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Research article

Botanical origins of honeys from pollen analysis during the main honey flow across agro-ecologies in kelala district, South Wollo, Ethiopia

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ABSTRACT

This study aimed to identify the botanical origins of honey through pollen analysis across agro-ecologies of Kelala district, South Wollo, Ethiopia. Fifteen honey samples were collected from traditional beehives, with 5 samples from each of the highland, midland, and lowland agro-ecologies. Qualitative and quantitative pollen analyses revealed that 22 honeybee plants belonging to 8 families, with no families of poisonous origin, and 3 growth forms were identified. Among these plant species, 18 were found in the midland and 13 were recorded in each of the highland and lowland agro-ecologies. The family Fabaceae, with 7 (31.81 %) of the identified honeybee plants, was highly dominant ($p < 0.03$) compared to the other families. Herbs, with 18 plant species, were highly dominant ($p < 0.001$) compared to shrubs and trees. In terms of plant species diversity, 10 plant species (*Bidens pachyloma, Guizotia scabra, Becium grandiflorum, Eleusine floccifolia, Lens culinaris, Lippia adoensis, Medicago polymorpha, Ocimum basilicum, Trifolium steudneri,* and *Zea mays*) were found in more than 50 % of the honey samples, with the first 2 in all the samples studied. Analysis of each honey sample showed that 8 (53.33 %) of the samples were monofloral, 3 (20 %) were bifloral, and the rest were multifloral. However, all the honeys produced due to agro-ecology (geographical origin) were monofloral. *Guizotia scabra* in the highland and *Bidens pachyloma* in the midland and lowland agro-ecologies were the predominant pollen producing species and contributors of monofloral honey. In conclusion, the safe and healthy monofloral honey produced across agro-ecologies suggests the suitability of the honey for human consumption and can potentially attract investors.

1. Introduction

Ethiopia has a rich biodiversity of flowering plants, many of which belong to honeybee flora, favorable climatic conditions, and abundant water resources sustaining about 5,982,336 honeybee colonies kept in beehives besides unidentified huge numbers existing in the wild. Subsequently, it ranks as one of the world's largest producers of honey and beeswax [1,2]. In 2022, the country produced approximately 52,034,413 kg of honey, with 93.3 % of the product coming from honeybee colonies managed in traditional beehives. The Amhara region, one of Ethiopia's ten regional states, contributed 10,172,964 kg of honey (19.55 % of the national product) from 1, 330,300 honeybee colonies in the same year. This enabled the region as the second largest honey producer after the Oromia region [2].

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Specifically, the Kelala district within the South Wollo zone of the Amhara region sustains 10,325 honeybee colonies and stands out as an area with high potential for beekeeping. The beekeeping in the district, predominantly practiced using traditional beehives (92.82 %) and an insignificant proportion of transitional (1.96 %) and frame beehives (5.22 %), presents several advantages over other agricultural activities. It provides income, ensures food security, combats poverty, creates job opportunities, and contributes to plant pollination and ecosystem conservation [1,3].

Honeybees rely on nectar and pollen from plants for their nourishment, and the availability of these resources is crucial for successful beekeeping [1,4,5]. However, all honeybee plants do not equally contribute to honeybees and honey production [6]. Some plants offer abundant nectar and pollen, while others provide only one of these resources [7]. Understanding the types, abundance, and quality of honeybee flora resources is essential for maximizing yields of honey and other honeybee products [1,8].

Pollen analysis in honey, known as melissopalynology, helps determine the botanical and geographic origins of honeys. Microscopic analysis of honey samples reveals the pollen content, aiding in identifying plants visited by honeybees [3,9,10]. As pollen grains from different botanical origins exhibit distinct morphological characteristics due to their genetic heredity, the qualitative and quantitative analysis of pollen grains through an optical microscope becomes relatively simple [5]. In fact, this traditional method of pollen analysis is time-consuming and may not effectively distinguish closely related plant species [11]. More recently, metagenomics methods, which utilize DNA sequencing, have been adopted [5]. These methods are capable of characterizing the composition of mixed pollen samples at a lower cost compared to the traditional melissopalynological approach [12]. However, the metagenomics methods have limitations since different genetic markers and sequencing platforms are used, lessening the comparability of independent studies [5]. Some plants, with specific characteristics in their cell structures, also complicate DNA extraction, causing difficulty in DNA analysis [13]. Despite these limitations, both methods are widely used and provide valuable information.

The honey pollen analysis involves identifying and counting pollen grains to determine the relative frequencies of each pollen type [5,14–16], thereby categorize honey samples into monofloral (dominated by ≥ 45 % pollen grains from a single plant species), bifloral (containing pollen from two plant species with a frequency of 15–45 % per species), or multifloral (containing pollen from multiple plant sources each with a frequency of <15 % per species). To aid in the identification process, a reference pollen slide collection (pollen library) is used to compare the morphology of pollen grains found in honey with those obtained directly from flowers [14,16,17]. By counting the pollen grains available on a microscope slide, researchers can assign a frequency class to each pollen type - predominant pollen (PP), secondary or accessory pollen (AP), important minor pollen (IMP), and minor pollen (MP), based on their

