes in the research area range from 519.25cm² to 1343.80cm², while larvae and pupae have respective sizes of 574.07cm² to 1407.05cm² and 618.76 to 1471.83cm². Dessie zuriya district had the highest pollen area, nectar area, honey area, and honey yield (15.28cm², 343.17cm², 386.13cm², and 22.13cm²), followed by Kalu (8.97cm², 236.02cm², and 18.88cm²), and Tehuledere (6.01cm², 142.54cm², 150.39cm², and 12.83cm², correspondingly). The development of queen cells and swarming behavior were unaffected by agro ecology. In general, compared to other ecotypes and races in the country, honeybee colonies in the study area generally performed better in terms of both



EVALUATION OF ABSCONDING RATE, FORAGING AND DEFENSIVE BEHAVIOUR IN *APIS MELLIFERA MONTICOLA*

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ABSTRACT

Honey bee colonies display a wide range of behavioural variations based on their genetic origin and environmental factors. It is crucial to evaluate the absconding, foraging, and defensive behaviour of different honey bee races to lay the groundwork for future selection and improvement in Ethiopia. For this study, we kept 90 colonies of *Apis mellifera monticola*, with 30 colonies/ agroecology, in improved box hives. These colonies were similar in terms of the resources contained in the hive. During the active seasons of September to December 2021 and April to June 2022, an assessment of the foraging activity, defensive behaviour, and absconding rate was done. The study revealed that colonies of *A.m. monticola* exhibited an average response time of 53.29 sec to disturbances, indicating their defensive behaviour. Additionally, these colonies demonstrated a willingness to pursue intruders for distances of up to 182.42 m. These colonies were excellent foragers, beginning their activities at 8:13 a.m. and returning to the hive by 10:04 p.m. Moreover, *A.m. monticola* showed an absconding rate ranging from 1.90 to 4.28% when faced with disruptions. To ensure optimal colony health and productivity, it is advisable to conduct further research on the selection of high-performing colonies.

Key words: *Apis mellifera monticola*, Absconding, defensiveness, evaluation, disease, foraging, ability, on-farm, agroecology, South Wollo, Ethiopia

Recent studies have focused on behaviours related to colony health and disease control, such as hygienic for honey bee colonies and is linked to their overall fitness, beekeepers generally prefer colonies that do

PLOS ONE

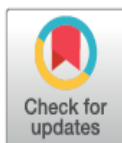
RESEARCH ARTICLE

Effect of various supplements on productive performance of honey bees, in the south Wollo Zone, Ethiopia

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Abstract

The productivity and well-being of honey bee colonies are greatly influenced by the nutrients present in the hives. A study was conducted to evaluate different supplemental feeds on honey bee productive performance during dearth periods. Thirty colonies were grouped into

Under review article

My Articles SUBMIT NEW MANUSCRIPT

SUBMISSION	TITLE	JOURNAL	STATUS	CHARGES
 234101480	EFFECTS OF DIFFERENT...	Journal of Apicultural Research	Revision Required	

Ref.: Ms. No. TJAR-2023-0193R1
234101480
EFFECTS OF DIFFERENT SUPPLEMENTATIONS ON HONEY BEE REPRODUCTIVE PERFORMANCE
Journal of Apicultural Research

Dear Dr Wubalem Alebachew,

Appendix 2: Analytical output of experimental data

Dependent Variable: FI FI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2063938.725	515984.681	553.53	<.0001
Error	685	638532.174	932.164		
Corrected Total	689	2702470.899			

R-Square	Coeff Var	Root MSE	FI Mean
0.763723	32.51526	30.53136	93.89855

Dependent Variable: EA EA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5119038.09	1279759.52	34.71	<.0001
Error	265	9769532.96	36866.16		

Corrected Total	269	14888571.05		
R-Square	Coeff Var	Root MSE	EA Mean	
0.343823	63.46439	192.0056	302.5407	

Dependent Variable: LA LA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5363470.79	1340867.70	43.75	<.0001
Error	265	8122642.65	30651.48		
Corrected Total	269	13486113.44			
R-Square	Coeff Var	Root MSE	LA Mean		
0.397703	41.63365	175.0756	420.5148		

Dependent Variable: PA PA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6019548.06	1504887.01	55.85	<.0001
Error	265	7141038.91	26947.32		
Corrected Total	269	13160586.97			
R-Square	Coeff Var	Root MSE	PA Mean		
0.457392	32.78683	164.1564	500.6778		

