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Original Research

Distribution of Stingless Bees and the Physico – Chemical characteristics of their Products at Digga and Wayu Tuqa Districts of East Wollega Zone, Oromia, Ethiopia

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Abstract

The study was conducted in Diga and Wayyu Tuqa districts of East Wollega zone, Oromia, to investigate the spread of stingless bees and evaluate their physicochemical qualities. The study involved 90 participants, including 5 who domesticated bees and 85 who collected honey from these insects. The analysis included sugar, pH, ash, moisture content, and free acidity on 18 honey samples. The study also examined average day temperature, honey yield, pollen yield, and propolis yield of stingless bees. The average temperature was 32.3°C, while the honey yield was 1.5L. The pollen yield was 500g in the midland agro-ecology, 350g in the lowland, and 554g in the highland. The average propolis yield was 74.5g in the midland, 59.35g in the lowland, and 93.45g in the highland. The study recorded data on honey composition in three agro-ecologies, with no significant difference (p>0.05) except for moisture content. Highlands had higher moisture content (MC), while lowlands had lower MC. The study found that stingless bees are adaptable and can thrive in agricultural ecosystems at various altitudes, but did not show any climatic preference. The study highlights the importance of honey composition in determining honey production.

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INTRODUCTION

Meliponiae and Apidae are the families in which stingless bees are classified (Culliney, 1983). Melipona is located in America, while Trigona and Meliponula are the most widespread genera under the subfamilies Meliponiae found in Africa (Souza et al., 2006). In contrast to Apis mellifera L., domesticated honey bees that reside in hives, Meliponini Apidae are wild, stingless bees (Souza et al., 2006). The majority of the world's stingless bee population, or Meliponini apidae, is located in tropical and subtropical regions (Michener, 2007). All stingless bees in Africa are highly organised social insects that share a single nest with numerous individuals, much like honeybees of the genus Apis. Because stingless bee colonies have a long lifespan by nature and no functional sting, they are excellent pollinators for crops grown in populated regions. They are significant from an ecological, economic, and cultural standpoint. For instance, stingless bees help pollinate wild and agricultural plants are a significant source of revenue (Quezada-Euan et al., 2018), and are used to cure a variety of illnesses (Kumar et al. 2012, Rao et al. 2016). In order to preserve food production resilience and increase output, more pollinator species must be employed in agriculture due to the mounting environmental strain and bee population decline those results from.

According to Kumar et al. (2012), meliponiculture is the practice of beekeeping using stingless bees in which bee keepers nurture, multiply, and exploit stingless bee colonies of different species for financial gain. Kumar et al. (2012) state that bee keepers can propagate colonies and produce goods including honey, pollen, cerumen, and propolis by managing stingless bee colonies in artificial hives. According to Cortopassi-Laurino et al. (2006), communities in Tanzania and Angola gather stingless bee honey from hollow logs and clay pots that they use as hives, despite the fact that the majority of stingless bee honey is collected from wild colonies in Africa (Eardley 2004). East Wollega is blessed with both man-made and natural forests and herbs, which serve as a haven for a variety of social insects, such as stingless bees. Nevertheless, honey hunters destructively remove the majority of stingless bee products from feral colonies (FAO, 2009; Eardley, 2004; Eltz et al., 2003). Stingless bees have been understudied despite their socioeconomic and ecological implications in Ethiopia (Arias et al., 2006; Eardley, 2004).

There is little information available regarding the nation's stingless habitat preferences, which include East Wollega. In the same way, there is no scientific proof on the stingless bees' preferred habits in the East Wollega zone's Diga and Wayu Tuqa districts. However, researching the existing indigenous knowledge on managing stingless bees could be a crucial first step towards domestication and the creation of meliponiculture, which could yield honey for food and medicine, cash income, and pollination for wild plants and crops (Eardley 2004). Additionally, it would stop natural stingless bee nests from being destroyed. Therefore, the present study was started with the following goals in mind: to describe the distribution of stingless bees in the East Wal-laga zone's Digga and Wayu Tuqa districts; to evaluate the stingless bees' production performances in the study districts; and to assess the physico-chemical qualities of the honey produced by the stingless bees in the study districts.

MATERIALS AND METHODS

Description of the Study Area

The study was carried out in the Oromia Regional State's Diga and Wayyu Tuqa districts of the East Wollega Zone. The districts of Diga and Wayyu Tuqa are situated roughly 12 and 11 km west and east, respectively, of Nekemte City, the East Wollega zone's capital. Nekemte is located approximately 331 km west of Finfinnee/Addis Ababa. The regions of Diga and Wayyu Tuqa were chosen because they are home to farmers that produce stingless bees, who have begun to domesticate the insects from their natural nests to artificial hives. Figure 1 shows a map with the study districts highlighted.