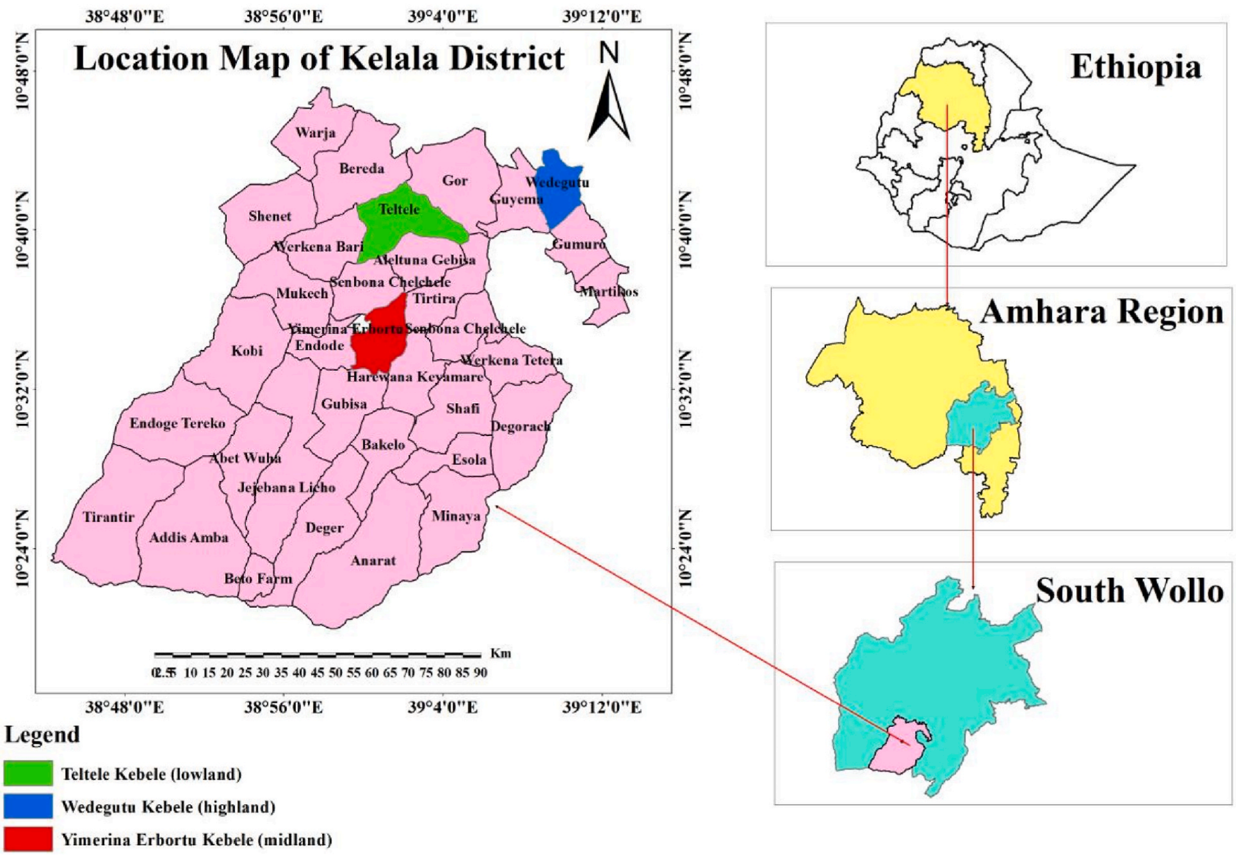

Fig. 1. Location map of Kelala district showing the study sites. [a,b] Different superscript letters associated with the percentage values refer to the significant variation among the corresponding frequencies at $p < 0.05$.

relative abundance of >45, 15–45, 3–15, and <3 %, respectively. Monofloral honey is perceived as high-quality product, being highly preferred by consumers, and subsequently earns higher price; moreover, it has several health benefits such as wound healing, antioxidant, anti-inflammatory, and anticancer activities [13,18]. A multifloral honey contains a variety of pollen and is considered to be rich in essential amino acids, aiding colony development. The presence of some toxic pollens is balanced by the favorable ones which makes multifloral honey safe for consumption [19,20].

Ultimately, this pollinic analysis helps infer the vegetation present in a specific area, understand any biodiversity change, and determine whether the honey is monofloral, bifloral, or multifloral [3,21,22].

Factors such as botanical and geographical origins, as well as handling conditions, affect the quality of honey [18] and these factors are considered to change through time. The decline in natural vegetation, including honeybee plants, due to population growth, urbanization, and climate change, has led to limited availability and low quality of pollen, affecting honeybee performance [1,23–26]. Identifying the existing honeybee plants in different geographical locations is crucial for improving the beekeeping sector.

In Ethiopia, studies have been conducted on the identification of honeybee plants through honey pollen analysis in various regions, such as those in Oromia region [8,15,27,28], as well as in southwest Ethiopia [29]. Kelala district in the Amhara region has a high potential for beekeeping, with the main honey flow season extending from the end of September to November. However, information related to the botanical origins and types of honeys produced in different localities (based on agro-ecologies) have not yet been documented.

The challenges affecting the natural vegetation, including honeybee forages, in the diverse agro-ecological zones of different parts of the country will also impact Kelala district. Furthermore, some plants are considered significant nectar or pollen sources for a specific location, differing from the food sources identified in other localities. Since the availability of floral sources, particularly pollen determines the survival and development of honeybee colonies [30], the identification of honeybee plants is essential for each locality over time. The study, therefore, aimed to identify the botanical origins and types of honeys produced during the main honey flow season, through honey pollen analysis, in different agro-ecologies of Kelala district, South Wollo, Ethiopia.

2. Materials and methods

2.1. Description of the study area

The study was conducted in Kelala district (10°24′00″ to 10°48′00″ N latitude and 38°48′00″ to 39°12′00″ E longitude), South Wollo, Ethiopia (Fig. 1). The district covers an area of 115,259.4 km^2 with an altitude ranging from 1226 to 2992 m above sea level (masl). According to the African Rainfall Climatology (ARC) satellite data for 2009 to 2018 (inclusive), the mean annual rainfall was 1092 mm. Meteorological Agency of Ethiopia's 10-year data (2009–2018) indicated that the minimum and maximum temperatures were 12.5 and 25 °C, respectively. The district is home to 2580 beekeepers managing 10,325 honeybee colonies, with 9584, 202, and 539 of the colonies being managed in traditional, transitional, and frame beehives, respectively. Kelala district is sub-divided into lower administrative units, nationally named as *kebeles*. It is characterized by three altitude-based climatic zones (agro-ecological zones): highland (*dega*), midland (*weina dega*), and lowland (*kolla*), with corresponding altitude ranges of above 2,400, between 1500 and 2,400, and below 1500 masl, respectively [1].

2.2. Selection of sites and honey sample collection

In the district, honeybee colonies are distributed throughout all agro-ecological zones of the district. Three sites (*kebeles*), namely, Wedegutu, Yimerina Erbortu, and Teltele were selected based on the beekeeping potential from the highland, midland, and lowland agro-ecologies, respectively. Since a significant number of the honeybee colonies (9584 or 92.82 %) were managed in traditional beehives and contributed substantial amounts of honey yield in the district, honey samples collected from this beehive type were considered for the study. On average, about 4 honeybee colonies were kept in traditional beehives per beekeeper. Therefore, experienced beekeepers who managed a minimum of 4 of their honeybee colonies in traditional beehives were listed in the selected *kebeles*, followed by randomly selecting 5 beekeepers per *kebele*, ensuring that the apiaries of the selected beekeepers were at least 3 km apart from each other to cover a broader foraging area of the honeybees.

Fifteen honey samples, produced by *Apis mellifera*, were collected from 15 different strong colonies belonging to the selected beekeepers. Each beekeeper was instructed to assign one of his strong colonies for honey sample collection during the main honey flow season (end of September to November). The beekeepers were further informed to harvest the honey samples following similar procedures: harvest on sunny days, but not on rainy days or during high relative humidity [10], harvest honeycombs fully sealed with honey and with no brood or empty cells, and collect the honey within one week of the peak honey harvesting period in plastic containers supplied by researchers with utmost cleanliness. Each honey sample, weighing 500 g, was purchased from the beekeepers at an agreed price in the next morning of the harvesting day since the harvesting was done at night as in most parts of the country.

Each collected honey sample was processed by breaking it into pieces, followed by straining using a sieve, allowing it to settle, and storing the homogenized sample in tightly closed plastic containers at 4 °C until analysis [5,10,31].