Dependent Variable: NA NA

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	46841.2963	11710.3241	46.82	<.0001
Error	265	66279.4444	250.1111		
Corrected Total	269	113120.7407			

R-Square Coeff Var Root MSE NA Mean
0.414082 39.72115 15.81490 39.81481

Dependent Variable: POA POA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	73911.7259	18477.9315	30.23	<.0001
Error	265	161965.0741	611.1890		
Corrected Total	269	235876.8000			

R-Square Coeff Var Root MSE POA Mean
0.313349 48.09774 24.72224 51.40000

Dependent Variable: HA HA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1260259.385	315064.846	43.18	<.0001
Error	265	1933755.815	7297.192		
Corrected Total	269	3194015.200			

R-Square Coeff Var Root MSE HA Mean

0.394569 36.16579 85.42360 236.2000

Dependent Variable: QC QC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.82962963	0.45740741	4.75	0.0010
Error	265	25.53703704	0.09636618		
Corrected Total	269	27.36666667			

R-Square Coeff Var Root MSE QC Mean
0.066856 399.1230 0.310429 0.077778

Dependent Variable: CS CS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	382.391204	95.597801	25.94	<.0001
Error	265	976.585648	3.685229		
Corrected Total	269	1358.976852			

R-Square Coeff Var Root MSE CS Mean
0.281382 40.33600 1.919695 4.759259

Dependent Variable: HY HY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	307.9583333	76.9895833	25.19	<.0001
Error	55	168.1197917	3.0567235		
Corrected Total	59	476.0781250			

R-Square	Coeff Var	Root MSE	HY Mean
0.646865	43.03628	1.748349	4.062500

Dependent Variable: GE GE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1597385.569	399346.392	138.42	<.0001
Error	55	158678.450	2885.063		
Corrected Total	59	1756064.019			

Dependent Variable: GR GR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	57045666.67	14261416.67	21.48	<.0001
Error	55	36517083.33	663946.97		
Corrected Total	59	93562750.00			

R-Square	Coeff Var	Root MSE	GR Mean
0.609705	46.82928	814.8294	1740.000

Dependent variable: NP NP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	55390162.90	13847540.73	22.22	<.0001
Error	55	34277825.12	623233.18		
Corrected Total	59	89667988.02			

R-Square	Coeff Var	Root MSE	NP Mean
0.617725	50.30584	789.4512	1569.303

Appendix 3: Analytical output of monitoring and evaluation data

Dependent Variable: EA EA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	72982650.2	36491325.1	149.34	<.0001
Error	627	153205385.0	244346.7		
Corrected Total	629	226188035.3			

R-Square	Coeff Var	Root MSE	EA Mean
0.322664	55.17224	494.3144	895.9476

Dependent Variable: LA LA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	74736877.8	37368438.9	144.82	<.0001
Error	627	161784343.7	258029.3		

Corrected Total 629 236521221.5

R-Square	Coeff Var	Root MSE	LA Mean
0.315984	53.36345	507.9658	951.8984

Dependent Variable: PA PA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	78399779.2	39199889.6	143.94	<.0001
Error	627	170750555.2	272329.4		
Corrected Total	629	249150334.4			

R-Square	Coeff Var	Root MSE	PA Mean
0.314669	51.89614	521.8519	1005.570

Dependent Variable: POA POA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9399.43175	4699.71587	213.51	<.0001
Error	627	13801.22857	22.01153		
Corrected Total	629	23200.66032			

R-Square	Coeff Var	Root MSE	POA Mean
0.405136	46.48846	4.691645	10.09206

Dependent Variable: NA NA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4469941.69	2234970.84	91.83	<.0001
Error	627	15259974.32	24338.08		
Corrected Total	629	19729916.01			

R-Square	Coeff Var	Root MSE	NA Mean
0.226557	68.13698	156.0067	228.9603

Dependent Variable: HA HA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5980629.97	2990314.98	87.77	<.0001
Error	627	21362459.34	34070.91		
Corrected Total	629	27343089.31			

R-Square	Coeff Var	Root MSE	HA Mean
0.218725	71.67788	184.5831	257.5175

Dependent Variable: hy hy

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	2	2673.100000	1336.550000	40.18	<.0001
Error	177	5887.450000	33.262429		
Corrected Total	179	8560.550000			

R-Square	Coeff Var	Root MSE	hy Mean
0.312258	32.13013	5.767359	17.95000

Dependent Variable: exrsw exrsw

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	441.269850	220.634925	3.92	0.0251
Error	60	3377.777722	56.296295		
Corrected Total	62	3819.047572			