Digga district

One of the 17 districts in Oromia Regional State's East Wollega zone is Digga district. It is situated 343 km west of Finfinnee/Addis Ababa and 12 kilometres west of Nekemte City, the capital of the East Wollega Zone. The district is situated between 1100 and 2300 metres above sea level (m.a.s.l.). The district's mean temperature ranges from 18 to 32˚C, while the district's mean annual rainfall falls between 1200 and 2000 mm. The natural and artificial vegetation in the Digga district can serve as the stingless bees' home and a source of honey and other items.

Wayu Tuqa district

The distance between the Wayu Tuqa district and Finfinne/Addis Ababa, the nation's capital, is approximately 324 km. It has an average yearly rainfall of 2400 mm and is situated at altitudes between 1700 and 2200 m.a.s.l. Some of Wayu Tuqa district's kebeles are right next to the protected woodland known as Komto. Wayu Tuqa is bordered to the west by the Guto Gida district and to the east by the Sibu Sire district. In the district, there are both man-made and natural forests that can serve as homes for the stingless and sources of vegetation.

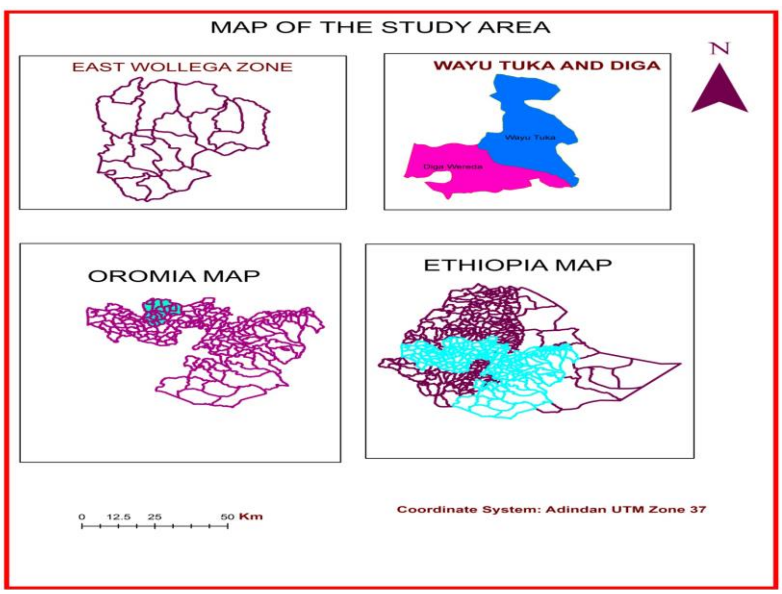

Figure 1. *Map of the Study area*

Data Sources and Methods of data Collection

Measurements of the physical characteristics of colonies, field observations of the study locations, and questionnaire surveys were used to gather primary data. Trained enumerators in "Afan Oromo" prepared and conducted the survey study's questionnaire. In addition to apiculture specialists and stingless bee producers (i.e., those participating in stingless bee domestication), key informants were also engaged in discussions. Five stingless bee honey producers and five apiculture specialists made up the key informants. Reports from the agriculture offices in both districts provided secondary data. Information about farmers' indigenous knowledge that was not gathered through interviews or secondary sources was obtained through field observation. To take images and double-check the actual procedures. Furthermore, stingless bee nests and their environments were studied.

Sampling methods

The regions of Diga and Wayu Tuqa were purposefully chosen for the study due to their prior involvement in the collecting of honey from stingless bees through domestication or hunting. The peasant organisations in the districts were divided into three agro-ecologies: lowland, midland, and highland. For this investigation, three peasant associations from each agro-ecology were chosen. In this study, ninety farmers participated in total. Out of the ninety farmers, five have been involved in the domestication of stingless bees. In order to obtain stingless bee nests, knowledgeable farmers in the research locations marked out places such as farmland and forestland, particularly those beneath trees.

Estimation of honey yield

Using a digital balance, honey was collected from highland, midland, and lowland areas. The honey from the underground nest was painstakingly collected by hand-digging the dirt using a variety of equipment, including shovels, axes, buckets, and sticks. In order to determine the direction and bottom of the nest sac, a thin, flexible stick is first inserted into the tunnel to begin the process of excavating the underground nest. The nest's depth is determined by the stick's length.