2.3. Honey pollen analysis

The honey samples collected across agro-ecologies were analyzed at Sekota Dry Land Agricultural Research Center (SDARC) laboratory to identify their botanical origins and the types of honey produced based on the frequency of each pollen type determined during the honey pollen analysis. The honey sample analysis was performed following the procedures recommended by the International Commission for Bee Botany [9]. Ten grams of each honey sample were mixed with 20 ml of warm distilled water (40 °C) in a pointed glass centrifuge tube, followed by centrifugation of the solution for 10 min at 1048 gravity (2500 revolutions per minute, rpm), decanting the supernatant, and collecting the residue into a conical tube. Then, 20 ml of distilled water was added to fully dissolve the remaining residue (sugar crystals), which was further centrifuged at 1048 gravity (2500 rpm) for 5 min, and the supernatant was decanted. The residue was then let to dry by evenly spreading with a micro spatula on a microscope slide. A drop of glycerine jelly was applied to the residue to make the pollen grains clear, and each sample was examined using an optical microscope (Carl ZEISS Axiolab 5 Microscopy GmbH, Germany) with a 100 × magnification power [5,10,31]. The frequency of occurrence of each type of pollen grain was calculated by counting 500 pollen grains from a single slide using a hemocytometer (counting chamber), and it was grouped into one of the four pollen frequency classes. The identified pollen grains in the honey samples which represented more than 45, 15–45, 3–15, and less than 3 % of the 500 pollen grains were then classified accordingly. Honey samples that contained the predominant pollen types (>45 %) were termed monofloral honeys; otherwise, they were considered either bifloral (with 15–45 % pollen from each of two plant species), or multifloral honeys, with less than 15 % of each pollen type [9,10,31,32].

Plant species revealed in more than 50 % of the honey samples are known as bee-favored [33]. Most scientific and common botanical names of plant species as well as their growth forms or habits (trees, shrubs, and herbs) were obtained from the detailed descriptions outlined in previous publications [1,23]. The number of plant species within a family was divided by the total number of honeybee plant species identified and multiplied by 100 to determine the relative abundance of botanical families [8].

2.4. Data analysis

Analyses of the collected data were performed using R software [34]. Frequency data associated with the levels of categorical variables (plant species belonging to different agro-ecology, growth forms, and families) were compared using the one-way Chi-Square (χ^2) procedure at $p < 0.05$ level of significance. The remaining data were summarized using descriptive statistics.

3. Results

3.1. Plant species identified through honey pollen analysis

The results of the current study show that 22 honeybee plants were identified based on the honey pollen analysis (Table 1). The relative diversity of the honeybee plants identified in the midland agro-ecology, which was 18 of the total 22, was higher than the 13 honeybee plants reported in each of the highland and lowland agro-ecologies (Tables 1 and 4). Regarding the plant species identified from each honey sample, one of the samples from the lowland agro-ecology (L4) with only 6 plants was the least rich in pollen types or had fewer diverse plant species unlike the maximum 12 plant species, with the highest richness of pollen types, identified in each of the honey samples collected from the midland agro-ecology (M1 to M5) as well as in one of the honey samples from the highland agro-ecology (H3).

Table 1
List of honeybee plants identified during the main honey flow season from honey pollen analysis and their contribution as pollen and/or nectar source, life form, and the families to which they belong across agro-ecologies of Kelala district, South Wollo, Ethiopia.

List of plant species	Local name (Amharic)	Family name	Agro-ecology	Life form	Contribution
Acacia albida	*Girar*	Fabaceae	Lowland	Tree	Pollen/nectar
Becium grandiflorum	*Matush*	Lamiaceae	Highland/midland	Shrub	Pollen/nectar
Bidens pachyloma	*Adey Abeba*	Asteraceae	All AEZs[a]	Herb	Pollen/nectar
Brassica carinata	*Gomenzer*	Brassicaceae	Midland	Herb	Pollen/nectar
Echinops macrochaetus	*Kosheshila*	Asteraceae	Midland/lowland	Herb	Pollen/nectar
Eleusine floccifolia	*Akrma*	Poaceae	All AEZs	Herb	Pollen
Guizotia abyssinica	*Nug*	Asteraceae	Midland	Herb	Pollen/nectar
Guizotia scabra	*Mech*	Asteraceae	All AEZs	Herb	Pollen/nectar
Hordeum vulgare	*Gebis*	Poaceae	Highland	Herb	Pollen
Lens culinaris	*Misir*	Fabaceae	Highland/midland	Herb	Pollen/nectar
Linum usitatissimum	*Telba*	Linaceae	Midland	Herb	Pollen
Lippia adoensis	*Kessie*	Verbenaceae	All AEZs	Herb	Nectar
Medicago polymorpha	*Waszima*	Fabaceae	All AEZs	Herb	Pollen/nectar
Ocimum basilicum	*Besobila*	Lamiaceae	All AEZs	Herb	Pollen/nectar
Pisum sativum	*Ater*	Fabaceae	Highland	Herb	Pollen
Rumex nervosus	*Embacho*	Polygonaceae	Midland/lowland	Shrub	Pollen
Senna siamea	*Yeferenji digita*	Fabaceae	Lowland	Shrub	Pollen
Sorghum bicolor	*Mashila*	Poaceae	Midland/lowland	Herb	Pollen
Triticum aestivum	*Sindie*	Poaceae	Midland	Herb	Pollen/nectar
Trifolium steudneri	*Maget*	Fabaceae	All AEZs	Herb	Pollen/nectar
Vicia faba	*Bakiela*	Fabaceae	Highland/midland	Herb	Pollen/nectar
Zea mays	*Bekolo*	Poaceae	All AEZs	Herb	Pollen

[a] AEZs = agro-ecological zones.

On the other hand, 10 (45.45 %) of the plant species (*Bidens pachyloma, Guizotia scabra, Becium grandiflorum, Eleusine floccifolia, Lens culinaris, Lippia adoensis, Medicago polymorpha, Ocimum basilicum, Trifolium steudneri,* and *Zea mays*) were found in more than 50 % of the honey samples studied, with the first 2 occurring in all the samples; whereas, the rest 12 (54.55 %) plant species were present in less than 50 % of the honey samples. Eight (36.36 %) of the honeybee plants found in more than 50 % of the honey samples (*Bidens pachyloma, Guizotia scabra, Eleusine floccifolia, Lippia adoensis, Medicago polymorpha, Ocimum basilicum, Trifolium steudneri,* and *Zea mays*) were distributed across all agro-ecologies (Tables 1 and 2).

Of the 22 honeybee plants identified, 13 (59.09 %) were major sources of pollen and nectar, 8 (36.36 %) were pollen source plants, and only 1 (4.55 %) plant was a nectar source (Table 1).