R-Square	Coeff Var	Root MSE	exrsw Mean
0.115544	124.3933	7.503086	6.031746

Dependent Variable: qcc qcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	78.0000000	39.0000000	2.60	0.0829
Error	60	901.4285714	15.0238095		
Corrected Total	62	979.4285714			

R-Square	Coeff Var	Root MSE	qcc Mean
0.079638	108.5296	3.876056	3.571429

Dependent Variable: swate swate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	866.66668	433.33334	2.60	0.0829
Error	60	10015.87294	166.93122		
Corrected Total	62	10882.53962			

R-Square	Coeff Var	Root MSE	swate Mean
0.079638	108.5296	12.92019	11.90476

Dependent Variable: absc_ra absc ra

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	66.666667	33.333333	1.06	0.3526
Error	60	1885.714177	31.428570		
Corrected Total	62	1952.380844			

R-Square	Coeff Var	Root MSE	absc_ra Mean
0.034146	196.2142	5.606119	2.857143

Dependent Variable: TtfFs_sec_ TtfFs(sec)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	17527.71429	8763.85714	630.49	<.0001
Error	60	834.00000	13.90000		
Corrected Total	62	18361.71429			

R-Square	Coeff Var	Root MSE	TtfFs_sec_ Mean
0.954579	4.850909	3.728270	76.85714

Dependent Variable: Tt_sec_mag_ Tt(sec)mag#

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	190946.5714	95473.2857	9134.12	<.0001
Error	60	627.1429	10.4524		
Corrected Total	62	191573.7143			

R-Square	Coeff Var	Root MSE	Tt_sec_mag_ Mean
0.996726	1.468598	3.233014	220.1429

Dependent Variable: TNoS__B_ TNoS (B)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2.5714286	1.2857143	0.15	0.8602

Error	60	510.8571429	8.5142857
Corrected Total	62	513.4285714	

R-Square	Coeff Var	Root MSE	TNoS__B_ Mean
0.005008	13.52680	2.917925	21.57143

Dependent Variable: TNoS_G_ TNoS(G)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	643.142857	321.571429	36.23	<.0001
Error	60	532.571429	8.876190		
Corrected Total	62	1175.714286			

R-Square	Coeff Var	Root MSE	TNoS_G_ Mean
0.547023	25.85337	2.979294	11.52381

Dependent Variable: TDF_m_ TDF(m)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1080.285714	540.142857	37.28	<.0001
Error	60	869.428571	14.490476		
Corrected Total	62	1949.714286			

R-Square	Coeff Var	Root MSE	TDF_m_ Mean
0.554074	2.029948	3.806636	187.5238

Appendix 4: Analytical outputs of honey quality data

Dependent Variable: FA FA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	310.40833	310.40833	3.49	0.0641
Error	118	10482.18333	88.83206		
Corrected Total	119	10792.59167			

R-Square	Coeff Var	Root MSE	FA Mean
0.028761	49.23853	9.425076	19.14167

Dependent Variable: MC MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.2013333	3.2013333	0.93	0.3361
Error	118	404.9453333	3.4317401		
Corrected Total	119	408.1466667			

R-Square	Coeff Var	Root MSE	MC Mean
0.007844	10.62617	1.852496	17.43333

Dependent Variable: pH pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02133333	0.02133333	0.28	0.5965
Error	118	8.93066667	0.07568362		
Corrected Total	119	8.95200000			

R-Square	Coeff Var	Root MSE	PH Mean
0.002383	8.789347	0.275107	3.130000

Dependent Variable: AC AC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.10740083	0.10740083	8.56	0.0041
Error	118	1.48085833	0.01254965		
Corrected Total	119	1.58825917			

R-Square	Coeff Var	Root MSE	AC Mean
0.067622	114.2143	0.112025	0.098083

Dependent Variable: HMF HMF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1693.505333	1693.505333	32.09	<.0001
Error	118	6227.906333	52.778867		
Corrected Total	119	7921.411667			

R-Square	Coeff Var	Root MSE	HMF Mean
0.213788	132.2896	7.264907	5.491667

Appendix 5: Some photos taken during data collection



App Fig.1: Washing of frames



App Fig. 2: Artificial honeycomb preparation



App Fig. 3: Infusion of stinging nettle and its leaf respectively



Fig. 4: Photo taken during monitoring and evaluation