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Evaluation of foraging patterns of bee species and characterization of their honey samples in two localities of Kheralu Taluka, Mehsana District, Gujarat

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ABSTRACT

In this work, Pollen analysis was carried out on two honey samples from two different localities (Dabhad village and Vaghavaadi village) of Kheralu taluka, Mehsana district, Gujarat. Melissopalynological examinations were made to isolate and identify pollen types in the honey. The aim of the study is to determine whether the honey samples are unifloral or multifloral. Moreover, to determine pollen types to understand the forage patterns of bee species. Pollen analysis was performed using the acetolysis method. A total of seventeen morphotypes of pollen were identified. *Brassica juncea* (L.) Czern., *Morus alba* L., *Sonchus oleraceus* L., *Solanum virginianum* L., *Foeniculum vulgare* Mill., *Ricinus communis* L., *Achyranthes aspera* L., *Tridax procumbens* (L.), *Amaranthus viridis* L., *Parthenium hysterophorus* L., *Cyanthillium cinereum* (L.) H.Rob., *Digera muricata* (L.) Mart., *Tagetes erecta* L., *Peristrophe bicalyculata* (Retz) Nees, *Eclipta prostrata* (L.), *Setaria glauca* (L.) Beauv. & unknown pollen of plant. Among these plants, the pollen of *Brassica juncea* (L.) Czern. was recorded with 53.03% and 41.71% in the Dabhad locality as well as the Vaghavaadi locality with the highest frequency percentage.

Keywords: Acetolysis, Pollen analysis, Bee plants, *Apis cerana*

INTRODUCTION

This work is mainly focused on Melissopalynology. Melissopalynology is the field of palynology that involves the study of pollen in honey samples. It also focuses on the implementation of these methods in apiculture. This helps us in identifying the source of the pollen captured in the honey.

Pollen is a granular cluster of male reproductive cells created in a flower's anthers. Pollen grains, as palynomorphs, encompass an unusual compound called "sporopollenin," which is observed in the outer wall (the exine) and helps make them impervious to acetolysis treatment during palynological studies (Shivanna *et al*., 1992). For honey bees, pollen is an important source of proteins, fatty acids, minerals, and vitamins (Moore *et al*., 1991 and Devender *et al*., 2015). Bees purposefully collect pollen grains to meet their protein needs and store them in pollen chambers in the hive (Bhattacharya *et al*., 2006).

Honey, the splendid sweet substance obtained from honeycomb, is primarily a viscous supersaturated solution of sugars. (Chefrour *et al*., 2009) Bees forage on flowering plants in search of pollen grains and nectar to use in the production of beebread (Proestos *et al*., 2005). The foraging pattern of bees varies from species to species, sometimes among varieties. It is a widely used sweetener all over the world. Honey has been an essential source of nourishment for humans since their inception (Freitas and Silva, 2006). Honey has been used as both a nutrient and medicine throughout human history. Apitherapy is an alternative medicinal branch that has developed in recent years. In which, diseases can be treated using honey and other bee products (Molan, 2001). The most significant element influencing honeybee behavior and honey quality is the vegetation that they inhabit (Wright *et al*., 2018 and Lau *et al*., 2019). The honey structure varies significantly and is mainly resolute by the floral source, but some other external variables like the processing technique of honey, variation of seasons, and some environmental conditions also play a role (Alvarez *et al*., 2010). Honey, a semi-liquid product having a unique combination of sugars and water. It also has minor constituents which give honey its unique properties, taste, flavor, and benefits. Enzymes, vitamins, minerals, pigments, and some biologically active substances like organic acids, and various lactones are the most significant of these components (Nafea *et al*., 2017) and this is the composition that, allows honey to be preserved for a long period of time with minor changes in the chemical composition, changes which do not alter its quality (Babarinde et al., 2011). Adulteration of honey is regarded as a serious problem, not only because of differences in flavor and taste but also because of proven health-promoting properties that differ between products of different botanical origins (Pasupuleti *et al*., 2017; Pimentel *et al*., 2022; Terzo *et al*., 2020).