3.2. Families and growth forms of honeybee plants

The identified honeybee plants were categorized into 8 families (Fig. 2). Fabaceae, which comprised 7 (31.81 %) of the identified species, was highly dominant ($p < 0.03$) than the other families, followed by Poaceae (22.72 %), Asteraceae (18.18 %), and Lamiaceae (9.09 %). Each of the remaining 4 families (Brassicaceae, Linaceae, Polygonaceae, and Verbenaceae) consisted of only one species with a relative frequency of 4.55 %. On the other hand, the plant species were grouped into 3 types of plant growth forms (habits): trees (5.56 %), shrubs (11.11 %), and herbs (83.33 %). Significantly higher ($p < 0.001$) numbers of the identified honeybee plants, therefore, belonged to the herbs than those in the other growth forms (Fig. 3).

3.3. Types of honeys produced based on pollen dominancy

The relative pollen grain frequency of identified honeybee plants from each honey sample and the average values due to geographical origins (agro-ecologies) are presented in Tables 2 and 3, respectively. Eight (53.33 %), 3 (20 %), and 4 (26.67 %) of the honey samples tested were monofloral, bifloral, and multifloral, respectively. The relative pollen grain frequency of the honey samples showed that *Guizotia scabra* in 3 of the samples (H1, H3, and H4) collected from the highland agro-ecology, and *Bidens pachyloma* in 2 of the samples (M1 and M3) from the midland agro-ecology and in 3 of the samples as well (L1, L3, and L4) from the lowland agro-ecology found to be predominant honeybee plants. Each of the two honey samples from the midland agro-ecology (M4 and M5) and one sample from the highland agro-ecology (L5) contained pollen grains with a frequency of 15–45 % from each of two honeybee plants (known as secondary or accessory pollen sources), *Bidens pachyloma* in common along with *Zea mays* (M4), *Guizotia scabra* (M5), and *Sorghum bicolor* (L5), providing bifloral honeys. The other 4 honey samples (H2, H5, M2, and L2) contained pollen grains with various frequencies of less than 45 %. Accordingly, 8 of the 15 honey samples (53.33 %) were regarded as monofloral honeys; whereas, 3 (20 %) of the samples were bifloral honeys, and the rest 4 (26.67 %) were multifloral honeys (Table 2).

Despite the three honey types reported in reference to individual honey samples, the overall result revealed that the honey produced in all agro-ecologies was monofloral (Tables 3 and 4). The plant species *Guizotia scabra* in the highland agro-ecology and *Bidens pachyloma* in the midland and lowland agro-ecologies were the predominant pollen producing species identified and contributors of monofloral honey such that the proportions of pollen grains counted for each species accounted for more than 45 % of the pollen grains considered in one microscope slide.

Plants classified as secondary pollen sources were *Becium grandiflorum* and *Bidens pachyloma* in the highland, *Guizotia scabra* and *Zea mays* in the midland, and *Sorghum bicolor* in the lowland agro-ecologies. *Becium grandiflorum* and *Guizotia scabra*, under the secondary pollen class in the highland and midland agro-ecologies, were respectively grouped in the important minor pollen category in the midland and lowland agro-ecologies; whereas, *Zea mays* and *Sorghum bicolor* were also present in the minor pollen class in the highland and midland agro-ecologies, respectively. The diversity of honeybee plant species under the 'important minor pollen' and 'minor pollen' classes was higher than those categorized under the 'predominant pollen' and 'secondary pollen' frequency classes (Table 4). Pictures of some of the honeybee plants and their pollen grains are presented in Fig. 4(a–f).

4. Discussion

4.1. Plant species identified through honey pollen analysis

The present study provides preliminary insights into the botanical and geographical origins of honeys in Kelala district. The botanical species of honeybee plants identified varied across the agro-ecologies (geographical locations), with more diversified plant species (18) in the midland agro-ecology than in the highland and lowland agro-ecologies (13 plants in each). The pollen availability in a particular area depends on the availability of floral resources and landscape diversity, both of which are directly associated with agro-ecology and affect the productivity of honeybees. These factors, along with seasonal fluctuations, limit the foraging radius of worker honeybees from a few hundred meters to several kilometres long [29,35,36].

Species richness was lowest in one of the honey samples from the lowland agro-ecology (L4), containing pollens only from 6 honeybee plants, while all the honey samples from the midland agro-ecology (M1, M2, M3, M4, and M5) and H3 from the highland agro-ecology, which contained pollens from a maximum of 12 floral types, are the highest rich in pollen types [37], and the rest honey samples contained pollen types in between. The availability of a diverse source of pollen diet is vital for the healthy nutrition of honeybee colonies [38,39] because of the variation in the type and amount of amino acids contained in the pollen of different plant species. Overall, a consistent supply of pollen from diverse sources of flora is beneficial to honeybee colonies [40].

The information on pollen occurrence, which describes the types of pollen collected by honeybees, and its abundance that well

Table 2
Relative frequency of pollen grain types (% distribution) of honeybee plant species identified in honey samples collected across agro-ecologies of Kelala district, South Wollo, Ethiopia.