Estimation of botanical origin of honey

Using the harmonised Melisopallynology procedures, the botanical origin of honey produced by stingless bees was ascertained (Von Der Ohe et al., 2004). Pollen analysis was used to discover which stingless bee honey plants were most liked. In a centrifuge tube, ten grammes of honey were dissolved in twenty millilitres of warm distilled water, maintained between twenty and forty degrees Celsius. After centrifuging the mixture for ten minutes at 3800 rpm, the supernatant was decanted. After adding 20 millilitres of distilled water to fully dissolve the residual sugar crystals, the mixture was centrifuged once more for five minutes at 3800 rpm, and the supernatant was extracted. A sterile microspatula was used to evenly spread the silt across the microscope slide, and the sample was then allowed to dry for a while. After that, a single drop of glycerin jelly was poured to the cover slip, and a pollen atlas was used to identify the pollen grains (Adgaba, 2002). The total number of distinct types of pollen grains detected in each sample was used to compute the proportions of each pollen type in each honey sample. A honey was classified as mixed if it had no predominant pollen, and as predominant pollen if the number of pollen grains measured was larger than 45% (Louveaux et al., 1978). Reference slides and a pollen atlas were used to identify the sources of the dominating pollen plants after specific images of the pollen finger prints were acquired from each slide (Adgaba, 2007).

Physico-chemical Analysis of Stingless bee Honey

For the physico-chemical analysis, eighteen honey samples (0.5L–2L) were gathered from lowland, midland, and highland regions. Depending on the amount of honey available, three samples—one from each nest—were taken from each agro-ecology. Measurements of the physical parameters, moisture content, PH and free acidity, total ash content, sugar content, and hydroxymethylfurfural (HMF) were all part of the phyisico-chemical study.

Measurements on physical parameters

Measurements of the physical parameters include the size of the colony, the nest, and any pertinent items that come from it. Using a class-made ruler, the width and height of the nests were measured in order to calculate the overall volume of the nest, which in turn gave the colony and nest product accommodation capacity of the nest. Using a digital thermometer, the temperature at the nest's entrance was determined. Products made by stingless bees, such as honey, pollen, propolis, etc., were measured and recorded using a digital balance.

Moisture content

An Abbé thermometer (ABBE-5 Bellingham Stanley. Ltd., United Kingdom) that can be thermostated at 20ºC and is routinely calibrated with distilled water was used to measure the moisture content of the samples that were collected.

Determination of pH and free acidity

The pH of honey was measured by immersing the pH metre electrode in the solution. The solution was further titrated with 0.1 M sodium hydroxide (NaOH) solution to pH 8.30 in order to quantify the free acidity. Using a 10-milliliter burette, the reading was taken precisely, down to the nearest 0.2 millilitre. Free acidity is measured in mill equivalents or mill moles of acid per kilogramme of honey. One mill mole is equivalent to ten millilitres of 0.1M NaOH. The Bogdanov (2009) approach is used to represent the result to one decimal place. Acidity is 10 V, where V is the volume of 0.1N NaOH in 10 g of honey.

Determination of Total Ash Content

The total ash content was calculated by carefully harvesting the honey to prevent soil mixing. In order to make the determination, honey samples were burned to constant mass at 600°C in a muffle furnace (BioBase JKKZ.5.12GJ, Shan- dong, China) (Bogdanov, 2009). Using the following formula, the percentage of weight of ash (WA) in grammes per 100 grammes of honey was determined:

$$WA\ (\%) = \left(\frac{M_1 - M_2}{M_0}\right) 100\%$$

Where: M_0= Weight of honey taken, M_1= Weight of ash + dish, M_2= Weight of dish,WA=Weight of ash

Determination of sugar content of honey

High performance liquid chromatography (HPLC-1260 Infinity Series, Agilent Technologies, Germany) was used to measure the sugar content of the honey. The 4.6 mm diameter, 250 mm length analytical stainless steel column that makes up the HPLC separation system contains 9 Amine-modified silica gel with particles ranging from 5 to 7 µm. By comparing the retention durations of the honey sugars—fructose, sucrose, and glucose—with those of the standard sugars, the honey sugars were identified (Bogdanov, 2009).

Determination of Hydroxyl Methyl Furfural (HMF)

The 6800 UV–Vis spectrophotometer (JENWAY, United Kingdom) was used to determine HMF. A small beaker was used to weigh a sample of honey, which was then combined with distilled water and placed into a volumetric flask (Bogdanov, 2009). Hydroxyl methyl furfural (HMF)/100 g honey = (A284 - A336) x 14.97 X 5/g sample, where A284 = absorbance at 284, A336 = absorbance at 336, 14.97 = constant, 5 = theoretical nominal sample weight, and g = mass of honey sample.