Consumers place a high value on the botanical and geographic provenance of honey, as these factors impact the product's physical, chemical, organoleptic, and bioactive qualities. Honey origins are also thought to be a valuable indicator of authenticity and quality (Kuldasheva *et al*., 2022). In recent years, Melissopalynology has attained a global status (Mander and Punyasena, 2014). This is supported by the fact that honey is increasingly utilized to cure a variety of disorders, in addition to being beneficial as a nutritional supplement (Molan, 2001). Its healing properties are due to the incorporation of pollen and nectar comprising bioactive components from medicinal plants foraged by bees (Kevan and Baker, 1983). Current article objectvs 1) To prepare a list of pollen types utilized by bee forage and to understand the forage pattern of bee species. 2) To identify the type of honey samples (Unifloral/Multifloral) with relative frequency classes and absolute pollen count of pollen type.

MATERIAL AND METHODS

Geographical location of the study area:

Mehsana is located in a western state of India, known as Gujarat. It is one of the 33 districts of Gujarat. Kheralu is the taluka of Mehsana district. It is 48 kilometers north of the district headquarters in Mehsana. The geographical area of the taluka is 334.24 sq. Kms. For the present study, two honey samples were collected from Dhabhad village and Vaghavaadi village, which lie at 23°57'24.9"N 72°36'58.3"E & 23°53'26.2"N 72°43'53.1"E geographical coordinates in Kheralu taluka (Shown in **Fig. 1**). It belongs to the sub-tropical forest area. Neem, Pongame, Babool, and Jujube are the majority of the trees grown in this area. The chief cereals are Wheat, Sorghum, and Bajara. Ricinus, Cotton, and Brassica are the predominant crops in this area. The samples were collected from the Farm area as well as the Roadside area of these villages with the help of the local community which is involved in honey harvesting.

