POLLEN ANALYSIS OF HONEY FROM BORANA ZONE OF SOUTHERN ETHIOPIA

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Received: 22 August 2018; accepted: 28 August 2019

Abstract

Nineteen samples of honey were collected from different localities of the Borana Zone and examined to identify the botanical origin of honey through honey pollen analysis. From nineteen honey samples, sixteen were identified as monofloral honeys. Twenty-eight plant species were identified as honey source plants and the identified plant species belonged to fourteen plant families. Out of twenty-eight bee plant species, 17.9% of them were found in the Fabaceae family followed by Asteraceae and Lamiaceae, each of them accounting for 14.3% of all honey plants species found in the samples. The Shannon-Wiener diversity index (H) showed that high diversity of plant species was found in eleven honey samples with a range of 1.07 (Bule Hora site 1) to 1.81 (Yabello site 2) on the basis of honey pollen analysis. Eight honey samples had lower diversity index values, ranging from 0 (Arero site 2 and Bule Hora site 3) to 0.84 (Gelana site1), which suggests the honey was obtained from a few dominant plant species. Accordingly, *Guizotia scabra*, *Haplocoelum foliolosum*, *Plectranthus assurgens*, *Terminalia brownii*, *Sesamum indicum*, *Satureja paradoxa*, *Croton macrostachyus* and *Acacia brevispica* were the major monofloral honeys produced from the area. This indicates that there is a huge potential for the production of monofloral honey. Since monofloral honey has a good market value and is preferred by consumers, the involvement of investors is recommended.

Keywords: Ethiopia, melissopalynology, monofloral honey, pollen

INTRODUCTION

Success in beekeeping depends upon the availability and abundance of bee flora resources (Addi et al., 2014). Plant species composition and their flowering duration differ from one place to another due to variation in topography, climate and cultural and farming practices (Fichtl & Addi, 1994). Extensive knowledge on types, population density and quality of floral reward (nectar and pollen) are prerequisites for successful beekeeping. The variability of honey types produced in a particular area depends upon the diversity of nectar-source plants (Sibel & Mustafa, 2007), which can be identified from honey with honey pollen analysis (melissopalynology). Melissopalynology is the branch of palynology which deals with the study of the botanical and geographical origin of honey through microscopic analysis of honey sediments in honey samples (Louveaux,

Maurizio, & Vorwohl, 1978). Pollen grains of each plant species have their own genetic code of inheritance and special structural patterns, which enable pollen grains of one species to be differentiated from another (Chauhan & Trivedi, 2011). This gives useful information about the botanical origin of honeys. Thus, the characterization of honeys based on pollen analysis is essential for both scientific and commercial interest (Sik et al., 2017).

Assessing the availability of bee flora resources and establishing a floral calendar in different agro-ecological zones of Ethiopia is an important tool for determining such beekeeping management operations as frequency of honey harvest and predicting the honey flow period of an area. An agro-ecological zone and season can affect various aspects of bee forage sources. Thus, the availability, length of flowering, flowering phenology, nectar and pollen production of different bee plants can

be determined by the agro-ecology of a given locality. This in turn affects the seasonal growth and productivity of bee colonies (Fichtl & Addi, 1994; Addi et al., 2014).

The Borana zone is dominated by savannah woodland plant communities and consists of woody and herbaceous vegetation (Layne, 1994). Wide ranges of landscape, favorable climatic condition and bee flora diversity of the Borena Zone favor the presence of large colony population of honeybees. Moreover, the area is dominated by pastoral and agro-pastoral communities and characterized by erratic rainfall and unsuitable for crop farming. In such areas, apicultural development has great potential to generate income and improve the livelihood of poor farmers. Also the farmers traditionally conserving and share the natural resources on a communal basis which creates a good opportunity for honeybees and beekeeping. As a result, government organizations and NGOs are

developing beekeeping in the area as part of the local strategy in poverty reduction. However, resources in the area have not been assessed yet to lay base for identifying high performing bee forages that could help in beekeeping development. Therefore, this study was focused on the identification of major and minor bee plants from honey pollen analysis.

MATERIAL AND METHODS

The study was conducted in the Borana Zone of the Oromia Regional State, Ethiopia (Fig. 1). The study sites were selected based on their potentiality for beekeeping. The honey samples were collected from the districts of Dugda Dawwa, Bule Hora, Abbaya, Gelana, Arero, Teltelle, Yabello and Melka Sodda.