Plant species	Highland					Midland					Lowland					NHS[5]
	H[2]1	H2	H3	H4	H5	M[3]1	M2	M3	M4	M5	L[4]1	L2	L3	L4	L5	
Acacia albida	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	4.60	7.60	0.0	0.0	9.80	3
Becium grandiflorum	14.2	15.6	11.6	17.8	23.8	4.4	2.2	3.2	1.2	5.0	0.00	0.00	0.0	0.0	0.00	10
Bidens pachyloma	17.0	22.4	17.2	13.8	15.6	74.0	32.2	53.4	37.8	39.6	47.60	37.60	67.0	50.6	43.20	15
Brassica carinata	0.0	0.0	0.0	0.0	0	0.4	0.0	1.2	0.0	1.4	0.00	0.00	0.0	0.0	0.00	3
Echinops macrochaetus	0.0	0.0	0.0	0.0	0	1.0	2.2	0.8	0.0	0.0	0.00	2.40	0.0	0.0	6.60	5
Eleusine floccifolia	0.8	1.4	1.0	0.8	0	0.0	3.0	4.2	5.0	5.8	0.00	0.40	3.2	1.4	0.00	11
Guizotia abyssinica	0.0	0.0	0.0	0.0	0	0.0	0.2	0.0	0.0	0.8	0.00	0.00	0.0	0.0	0.00	2
Guizotia scabra	50.2	43.6	48.2	54.0	39	7.6	18.4	17.6	13.8	19.6	9.40	5.80	3.4	12.6	7.80	15
Hordeum vulgare	6.6	0.0	6.2	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.0	0.0	0.00	3
Lens culinaris	4.6	5.6	2.0	4.8	4	4.8	6.2	0.0	0.0	6.0	0.00	0.00	0.0	0.0	0.00	8
Linum usitatissimum	0.0	0.0	0.0	0.0	0	2.0	0.0	1.2	0.8	0.0	0.00	0.00	0.0	0.0	0.00	3
Lippia adoensis	0.0	2.0	1.8	1.2	0	2.8	5.6	4.2	3.2	1.2	0.00	16.20	0.0	23.2	8.60	11
Medicago polymorpha	0.6	2.2	1.6	0.0	2.6	1.4	5.2	0.0	4.8	4.6	0.00	0.00	4.0	0.0	0.00	9
Ocimum basilicum	1.0	3.4	1.8	1.2	2.6	0.0	0.0	0.0	2.0	0.0	8.40	1.60	0.0	0.0	0.00	8
Pisum sativum	0.0	1.4	3.2	1.2	2.2	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.0	0.0	0.00	4
Rumex nervosus	0.0	0.0	0.0	0.0	0	0.6	0.0	0.4	0.4	0.6	2.60	0.00	1.4	0.0	0.00	6
Senna siamea	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.60	0.80	0.6	0.0	1.00	4
Sorghum bicolor	0.0	0.0	0.0	0.0	0	0.0	0.0	1.2	1.8	0.0	19.80	21.00	14.0	10.2	19.00	7
Triticum aestivum	0.0	0.0	0.0	0.0	0	0.2	1.6	0.0	0.0	1.2	0.00	0.00	0.0	0.0	0.00	3
Trifolium steudneri	0.0	1.6	2.6	0.8	0	0.0	0.4	1.6	0.0	0.0	2.40	3.00	0.0	2.0	0.60	9
Vicia faba	4.4	0.0	2.8	3.4	5.4	0.8	0.0	0.0	1.2	0.0	0.00	0.00	0.0	0.0	0.00	6
Zea mays	0.6	0.8	0.0	1.0	0.6	0.0	22.8	11.0	28.0	14.2	4.60	3.60	6.4	0.0	3.40	12
No. of plant species	10	11	12	11	10	12	12	12	12	12	9	11	8	6	9	

[2]H = honeys sampled from the highland agro-ecology.
[3]M = honeys sampled from the midland agro-ecology.
[4]L = honeys sampled from the lowland agro-ecology.
[5]NHS = number of the honey samples containing the plant species.

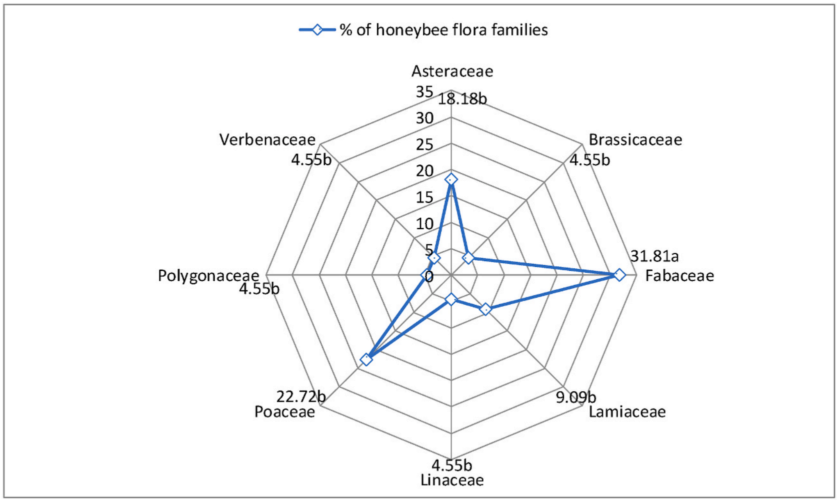

Fig. 2. Percentage of honeybee plant species from different botanical families identified from honey samples collected in Kelala district, South Wollo, Ethiopia. [a,b] Frequency values associated with different superscript letters differ significantly at $p < 0.05$.

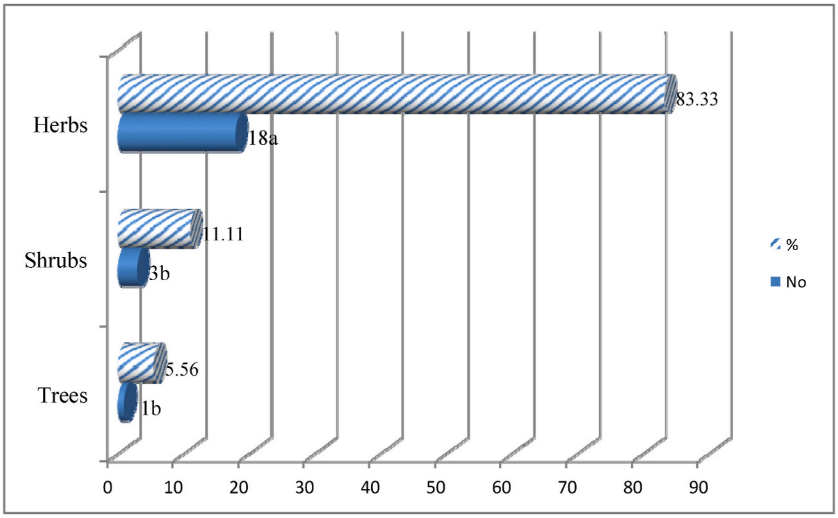

Fig. 3. Percentages and frequencies of identified honeybee plants classified based on their growth form at Kelala district, South Wollo, Ethiopia.

describes the types of pollen on which honeybees depend, helps understand the pollen source availability for honeybee nutrition [41]. The identification of *Bidens pachyloma* and *Guizotia scabra* in all the honey samples collected across the agro-ecologies of the study area suggests that honeybee colonies have a better preference for these plants for pollen collection. Moreover, these plants are widely distributed probably due to their wider climatic and edaphic adaptation. *Bidens pachyloma* is endemic to Ethiopia and easily adapts to various soil types on short grassland, mountainous areas with gentle slopes, river banks, margins of arable land, and roadsides at an altitude ranging from 2200 to 3300 m. It flowers at the end of the main rainy season (late September), which coincides with the main honey flow season in most parts of the country including the study area (Kelala district), and supplies large quantities of pollen for brood rearing and adult honeybees. Similarly, *Guizotia scabra* grows on short grasslands, meadows, forest margins, roadside ditches, and waterways in various agro-ecologies, and flowers all year round. *Bidens* species and *Guizotia scabra* have been reported as weeds with vital importance to honeybees and honey production in different parts of the country [1].

Herbaceous plants growing as weeds on cultivated lands and neglected open areas, and those used as ornamentals are important honeybee flora sources [42]. In Ethiopia, species belonging to *Bidens, Guizotia, Echinops,* and *Vernonia* are recognized as important honeybee plants due to their extensive distribution and abundant pollen production. *Becium grandiflorum* is a secondary pollen source in the highland agro-ecology but an important minor pollen source in the midland agro-ecology, and it is endemic to the Ethiopian highlands, growing on degraded lands and flowering throughout the year [1]. These plant species and others widely used by honeybees imply their valuable importance for honey production and the need for proper conservation of the honeybee flora.