Data Analysis

SPSS version 20 and SAS version 9.0, respectively, were used to analyse qualitative and quantitative data that were obtained through various methods. The model fitted for quantitative data was:

$Y_{ij}=\mu + t_i + e_{ij}$,

where µ represents the overall mean, t_i the i^{th} treatment effect, e_{ij} the error term, and ti the agro-ecological effect. The means of every agroecological group were compared following an alpha 0.05 test.

The following formula was used to rank the various stingless bee enemies and factors affecting nest size: rank index=sum of (5 * number of households ranked as first + 4 * number of households ranked as second + 3 * number of households ranked as third + 2 * number of households ranked as fourth + 1 * X number of households ranked as fifth) for an individual reason divided by the sum of (5 * number of households ranked as first + 4 * number of households ranked as second + 3 * number of households ranked as third + 2 * number of households ranked as fourth + 1 * X number of households ranked as fifth) for overall reasons.

RESULTS AND DISCUSSION

Socio-economic Profiles of Respondents

Socio-economic Profiles of Respondents As shown in Table 1, hunting for stingless bee nests typically entails looking for wild stingless bees in forests, sometimes far from residential areas, and it is customarily taboo for women to wander through forests alone. As a result, all (100%) respondents were male. In actuality, according to a few study participants, rural women who frequently gather firewood from the forest play a part in identifying the nests of their male relatives, the stingless bees. Furthermore, it is commonly believed that males are responsible for stingless bee nest searching and for setting up hollow wooden logs in huge trees to attract honeybees (Abebe, 2011; Shenkute et al. 2012). Just 7.8% of respondents were unmarried, while the bulk (92.2%) were married. About 52.2, 32.2%, and 4.4% of the respondents, respectively, attended primary, secondary, and predatory schools in terms of education. Merely 4% of the participants in the present study were engaged in or attending tertiary education, whilst approximately 6.7%

of the respondents had no formal educational enrollment. Farmers in the study areas can be trained to domesticate bees with minimal technical assistance or capacity building on beekeeping technologies based on their educational background and experience seeking stingless bee nests. Every respondent was a farmer with prior experience collecting honey from stingless bees. Five or so of them had begun domesticating stingless bees.

Table 1

Socio-economic profiles of respondents

Variables	Agro ecologies			Total	Proportion %
	Midland	Lowland	Highland		
Number of respondents[¥]	30	30	30	90	100
Marital status	30	30	30	90	100
Married	26	27	30	83	92.22
Single	4	3	0	7	7.78
Educational status					
Primary (1-8 grade)	12	8	27	47	52.22
Secondary (9-10 grade)	12	14	3	29	32.22
High School (11-12 grade)	1	3	0	4	4.44
Diploma	0	1	0	1	1.11
Degree and above	1	2	0	3	3.33
Not enrolled in formal education	4	2	0	6	6.67

[¥]*Respondents were all males*

Perception of Respondents about stingless bees

There was variation in the colour of the stingless bee honey collected from the various agro-ecologies during the current study. This is supported by the fact that 38.9% of respondents said the honey from stingless bees was golden, 35.6% said it was black, and 25.56% said it was light red. Teferi et al.'s colour study results from 2023 also showed that the samples of stingless bee honey ranged in colour from water white to dark amber. The authors reported that the honey collected from Diga district and other districts in Western Oromia Regional state had a light amber or yellowish orange colour. However, the same authors observed that a water white colour was present in the sample taken from the Gechi area in the Illubabor zone. In accordance with the USDA's (1985) requirements for colour designations for extracted honey, around 50% of the collected samples were classified as light amber, 21.5% as extra light amber and amber, and 7% as water white. This suggests that stingless bee honey changes according to a number of variables. The numerous floral kinds produced by stingless bees from various agro-ecologies and production methods, as well as light exposure, temperature, storage conditions, and enzyme reactions, could all contribute to the variance in colour of honey.

Study participants reported that the honey produced by Meliponula beccarii stingless bees tastes delicious. However, stingles bee honey has a higher acid content than honey from honey bees, according to laboratory study done at the Holeta Bee Research Centre (HBRC). This would have made the stingles bee honey taste sour rather than sweet. About 33.3% of the participants in the current study reported seeing three casts in the stingless bee colony—the queen, drones, and workers—just like in stinging honey bee colonies. Nonetheless, over 28.9% of respondents were unaware of this classification, and roughly 37.8% of respondents deny its existence.