The honey samples were strained using double sieves and cheese cloth at the Holeta Bee Research Center (HBRC) laboratory to get pure

Fig. 1. Map of Ethiopia showing the regions, zones of Oromia and districts of the study area.

A total of nineteen crude honey samples were collected during honey flow seasons in October and November from beekeepers' home. For each honey type three samples were purchased. Each sample weighed 500 g.

honey from crude honey. The samples were then stored at 4°C for further analysis. For honey pollen analysis, the method recommended by the International Commission for Bee Botany (Louveaux, Maurizio, & Vorwohl, 1978) was adopted. Ten grams of each honey was dissolved in 20 ml of warm water (40°C). The solution was centrifuged for 10 min at 2500 r/min, the supernatant solution was decanted, and the sediments were collected into a conical tube for the study (Erdtman, 1960). The sediments were rinsed with distilled water to enhance further extraction of pollen from honey, centrifuged for 5 min at 2500 r/min, and preserved for study. To analyze the pollen content of the honey samples, two slides were prepared from each sample and photographed under a light microscope. Pollen types were identified by comparison 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 (Anklam, 1998; Oliveira, Van Den Berg, & Santos, 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. The types of pollen were allocated to one of four frequency classes for nectar source plants: predominant pollen types (>45% of the total pollen grains counted); secondary pollen types (16%-45%); important minor pollen types (3%-15%); and minor pollen types (3%) (Louveaux, Maurizio, & Vorwohl, 1978). Honeys with predominant pollen types were considered as monofloral. Finally, descriptive statistics and Shannon-Wiener Index (H') were used to estimate plant species diversity in honey pollen analysis (Shannon & Weaver, 1949; Ramirez-Arriaga, Navarro-Calvo, & Diaz-Carbajal, 2011), according to the following equation:

$$\text{Shannon Index } (H') = - \sum_{i=1}^s P_i * \ln P_i$$

H' is Shannon-Wiener diversity index, s is the number of species (pollen type), P_i is the proportion of individuals or the abundance of the i^{th} species (pollen type), $\ln$ is the natural

logarithm, and Σ is the sum from species 1 to species S .

The relative abundance of botanical families was calculated as a number of plant species found in the family divided by the total number of species and multiplied by 100. Clustering of botanical origin of honey source identified from honey pollen analysis was analyzed using PCA plot of R-software (R Core Development Team, 2011).

RESULTS

The results of honey pollen analysis are presented in Tab. 1. In total twenty-eight honeys-source plants were identified. The predominant pollen types were recorded for *Guizotia scabra*, *Haplocoelum foliolosum*, *Plectranthus assurgens*, *Croton macrostachyus*, *Terminalia brownii*, *Sesamum indicum*, *Acacia brevispica* and *Satureja paradoxa*. The secondary pollen source plants recorded were *Trifolium* spp., *Eucalyptus* spp. and *Ocimum* spp. The diversity of important minor and minor honey source plant species were higher than predominant species and secondary pollen sources.

The analysis of plant species from pollen count indicated that the highest species diversity was found for honey samples collected from Arero site 3 and Yabello site 2. The only species was identified in honey from Arero site 2 and Bule Hora site 1 (Fig. 2). This indicated that honey samples collected from Arero site 3 and Yabello site 2 were produced from many plant species, while those gathered in Arero site 2 and Bule Hora site 1 were from a few dominant bee plant species.

The twenty-eight pollen types identified in the honey samples were classified into fourteen plant families. From these twenty-eight species, 42.9%, 35.7%, and 21.4% were trees, herbs and shrubs respectively (Tab. 2). This result also indicated that weeds (*Guizotia scabra*, *Hypoestes forskalii*, *Hypoestes triflora* and *Trifolium* species) and some cultivated crops (*Sesamum indicum*) also greatly contribute to honey production.

Table 1.