Nearly 60 % of the honeybee plants identified in the current study were pollen and nectar sources, which corresponds to the fact

Table 3
Overall mean of relative frequency of pollen grains (% distribution) of honeybee plant species identified in the highland, midland, and lowland agro-ecologies of Kelala district, South Wollo, Ethiopia.

Plant species	Highland (%)	Midland (%)	Lowland (%)
Acacia albida	–	–	4.4
Becium grandiflorum	16.6	3.2	–
Bidens pachyloma	17.2	47.4	49.2
Brassica carinata	–	0.6	–
Echinops macrochaetus	–	0.8	1.8
Eleusine floccifolia	0.8	3.6	1
Guizotia abyssinica	–	0.2	–
Guizotia scabra	47	15.4	7.8
Hordeum vulgare	3.4	–	–
Lens culinaris	4.2	3.4	–
Linum usitatissimum	–	0.8	–
Lippia adoensis	1	3.4	9.6
Medicago polymorpha	1.4	3.2	0.8
Ocimum basilicum	2	0.4	2
Pisum sativum	1.6	–	–
Rumex nervosus	–	0.4	0.8
Senna siamea	–	–	0.6
Sorghum bicolor	–	0.6	16.8
Triticum aestivum	–	0.6	–
Trifolium steudneri	1	0.4	1.6
Vicia faba	3.2	0.4	–
Zea mays	0.6	15.2	3.6

Table 4
Classification of honeybee plants in reference to honey pollen grains count across agro-ecologies in Kelala district, South Wollo, Ethiopia.

Pollen frequency class/category	Agro-ecological zones		
	Highland	Midland	Lowland
Predominant pollen source (>45 %)	*Guizotia scabra*	*Bidens pachyloma*	*Bidens pachyloma*
Secondary pollen source (15–45 %)	*Becium grandiflorum* *Bidens pachyloma*	*Guizotia scabra* *Zea mays*	*Sorghum bicolor*
Important minor pollen source (3–15 %)	*Hordeum vulgare* *Lens culinaris* *Vicia faba*	*Becium grandiflorum* *Eleusine floccifolia* *Lens culinaris* *Lippia adoensis* *Medicago polymorpha*	*Acacia albida* *Guizotia scabra* *Lippia adoensis* *Zea mays*
Minor pollen source (<3 %)	*Eleusine floccifolia* *Lippia adoensis* *Medicago polymorpha* *Ocimum basilicum* *Pisum sativum* *Trifolium steudneri* *Zea mays*	*Brassica carinata* *Echinops macrochaetus* *Guizotia abyssinica* *Linum usitatissimum* *Ocimum basilicum* *Rumex nervosus* *Sorghum bicolor* *Trifolium steudneri* *Triticum aestivum* *Vicia faba*	*Echinops macrochaetus* *Eleusine floccifolia* *Medicago polymorpha* *Ocimum basilicum* *Rumex nervosus* *Senna siamea* *Trifolium steudneri*
No. of honeybee plants identified	13	18	13

that honeybees choose the same plants providing both pollen and nectar, constituting the entire diet of honeybees, although the nutritional contents and quantities of these honeybee foods differ substantially among plant species [43]. Ten (45.45 %) of the plant species (*Becium grandiflorum, Bidens pachyloma, Eleusine floccifolia, Guizotia scabra, Lens culinaris, Lippia adoensis, Medicago polymorpha, Ocimum basilicum, Trifolium steudneri,* and *Zea mays*), which were revealed in more than 50 % of the honey samples, are said to be honeybee-favored plants [33]. Except for *Becium grandiflorum* and *Lens culinaris*, the honeybee-favored plants were distributed in all the agro-ecologies probably due to their wider climatic and edaphic adaptation than the other honeybee plants mentioned.

4.2. *Families and growth forms of honeybee plants*

The family Fabaceae, comprising 7 honeybee plants (31.81 %), was the most significant representation of the pollen types compared to the other families identified. This family included plant species like *Acacia albida,* which are resistant to arid climatic conditions, as well as pulse crops such as *Lens culinaris, Pisum sativum,* and *Vicia faba*. It is one of the largest families in Ethiopia, comprising 108 genera and 639 species, where pulse crops, many forage species, and leguminous browse trees of economic importance for honeybees are included. Except for *Acacia albida*, there were no other tree honeybee forages revealed in this study, probably