Distribution of Stingless Bees in different agro-ecologies

The suitability of the habitat has a major impact on the distribution of stingless bee species and honey bee species in African savanna ecosystems (Kajobe, 2007; Naug, 2009). The results of this study showed that lowland, midland, and highland agroecologies are home to stingless bees (Table 6). In terms of choosing nest sites, stingless bees are categorised as generalist feeders. They consumed a variety of flowers, suggesting that they may live in a variety of agro-ecologies. According to the recent study, stingless bees often prepare their nests in subterranean dirt. This is consistent with research by Eardley (2010), who found that stingless bees in Tanzania and South Africa build their nests underground and in abandoned termite mounds. Additionally, stingless bees have the ability to build their nests inside of hives, which signifies the beginning of meliponi culture in the research locations. The recent investigation of the stingless bee's nesting place is consistent with Darchen's (1969) findings that Hypotrigona, Clepto-trigona, and M. Nebulata and M. Termite mounds have been observed as breeding sites for beccarii. Respondents to the pre-sent study stated that stingless bee natural nests are typically located beneath the canopy of trees like Croton macrostachyus, Vernonia rueppellii, Vernonia amygdalina, etc. The distribution of stingless bees within the African savanna ecosystems is generally influenced by the habitat and nesting requirements that vary between species, according to literature reviews (Kajobe, 2007). They can build their nests in both natural and man-made structures, such as cracks, fallen trees, earthen banks, and rocks. In this investigation, stingles

For nesting, 13 bees preferred areas in forests. Due to the trees' roots, it was challenging to extract honey and other nest products because of their affinity for forested areas, especially those under tree canopy (Figure 2).

Figure 2. Stingless bees' nest under a tree

Major Factors affecting nest size of stingless bees' colony

The size of a colony can have a major influence on the nest sizes (both depth and width) of stingless bees. Stingless bees nest beneath trees. The use of agrochemicals is the primary factor influencing a colony's nest size, as Table 2 illustrates. The availability of flowers, or sources of flora, stingle bees' colony sizes, and their enemies accounted for the second, third, and fourth significant elements. correspondingly.

Table 2

Factors affecting population of stingless bees' colony

Variables	Relative degree of importance					Total	Index	Rank
	1st	2nd	3rd	4th	5th			
Ecology	20	10	5	4	2	165	0.26	2
Flower availability	10	11	1	0	0	97	0.15	3
Enemies	11	4	0	0	0	71	0.11	5
Chemicals	30	15	10	20	15	295	0.47	1

Proportion of Stingless Bee Products Collected From different Agro-ecologies

In the current study, 1.28lit of honey/nest (0.83lit to 1.5lit) on average was gathered (Table 3). In comparison to the midland and highland agro-ecologies, the lowland produced less honey. The amount of honey produced by the highland and midland agro-ecologies was about equal. In this investigation, the average amount of pollen and propoplis harvested/nest was 468 grammes and 75.77 grammes, respectively. In comparison to the other agro-ecologies, lowland agro-ecology had the lowest yields of propolis and pollen, while highland agro-ecology had the largest yields of both products. The variations in the flora and water availability between the agro-ecologies could be the cause of the discrepancy. Because stingless bee nests are built underground, these bees typically visit bushes and herbs that are close to their nests. In contrast to lowlands, the majority of plants are more common in highland and mid-altitude regions. This must be an additional factor contributing to the bees' increased output in high- and mid-altitude regions.

Table 3

Stingless Bees' Products from the different agro-ecologies

Variables	Agroecologies			Overall Average
	Midland	Lowland	Highland	
Average honey/nest (L)	1.5	0.83	1.5	1.28
Average pollen/nest (g)	500	350	554	468
Average propolis/nest (g)	74.5	59.35	93.45	75.77