Predominant, secondary, important minor and minor honey source plants in districts of Borana Zone based on honey pollen analysis category

District	Predominant pollen source (> 45%)	Secondary pollen source (16-45%)	Important minor pollen source (3-15%)	Minor pollen source (< 3%)
Abbaya	<i>Guizotia scabra</i>	<i>Trifolium</i> spp.	<i>Acacia brevispica</i> <i>Hypoestes triflora</i>	<i>Aloe debrana</i> <i>Croton macrostachyus</i> <i>Ocimum basilicum</i>
Arero	<i>Haplocoelum foliolosum</i>		<i>Aloe debrana</i> <i>Acacia senegal</i> <i>Ocimum basilicum</i> unknown pollen	<i>Hypoestes triflora</i> <i>Satureja paradoxa</i> <i>Plectranthus assurgens</i> <i>Vernonia amygdalina</i> <i>Hypoestes forskaolii</i>
Bule Hora	<i>Plectranthus assurgens</i> <i>Croton macrostachyus</i>	<i>Eucalyptus</i> spp. unknown pollen	<i>Guizotia scabra</i> Unknown pollen	<i>Hypoestes triflora</i>
Dugda Dawwa	<i>Croton macrostachyus</i> <i>Guizotia scabra</i>	<i>Terminalia brownii</i> unknown pollen	<i>Ocimum basilicum</i> <i>Plectranthus assurgens</i> <i>Hypoestes forskaolii</i> unknown pollen	<i>Carthamus tinctorius</i> <i>Acacia persiciflora</i> <i>Vernonia amygdalina</i> <i>Aloe debrana</i> unknown pollen
Gelana	<i>Terminalia brownii</i> <i>Sesamum indicum</i>	<i>Guizotia abyssinica</i> unknown pollen	<i>Hypoestes triflora</i> <i>Croton macrostachyus</i> <i>Coffea arabica</i> unknown pollen	<i>Plectranthus assurgens</i> <i>Ocimum basilicum</i> <i>Acacia persiciflora</i> <i>Eucalyptus</i> spp.
Melka Sodda	<i>Acacia brevispica</i>	<i>Acacia brevispica</i> <i>Terminalia brownii</i>	<i>Syzygium guineense</i> <i>Terminalia shimperana</i> <i>Vernonia auriculifera</i> <i>Guizotia scabra</i>	unknown pollen
Teltelle	<i>Guizotia scarba</i>	unknown pollen <i>Terminalia shimperana</i>	<i>Hypoestes forskaolii</i> <i>Terminalia brownii</i> unknown pollen	<i>Acacia brevispica</i> <i>Trifolium</i> spp. unknown pollen
Yabello	<i>Satureja paradoxa</i> <i>Haplocoelum foliolosum</i>	<i>Ocimum</i> spp. unknown pollen	<i>Guizotia scarba</i> <i>Rhus glutinosa</i> <i>Dombeya aethiopica</i> <i>Hibiscus</i> spp. unknown pollen	<i>Ocimum basilicum</i> <i>Sesamum indicum</i> <i>Pterolobium stellatum</i> <i>Plectranthus assurgens</i> <i>Vernonia amygdalina</i> <i>Aloe debrana</i> <i>Hypoestes triflora</i>

Table 2.