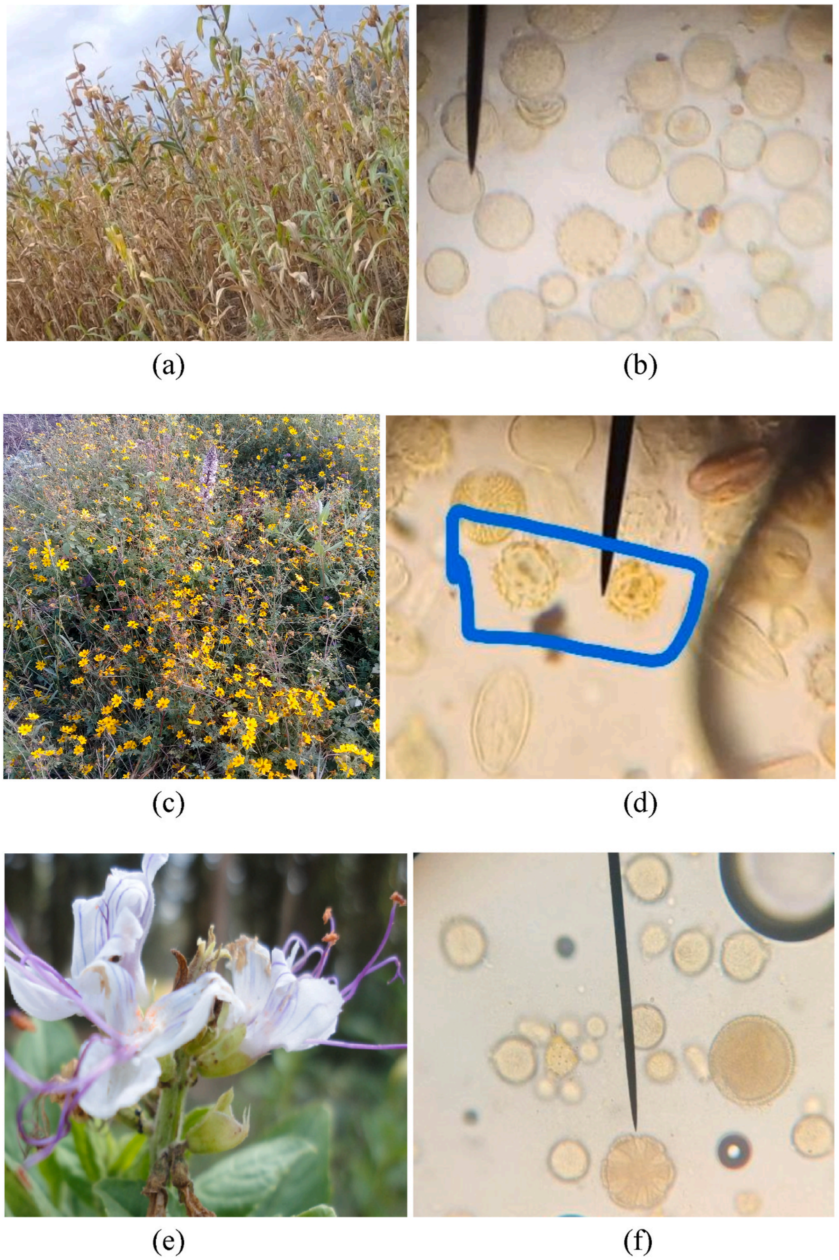

Fig. 4. Sample honeybee plants with their pollen grains: *Sorghum bicolor* (a) and its pollen grains (b); *Bidens pachyloma* (c) and its pollen grains (d); and, *Becium grandiflorum* (e) and its pollen grains (f).

because their flowering period did not coincide with the main honey flow period of the study district, or they were not primarly preferred by honeybees. The diversity of nectar and pollen source plants varies across seasons and sites [43]. In Austria, for instance, trees which were most abundant and provided 59.7 % of the pollen grains in April to May, sharply declined in pollen production from June to August, and the production completely disappeared in September, while that of herbs increased tremendously [41]. On the other hand, reports indicate that honeybees used to forage on only a fraction of the flowers available in their surroundings [44,45]. Only 11 % of the available flowering plants were foraged by worker honeybees in studies conducted in Wales [44], and less than 50 % and Southern Finland [43].

The families Poaceae and Asteraceae, next to that of Fabaceae, were reported to comprise abundant honeybee plants. Poaceae is the second largest family in Ethiopia with 149 genera encompassing 570 species. *Hordeum vulgare, Sorghum bicolor, Triticum aestivum,* and *Zea mays* belong to this family, providing pollen for brood rearing and honeybee colony development, and are among the staple foods for human beings. The Asteraceae family, consisting of 130 genera and 459 species in Ethiopia, possesses plant species that are excellent sources of pollen and nectar. The plant species belonging to *Echinops, Guizotia,* and *Bidens* are classified under the family

Asteraceae and are known for their wider geographical distribution and plentiful pollen sources in different parts of the country [1]. It has been indicated in an earlier study that Asteraceae (38 %) and Fabaceae (13.5 %) were the largest plant families of the total 42 plant families discovered [46]. Similarly, in a study conducted in Austria, 85 families were detected where Fabaceae and Asteraceae were mentioned as the most important families providing pollen to honeybees [41]. Asteraceae and Fabaceae were also the largest plant families recognized in Iran [47]. The higher contribution of Fabaceae, Poaceae, and Asteraceae in this study is expected since they are the most species-rich taxonomic families in Ethiopia [1].

Some honeybee plant species from the families Acanthaceae, Ericaceae, Euphorbiaceae, Phytolacaceae, Ranunculaceae, and Solanaceae are well known for their toxicity to honeybees [48,49]. None of these plant families was identified in the present study, suggesting that the identified honeybee plants are not harmful to honeybee colonies. Moreover, the honey produced in the study district (Kelala) can be considered safe and suitable for human consumption.

The family Fabaceae consists of trees, shrubs, and herbs, and is the only family in which a tree has been presented among the entire plants identified. The family Asteraceae primarily comprises herbs and shrubs, and trees are less common [1]. However, all the plant species documented under this family during the current study (*Bidens pachyloma, Echinops macrochaetus, Guizotia abyssinica,* and *Guizotia scabra*) were herbs. In the southwest Jimma zone of Ethiopia, herbs were dominant (44 %) but were followed by trees (34 %), with shrubs contributing the least (22 %) [28]. However, entirely different proportions of floral growth forms were reported in the southwestern region (Jhansi) of northern India, where trees were the most dominant (56 %), followed by herbs (28 %) and shrubs (16 %) [50]. The variations in the proportions of the types of pollen source plants among various studies are primarily attributed to the differences in the seasons of honey sample collection and geographical locations [43].

4.3. Types of honeys produced based on pollen dominancy

The types of honeys produced are based on pollen dominancy. Honey produced in a particular area is influenced by the honeybee flora of the locality and the foraging behavior of honeybees [36]. Regarding individual honey samples, slightly more than half (53.33 %), one-fifth (20 %), and about one-fourth (26.67 %) of all the honeys sampled were monofloral, bifloral, and multifloral honeys, respectively. However, the overall values revealed that all the honeys due to geographical origin (agro-ecology) were found to be monofloral. Inconsistent with the present finding, a recent study conducted across the different agro-ecologies in the southwestern part of Ethiopia reported that 93.06 % of the honey samples investigated were multifloral and 6.94 % were monofloral [29].

Pollen grains predominantly originating from a unique plant species (45 % of all nectariferous pollen grains counted) fall into the predominant pollen class, and the honeys are referred to as monofloral honeys [10]. Accordingly, the honeys in the midland and lowland agro-ecologies can be named *Bidens* honeys, and those in the highland agro-ecology can be referred to as *Guizotia* honeys, due to the predominant pollen types contributed from these plants [3]. In line with the current study, *Guizotia scabra* was reported as one of the predominant pollen source plants providing monofloral honeys in Guji zone [51] and Godere district [52] in Ethiopia. In another study [29], *Guizotia* species in all the agro-ecologies and *Bidens* species in the lowland agro-ecology were identified as secondary pollen type plants, dictating the better contribution of these plants to honeybees in different localities of the country.

Wild plants, cultivated crops, and plants in gardens are the three main sources from which the pollens in honey originate [53]. *Bidens* species and *Guizotia scabra* are weeds of vital importance as honeybee forages, contributing to honey production in Ethiopia. The other wild-growing plants recognized in the study area, including *Acacia albida, Becium grandiflorum, Echinops macrochaetus, Eleusine floccifolia, Lippia adoensis, Medicago polymorpha, Rumex nervosus, Senna siamea,* and *Trifolium steudneri* are also honeybee forages well adapted in different parts of the country [1,23,49]. *Sorghum bicolor* in the lowland and *Zea mays* in the midland agro-ecologies were among the plants classified as secondary pollen sources, where along with other cultivated crops in the important minor pollen and minor pollen classes (*Brassica carinata, Guizotia abyssinica, Hordeum vulgare, Lens culinaris, Linum usitatissimum, Ocimum basilicum, Pisum sativum, Triticum aestivum,* and *Vicia faba*) were valuable floral sources for honeybees, and comprised 50 % of the identified plant species. The contribution of cultivated crops as floral sources for honeybees was also reported in earlier studies in Ethiopia [29] and Kenya [54], and such information reflects the local agricultural practices in addition to the floral diversity and species composition of honeybee plants [9].