Characteristics of Nest Physical Parameters at different agro ecologies

The average mean temperature at the nest gate was 32.0°C, 32.3°C, and 35.2°C for the highland, midland, and lowland agro-ecologies, respectively (Table 4). The mean differences in temperature between lowland and midland, as well as between highland and lowland, were 2.9°C and 3.2°C, respectively. The pre-sent study found an inverse association between the temperature of the nest and the moisture level of the honey. The moisture content of the honey dropped as the temperature in the nest rose. In all agro-ecologies, the average diameter of a nest's gate was 1 cm. The same gate sizes across all agro-ecologies could be attributed to their shared function (entrance only). The necessity for the colony to forbid predator entry may be related to the gate's nar-row size. The nest's average horizontal diameter in lowland areas was 14 cm, while in midland and fifteen agro-ecologies in the highlands. The average vertical heights of the nests in the low-land, mid-land, and high-land agro-ecologies were 18 cm, 22 cm, and 23 cm, respectively. It is evident from the current data that the vertical nest size reduced as altitude (from high land to low land) dropped. The explanation appears to be that, in contrast to midland and highland agro-ecologies, soil moisture content declines with altitude, making it more difficult for bees to delve deeply in lowland environments. Agro-ecological temperature differences could be the second likely cause of the variation in nest dept. The temperature may rise as nest depth grows, which could impact the health of the bees. In the midland, lowland, and highland ecologies, the corresponding depths to reach the coral were 0.45–1 m, 0.3–1 m, and 0.5–1 m, respectively.

Table 4

Characteristics of Nest Physical Parameters at different agro-ecologies

Variables	Agro ecologies		
	Midland	Lowland	Highland
Average nest temperature in (^{o}C)	32.3	35.2	32
Average diameter of gate of nest (cm)	1	1	1
Average horizontal diameter of nest (cm)	15	14	15
Average vertical diameter of nest (cm)	22	18	23
Depth to reach colony (m)	0.45-1	0.3-1	0.5-1

Purpose of harvesting stingless bee's honey in different agro-ecologies

Objectives of hunting and collecting stingless bee honey include generating income, consuming, and using it medicinally. According to every respondent in the current survey, they hunt and harvest stingle bee honey for its therapeutic properties. Furthermore, stingless bee honey is more costly than regular bee honey. Approximately 80% of those surveyed said that stingles bee honey ranges in price from 400 to 600 ETB per kilogramme. Similarly, Indian researchers Kumar et al. (2012) stated that Stingless bee honey is more in demand on the market and thought to have medicinal potential than Apis mellifera honey. Regarding yield, roughly 33.3% and 66.7% of the respondents, respectively, stated that they had harvested 1.5 to 2 kg and 0.5 kg to 1.5 kg of honey per nest. The yield that was achieved in this investigation is consistent with Cortopassi-Laurino et al. (2006). In order to extract honey from stingless bee hives, participants mentioned 16 that they employ a variety of tools, such as sticks, spades, "gaso," "gajara," and glass or plastic bottles, among other things. A slender, straight stick that is flexible is inserted into the entry hole of the underground nests by honey hunters, who use it as a guide to descend to the nest's bottom. After that, the complete contents of the nest are carefully removed and placed on a large leaf or plastic sheet, and the outside of the nests are cleared of dirt and other debris. Although honey hunting is year-round and can be done at any time, November and December are the busiest months for harvesting honey.

Major Enemies of stingless bees in the study areas

Wasps, honey badgers, ants, and birds rank among the principal enemies of stingless bees in the study areas in order of significance, according to respondents. Man is also mentioned as a bee's enemy. According to the respondents, man is not only robbing the bees of their produce but also destroying the entire hive. Additionally, dangerous pesticides that are sprayed by humans on agricultural fields and the environment have the potential to kill a large number of stingless bees and contaminate their sources of food. There are no identified stingless bee diseases in the research locations, according to respondents. Respondents to the current study stated that stingless bees do not favour gathering nectar from rapeseed and that using less or not using rapeseed prevented the bees from contracting infections. Nonetheless, it is important to view responders' suggestions that stingless bees don't appreciate rapeseed and that diseases carried by the seed should be taken seriously.

Table 5

Major Enemies of stingless bee in the study areas

	Relative degree of importance’s						
Enemies	1st	2nd	3rd	4th	Total	Index	Rank
Ants	34	13	28	15	246	0.30	3
Gajii (wasps)	47	25	15	0	293	0.35	1
Man	0	21	23	15	124	0.15	5
Birds	27	24	0	0	180	0.21	4
Honey badger	35	15	14	8	292	0.25	2

Domestication of stingless bees, inspection a nd transferring methods

A small number of farmers who have reported on stingless bee domestication have mentioned the need of prehand preparation of box hives coated in mud and cow dung. Farmers who first began domesticating stingless bees claim that hives made of mud were also created, however the bees had a relatively short lifespan. Stingless bees are benign, easy to see, and harmless. The hive is inspected beginning on its exterior and moving inside. There are no plants grown or sown especially for stingless bees. Rather, stingless bees gather pollen from various plants, fruits, vegetables, and spices that are in the vicinity of their nests. The respondents were unsure as to whether stingless bees have a derth period or not. The process of transferring a colony involves removing the entire contents of a nest from its original habitat.