List of honeybee plants identified from honey pollen analysis in Borana Zone

No.	Scientific name of plant	Family name of the plant	Vernacular name	Life form	Rewards to honeybee
1	<i>Acacia brevispica</i>	Fabaceae	Hamaressa	Tree	Nectar & pollen
2	<i>Acacia persiciflora</i>	Fabaceae	Burra/bate	Tree	Nectar & pollen
3	<i>Acacia senegal</i>	Fabaceae	Saphansa	Tree	Nectar & pollen
4	<i>Aloe debrana</i>	Aloaceae	Hargisa	Herb	Nectar & pollen
5	<i>Carthamus tinctorius</i>	Asteraceae	Sufi	Herb	Nectar & pollen
6	<i>Coffea arabica</i>	Rubiaceae	Buna	Shrub	Nectar & pollen
7	<i>Croton macrostachyus</i>	Euphorbiaceae	Bakannisa	Tree	Nectar & pollen
8	<i>Dombeya aethiopica</i>	Sterculiaceae	Dannisa	Tree	Nectar & pollen
9	<i>Eucalyptus camaldulensis</i>	Myrtaceae	Bargamo Dima	Tree	Nectar & pollen
10	<i>Eucalyptus globulus</i>	Myrtaceae	Bargamo Adi	Tree	Nectar & pollen
11	<i>Guizotia scabra</i>	Asteraceae	Tufo/Hada	Herb	Nectar & pollen
12	<i>Haplocoelum foliolosum</i>	Sapindaceae	chana	Tree	Nectar & pollen
13	<i>Hibiscus</i> spp.	Malvaceae	Inchinni	Shrub	Nectar & pollen
14	<i>Hypoestes forskoolii</i>	Acanthaceae	Dergu	Herb	Nectar & pollen
15	<i>Hypoestes triflora</i>	Acanthaceae	Dergu	Herb	Nectar & pollen
16	<i>Ocimum basilicum</i>	Lamiaceae	Urgo/kajima/Basobila	Herb	Nectar & pollen
17	<i>Ocimum urticifolium</i>	Lamiaceae	Damakase	Shrub	Nectar & pollen
18	<i>Plectranthus assurgens</i>	Lamiaceae	Ajoftu	Herb	Nectar & pollen
19	<i>Pterolobium stellatum</i>	Fabaceae	Harangama	Shrub	Nectar & pollen
20	<i>Rhus glutinosa</i>	Anacardiaceae	Tatessa	Tree	Nectar & pollen
21	<i>Satureja paradoxa</i>	Lamiaceae	Teneddam	Herb	Nectar & pollen
22	<i>Sesamum indicum</i>	Pedaliaceae	Salit	Herb	Nectar & pollen
23	<i>Syzygium guineense</i>	Myrtaceae	Badessa	Tree	Nectar & pollen
24	<i>Terminalia brownii</i>	Combretaceae	Birdessa	Tree	Nectar & pollen
25	<i>Terminalia shimperana</i>	Combretaceae	Dabaka	Tree	Nectar & pollen
26	<i>Trifolium</i> spp.	Fabaceae	Sidisa	Herb	Nectar & pollen
27	<i>Vernonia amygdalina</i>	Asteraceae	Ebicha	Shrub	Nectar & pollen
28	<i>Vernonia auriculifera</i>	Asteraceae	Rejji	Shrub	Nectar & pollen

The highest number of melliferous plant species was recorded for the Fabaceae family (five species and 17.9% of a total species number found in the collected samples), followed by the Asteraceae and Lamiaceae families (four species), Myrtaceae family (three species), and the Acanthaceae and Combretaceae represented by two species. The other botanical families were represented by only one species (Fig. 3). Based on the diversity of honeybee plant species obtained from honey pollen analysis, the study

sites were classified into four clusters. Cluster A includes Bule Hora site 1, Gelana site 2 and Melka sodda site 1 and 2. Cluster B includes Abaya 1, Abaya 2, Arero 1, Bule Hora 1, Dugda Dawwa 2, Gelana 1 and Teltelle site 1. Cluster C includes Bule Hora 2, Bule Hora 3, Dugda Dawwa 1 and Yabello site 1. Cluster D includes Abaya 2, Arero 2 and 3, Dugda Dawwa 1, Melka sodda 2 and Yabello site 2 (Fig. 4). Clusters B and C were almost similar in species diversity, while cluster D was different from the others.

Fig. 2. Diversity of plant species from honey pollen analysis of different sites (H' is Shannon-Wiener Diversity Index and Hmax is maximum number species diversity).

Fig. 3. Percentages of plant species from different botanical families identified in honey samples collected in the Borana Zone.

Fig. 4. Clusters of the study sites based on the species diversity from honey pollen analysis using PCA component plot analysis. Component 1 and 2 are PCA axis, a number in the clusters represent the sampling site, letters represent number of clusters and the diagram in the figure showed a level of similarity among one another.

Fig. 5. Major bee forage plants that provide monofloral honeys in the districts of the Borana Zone.

On the basis of honey pollen analysis, sixteen monofloral honeys were identified. When the data were pooled together at a district level, only eight monofloral honeys originating from eight plant species were produced in different parts of the zone (Fig. 5). *Guizotia scarba* provided monofloral honey in the Abbaya, Dugda Dawwa and Teltelle districts due to its growing in abundance in a wide range habitats: cultivated fields, forest margin and open grasslands. Whereas the monofloral honey of *Haplocoelum foliolosum* was collected from the Arero and Yabello districts, *Plectranthus assurgens* (Bule Hora), *Terminalia brownii* (Gelana), *Sesamum indicum* (Gelana), *Satureja paradoxa* (Yabello) and *Croton macrostachyus* were from the Bule Hora and Dugda Dawwa districts. The harvesting season was October-December for monofloral honey of *Guizotia scarba*, *Haplocoelum foliolosum*, *Plectranthus assurgens*, *Terminalia brownii*, *Sesamum indicum* and *Satureja paradoxa*, while April-June for *Croton macrostachyus* and March-May for *Acacia brevispica*.