All the honeybee plants identified in the current study were important because they were not among the plants described as poisonous to honeybees [1,48,49]. Honeybee plants known for their toxicity to honeybees are commonly found within the families of Acanthaceae, Ericaceae, Euphorbiaceae, Phytolacaceae, Ranunculaceae, and Solanaceae [48]. None of these plant families was recovered in the current honey pollen analysis.

Honey, the natural product of honeybees, has diverse compositions influenced by the type of flora of a specific locality and the behavior of honeybees foraging for nectar and pollen [1,36]. Studies conducted in the central region of Shanxi province of north China [33], and in the Borana [8] and Guji [51] zones in Ethiopia indicated the high diversity of honeybee plants in multifloral honeys than in monofloral ones. In contrast, higher plant species diversity was reported in one of the sites in the Borana zone that produced monofloral honey [8]. Honeybees collect a wide variety of pollen types, but they generally focus on only a few or a fraction of the available flowering plants in their surroundings to collect nectar and pollen [43,55]. Factors that likely contribute to this species' specific preference for a few of the honeybee plants depend on the honeybee species' affinity, the flowering vegetation strata, and the environmental conditions [56]. The production of monofloral honey, regardless of the extent of plant species diversity, could therefore be associated with the high selection behavior of worker honeybees for food sources (pollen and nectar) and exhaustively exploiting the selected flowers until the food sources become depleted.

Nowadays, many honey consumers worldwide have a high preference for monofloral honeys over honeys of multifloral type. Accordingly, honey traders and processors are inclined to deal with monofloral honeys [3]. On the other hand, a multifloral pollen source (diet) is important for the development of honeybee colonies as it contains various types of pollen that provide essential amino acids. Besides, it minimizes the risk of collecting a high proportion of poisonous pollen as it is balanced by the presence of a variety of favorable pollens, unlike the predominant pollen source in monofloral honey [19].

The pollen analysis from 12 honey samples collected from Gera forest in the Oromia region of Ethiopia showed that all the honeys were monofloral [27]. However, they exhibited entirely different plant species compared to those identified in the current study. Moreover, it was found that 84 % of the honey samples collected from 19 localities in the Borana zone of southern Ethiopia were monofloral, despite originating from 28 diverse honeybee plant species [8]. Only about 4 of the honeybee plant species reported in this latter study were similar to those listed in the current study. The analysis of 87 honey samples from the French Guiana Atlantic coast also indicated that the predominant pollens were obtained from 12 plant species such as *Mimosa pudica, Cocos* sp., *Rhyncospora* sp., *Avicennia germinans, Paspalum* sp., and *Spermacoce verticillata* [57], all of which were entirely different from plants identified in any of the pollen frequency class in the current study. This indicates the inconsistency of botanical origins of honeys in different studies and the need for undertaking pollen analysis to discern the botanical and geographical origins of honeys of a specific locality. These wide variations in the types of honeybee plants across locations (various studies) mainly arise due to the differences in climatic and edaphic conditions which favor some species to thrive better in a specific locality than in others [1,23]. Such variations consequently resulted in the production of different types of honeys in the country [1,2], dictating the need to identify the botanical origins of honey of a specific location at different time intervals to characterize the honeys produced. Identifying the pollen types exploited by honeybees is also helpful for the proper maintenance and management of the plant species. The extensive national afforestation (green legacy), that has been implemented by the government of Ethiopia over the past five years, is expected to continue in the upcoming years. This initiative can potentially promote the supply of nectar and pollen sources for honeybee colonies through the introduction of new species and the conservation of the existing flora.

Various proportions of multifloral honeys were reported from the samples examined in several studies. To this regard, mention can be made of those in southwest Ethiopia with 93.06 % of the samples being multifloral [29], 26.32 % in the central region of Shanxi province of north China [33], 100 % in the central Alborz region and the mountainous Zagros ecosystems of Iran [46], 100 % in Estonia [58], 25 % in Malaysia [59], and 100 % in the regions of Konya, Karaman and Ulukışla of South Anatolia [60].

The higher plant species diversity in the minor pollen group and the decline in variability towards the dominant pollen group were consistent with the findings reported in several other studies [8,60,61]. The selective foraging behavior of worker honeybees and the high potential of plants to provide abundant food sources enable the production of monofloral honeys, such that the plant species in the predominant pollen group are the least diverse. However, plants in the minor pollen group do not provide good nectar and/or pollen or are less preferable to honeybees [5,10].

The predominant pollen source in the midland and lowland agro-ecologies, *Bidens pachyloma,* was grouped as a secondary pollen source in the highland agro-ecology, and *Guizotia scabra,* which was described as the predominant pollen source in the highland agro-ecology, was categorized under the secondary pollen class in the midland agro-ecology. This implies that dominancy due to pollen grain count is not consistent across locations, probably due to the variation in the abundance of the plant species in different places. A plant in the secondary or lower pollen grain count category may also be grouped as a predominant pollen source in the absence of other plants that are highly preferred by honeybees. Thus, undertaking honey pollen analysis across geographical origins (agro-ecologies) not only dictates the available honeybee plants but also their potential for honey production.

5. Conclusions

The present study highlighted the botanical and geographical origins of honeys in different agro-ecologies of Kelala district. The study identified more diversified honeybee plants in the midland agro-ecology than in the highland and lowland agro-ecologies. The identified honeybee flora were dominated by herbs than shrubs and trees. Cultivated crops contributed 50 % of the floral sources for honeybees. Fabaceae, Poaceae, and Asteraceae were the families containing more diversified floral sources for honeybees as they belong to species-rich taxonomic families in Ethiopia. *Guizotia scabra* in the highland and *Bidens pachyloma* in the midland and lowland agro-ecologies were the predominant pollen sources, suggesting the contribution and high potential of these species for monofloral honey production in the study area. Moreover, the honeys produced across agro-ecologies were safe and healthy, signifying their suitability for human consumption and potential market value. We also recommend further study to identify the major botanical origins and honey types produced in the other honey flow seasons. Additionally, the recent and DNA-based metabarcoding technique, with no available data on its use in Ethiopia, can be employed using a combination of multiple markers to complement the time-consuming conventional melissopalynology method or as an alternative approach.

CRediT authorship contribution statement

Oumer Hussein: Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ali Seid:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization.

Data availability statement

Data associated with this study are not deposited into a publicly available repository. The data will be made available upon request.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because the study did not use animals (honeybee colonies), rather it used honey samples for pollen analysis.

Additional information

There is no additional information to be mentioned concerning this manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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