Botanical origin

Major plant species found in the pollen samples included Vernonia abyssinica, Bidens prestinaria, Eucalyptus globulus, Brassica carinata, and Plantago lanceolata L. (See Table 6). According to the pollen analysis, Meliponula beccarii prefers to visit herbaceous plants over tree plants, in contrast to Apis mellifera, which feeds on all types of shrubs, trees, and herbs. Stingless bees visit Eucalyptus globulus trees because it is continuously available throughout the year. Due to stingless bees' poor flight rate or their inability to get to blooms that Apis mellifera L. According to Cairns et al. (2005). The majority of stingless bee nests in the current study regions are found in fallow and farmed fields, which support the development of herbaceous vegetation. This is because, according to Jalil et al. (2017), stingless bees are not picky when creating a colony hive. The authors claim that because of their small size, stingless bees are uniquely suited to pollinate small flowers, something that relatively large honey bees are unable to accomplish.

Physico-Chemical Analysis

The main physical and chemical measures made in this study are the amount of honey moisture, the pH and free acidity, the amount of total ash, the amount of sugar, and the amount of hydroxyl furfuraldehyde (HMF).

Moisture content

The honey samples analysed in the current study had moisture contents ranging from 30% to 33.8%. The honey mostutre content varies significantly ($p<0.05$) between the three agro-ecologies (Table 6). In generl, honey moisture content is a physical parameter that is connected to the climate and honey maturation level. The variations in the research regions' water availability and flower species availability could also be the cause of the discrepancy. Studies on honey's moisture content or proportion have also shown that location has a major impact; moisture proportions of 25% to 42% have been documented (Souza et al., 2006; Nascimento et al., 2015). It's possible that stingless bees are naturally capable of figuring out the ideal moisture level for ripe honey. The lowland in the current study had the lowest moisture content (30%), whereas the highland had the highest moisture level (33.8%). There was a 3.8% moisture differential between the highland and lowland agro-ecologies. Conversely, honey collected from lowland regions was sweeter than honey from highland regions. Differing water contents could be the cause of the discrepancy. The moisture level of honey has a significant role in determining its stability and longevity. Increased moisture content may cause unfavourable yeast fermentation of the honey, which would produce carbon dioxide and ethyl alcohol. A sour flavour and runny consistency are also caused by the breakdown of ethanol into acetic acid and water, rendering the honey unfit for human consumption (Nascimento et al., 2015).

Table 6

The Physico-Chemical characteristics of honey from stingless bee across agro ecologies

Parameters	ML	LL	HL	Overall range	SE	P-value
Moisture Content (%)	31.36[b]	30.93[b]	33.23[a]	30-33.8	0.40	0.011
pH	3.93	3.80	3.86	3.7-4.0	0.03	0.394
Ash test (%)	0.26	0.24	0.22	0.17-0.27	0.01	0.45
Free Acidity (meq/kg)	60.22	56.69	52.51	48.95-64.08	1.57	0.075
HMF (mg/100g)	4.04	3.7	3.8	1.63-6.08	0.48	0.968
Fructose (%)	26.6	28.6	27.8	23.85-32.21	0.93	0.727
Glucose (%)	25.7	24.7	22.8	21.75-26.92	0.99	0.541
Sucrose (%)	2.58	4.38	2.43	1.05-4.83	0.40	0.060

Different superscripts indicate significant differences; ML = Midland; LL = Lowland; HL = Highland; Meq/kg = milliequivalents per kilogram; HMF = Hydoxymethylfurfural

Stingless bee honey generally has a higher moisture content than Apis mellifera honey. This might be because stingless bee honey has a high hygroscopicity (Alves et al., 2005; Nascimento et al., 2015). According to the authors, bee species' inherent characteristics and the materials they utilised to build their honey storage can also have an impact on the moisture content of honey. Because stingless bee honey has a high moisture content, it should be stored in a refrigerator to prevent yeast deterioration and changes to its physical and chemical characteristics.

Sugar content of Stingless bee's honey

In the current investigation, there was no discernible variation in sugar content across the agro-ecologies ($p > 0.05$) (Table 6). Compared to A, stingless bee honey has a lower reducing sugar concentration. honey from mellifera. The amounts of fructose, glucose, and sucrose that were obtained in the current study range from 23.85% to 32.21%, 21.75% to 26.92%, and 1.05% to 4.83%, respectively. With the exception of glucose, which was higher in highland agro-ecologies, the proportions of these simple sugars were highest in low-land agro-ecologies. The decreased sugar content and acidic pH of stingless bee honey contribute to its taste, indicating a strong consumer preference for this kind of honey (Nascimento et al., 2015). The sucrose content found in this study is greater than the range of 0.52-2.86% from Venezuelan honey produced by the Melipona species that Bogdanov and Kilchenmann (1994) reported. The botanical source of nectar has an impact on the sucrose content of honey, suggesting that the sugar composition of honey is dependent on both the sugars found in the flora and their conversion by bee-secreted enzymes.