DISCUSSION

Based on the pollen analysis, the bee flora of the Borana Zone has high species diversity due to the identified bee forage species belonging to *Acacia-commiphora* woodland vegetation which is one of the richest and most diverse vegetation types in the semiarid lowlands of Ethiopia. This study indicated that 84% of honey was monofloral even though the diversity of bee flora was high. Similarly, a study conducted in North Algeria and the Guji Zone of Ethiopia reported that from collected honey samples 40% and 79% were monofloral honeys respectively (Samira, Boumedienne, & Abdelkader, 2013; Bareke & Addi, 2018). On the other hand, Puusepp & Koff (2014) indicated that their honey in Estonia was 100% multifloral.

According to the number of pollen types and the share of each species in the total pollen count, sixteen honey samples were identified as monofloral honeys and three samples were identified as multifloral honeys. In multifloral

honey, there was a high diversity of honeybee plant species as compared to monofloral honey. This is similar with the study conducted by Bareke & Addi (2018) in the Guji Zone of Ethiopia that there was a high diversity of honeybee plants in multifloral honey. However, the highest diversity of honeybee plants was found in the monofloral honey sample collected from the Yabello site 2 ($H=1.81$). After the rain season, *Satureja paradoxa* was the most (abundant (45.8%) honeybee plant species in this area, coexisting with many other honeybee plant species that were less abundant in the area. Due to this, the highest diversity was recorded for the Yabello site 2.

The Shannon-Weaver diversity index showed high diversity of plant species in eleven honey samples with a range of 1.07 in the Bule Hora site 1 to 1.81 in the Yabello site 2. The higher diversity of these bee forages is due to the location of the study site in one of the Eastern Biodiversity Hotspot areas which is known for higher plant diversity. Eight honey samples have lower diversity index values ranging from 0 in the Arero site 2 and Bule Hora site 3 to 0.84 in the Gelana site 1, which suggests the low diversity of bee plant species is due to the limited distribution of the bee forages and clearing of important bee forages for various purposes.

Microscopic analysis of honey sediments revealed that plant species variability is greatest in the group of minor pollen sources, followed by the important minor pollen, secondary, and dominant groups. The low number of species in the dominant pollen group is due to the foraging fidelity of honeybees or dominance of few plant species. Whereas, minor pollen groups are either less abundant or do not provide good nectar or pollen, both for honeybees. Sabo et al. (2011) also reported that there is little variability among pollen species in the dominant groups, while more among minor pollen, important minor pollen and secondary pollen groups. Honeybees are a good resource for palynological studies by revealing the flowering sequences of plants within the foraging radius and giving direct evidence of the in-situ vegetation. An analysis

of the pollen content of honey is used to investigate the provenance and provide a quantitative measure of floral origin (pollen percentage) for use in the market (Louveau, Maurizio, & Vorwohl, 1978). It is very effective to determine and control the geographical origin of honeys and provides information about the microclimate condition which affects the honey quality (Von der Ohe et al., 2004).

The main problem faced identifying the botanical origin of honey based on honey pollen analysis and classifying the pollen count for predominant pollen source, secondary pollen source, important minor pollen source and minor pollen source was that a given honeybee plant is predominant in one district but an important minor in the other districts. This may be due to variation in the abundance of the species. For example, in the Bule Hora District *Guizotia scabra* was not widely distributed and not considered as an important minor pollen source (13.5%) whereas in Abayya District it was a major honeybee plant (61.97%). This indicated that the abundance of a given honeybee plant has a great impact on honey production potential. Hence, identifying the honey production potential of bee plant species is important in addition to honey pollen analysis.

In this study, *Guizotia scabra*, *Haplocoelum foliolosum*, *Plectranthus assurgens*, *Terminalia brownii*, *Sesamum indicum*, *Satureja paradoxa*, *Croton macrostachyus* and *Acacia brevispica* were identified as sources of monofloral honeys. This indicates that there is a huge potential for the production of monofloral honey in the Borana Zone. Since monofloral honey has good market value and favoured by consumers, the involvement of investors is recommended.

ACKNOWLEDGEMENTS

The authors are thankful to the Holeta Bee Research Center and Oromia Agricultural Research Institute for providing the required facilities and logistics. Our sincere thanks also to Konjit Asfaw for helping us in the laboratory.

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