Free acidity

In the current investigation, the free acidity of stingless bee hone did not significantly differ (p>0.05) among the three ecologies. The current analysis yielded free acidity values ranging from 48.95% to 64.08% (Table 6). A discrepancy could result from improper management of the honey-harvesting process or from transferring the product for laboratory testing. Free acidity is one of the quality indicators of honey. It indicates whether the honey is fermented or not and correlates to the presence or absence of organic acids in the product. Furthermore, acidity rises with fermentation and is correlated with the honey's maturity level (Alves et al., 2005). In comparison to A, stingless bee honey often has a higher acidity. honey from mellifera. The characteristic sour flavour of stingless bee honey is caused by free acidity (Bogdanov and Kilchenmann, 1994; Nascimento et al., 2015). When compared to honey from A, stingless bee honey has a higher antibacterial efficacy and therapeutic benefit due in part to its high acidity. mellifera (Garedew et al., 2003; Bogdanov et al, 1996).

pH values

The measurement of a solution's acidity or alkalinity, or pH value, serves as a physical-chemical metric that is linked to the microbial development of any given food. The current study yielded pH values ranging from 3.8 to 3.9 (Table 6), with no significant differences (p>0.05) seen between the agro-ecologies. Various publications have observed varying pH levels for stingless bee honey, ranging from 3.3 to 4.7 (Bogdanov et al., 1999; Alves et al., 2005). The pH range of stingless honey bee honey was reported by Nascimento et al. (2015) from Brazil to be 2.93 to 4.08. The flora that bees use, the storage conditions, and the geographic areas in which the honey is produced can all have an impact on the pH values of honey (Terrab et al., 2004).

Ash (%)

The study's ash contents, which range from 0.17 to 0.27. The amount of ash in honey samples is thought to be a good predictor of their purity. The amount of minerals in honey is indicated by its ash content, which is determined by the nectar's botanical source and the kind of soil seen in the nests of stingless bees (Nascimento et al., 2015; Garedew et al., 2003). Meliponinae honey produced in Brazil has ash concentrations ranging from 0.140 to 0.327%, according to Nascimento et al. (2015). However, Bogdanov et al. (1996) found that the ash percentage in Venezuelan stingless bee honey ranges from 0.12 to 0.76%.

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is described as a naturally occurring breakdown product of fructose that forms gradually during honey storage and much more quickly upon heating. It is commonly used as a gauge of the freshness of honey (FAO, 2009). The measurement of the amount of hydrogen methylfurfural (HMF) in honey serves as a benchmark for determining the degree of heating. The quality of the honey decreases with increasing Hydroxymethylfurfural (HMF) value. Honey's concentration of Hydroxymethylfurfural (HMF) increases with storage and extended heating. Certain nations impose limitations on the amount of Hydroxymethylfurfural (HMF) that can be found in imported honey, such as 40 mg/kg. Honey that exceeds these standard limits is not recognised.

CONCLUSIONS

Stingless bees build their nests in farm land, fallow land areas and open or grazing areas. Their nests are different in size among agro-ecologies. However, there was no observed difference with regard to the entrance gate holes of the nests. The average nest temperature was highest in the lowland and lowest in highland. On the other hand, moisture content of honey from stingless bees was lowest at lowland agro-ecology. Similarly, Stingless bees' products are lower in lowland agro-ecology compared to the midland and highland agro-ecologies. *Guizotia abyssinica, Vernonia abyssinica, Bidens prestinaria, Eucalyptus globulus, Brassica carinata* and *Plantago lanceolata L*. were the major plant species most used by stingles bees in the study areas. Wasps, honey badger, ants and birds are some of the major enemies of the stingless bees in the study areas in their order of importance. In addition, man is also reported as an enemy of the bees due to its destractive role during honey harvesting. Stingless bees prefer herbaceous types of flora than types of tree which indicates their short distance flight from their nest. From the current study, it can be concluded that stingless bees survive in lowland, midland and highland agr-ecologies, but they less prefer the lowland weather may be due to moisture stress.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENTS

The data of this study are available from the corresponding author upon request.

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