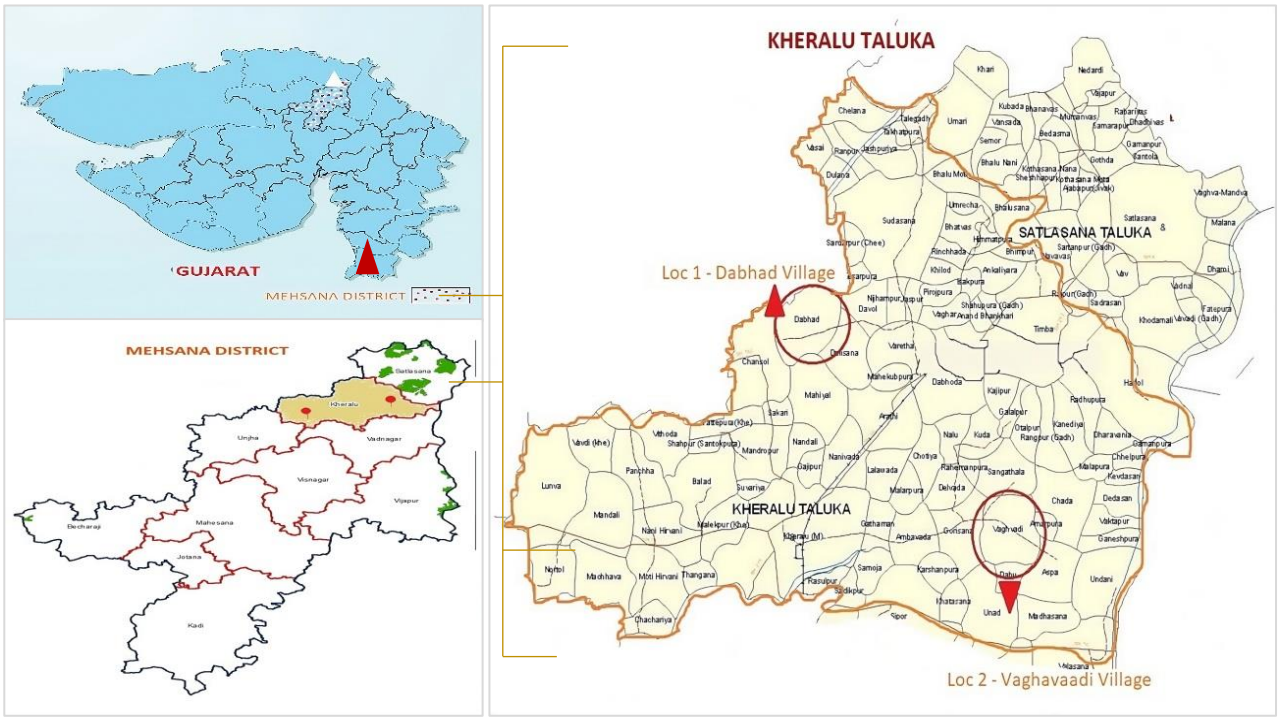

Fig. 1. Structure of map showing both location (Loc-1 Dabhad Village & Loc-2 Vaghavaadi Village) sites

Honey sampling:

For the present study, two samples were collected, for quantitative pollen analysis from two different villages of Kheralu taluka. A limited amount of samples was collected. The honey was filtered through a single thick white cloth to remove suspended particles like dirt, bee wax, and other impurities. Samples were collected in airtight glass bottles from the place of honey extraction and after that, it was stored at room temperature. The *International Commission for Bee Botany*'s (ICBB) suggested technique for pollen analysis was used. 20 ml of warm water (40-50 °C) was used to dissolve 10g of the honey sample. The resultant honey solution was centrifuged for a duration of 10 min. at 2000 rpm setpoint; the supernatant solution was decanted after adding 10 ml of warm water to dissolve the remaining soluble components, then again centrifuged for 5 min. at 2000 rpm.

The sediments were placed in a conical tube for collection after the supernatant solution was decanted.

Acetolysis:
The sediment/residue was treated with an acetolysis mixture (i.e., Acetic anhydride: conc. Sulphuric acid = 9:1 v/v) for approximately 30 min. at room temperature. Then, the residue was rinsed with the distilled water, and centrifuged for 5 min. at 2000 rpm, and after adding 1-2 ml of 30% glycerine with the residue to preserve for further study. Acetolysis method (Nair *et al*., 2013) has been followed for this study.

Pollen analysis:
For analysis of pollen content of the collected honey samples, three slides were prepared from the sample, and by comparing the types of pollen under 100 times magnification to reference slides of pollen gathered directly from plants in the study region, the pollen types were determined.

Neubauer chamber for counting pollen:
Pollens were counted under a light microscope at 100 times magnification over an Improved Neubauer Chamber (Hemocytometer - a counting chamber).

Absolute pollen count (APC):
Based on the average number of different observations, the pollen sediment contained in 10g honey was calculated. The sample has been classified into five groups as proposed by Louveaux *et al*., 1978:

Group I	:	< 20,000 pollen grains,
Group II	:	20,000 - 1, 00,000 pollen grains,
Group III	:	1, 00,000 - 5, 00,000 pollen grains,
Group IV	:	5, 00,000 - 10, 00,000 pollen grains,
Group V	:	> 10, 00,000 pollen grains.

Quantification of pollen type and frequency (%):
The percentage frequency of the pollen taxa in the sample was calculated for the presentation of frequencies of pollen grain in honey, the system adopted by Louveaux *et al*., (1978) has been used. The percentage frequency of each pollen type was calculated using the following formula:

$$\boldsymbol{Frequency}\ (\%) = \frac{\boldsymbol{Number\ of\ pollen\ grain\ of\ a\ taxon}}{\boldsymbol{Total\ no.\ of\ pollen\ grain\ of\ all\ taxa}} \times \mathbf{100}$$

Table 1. Honey classification based on pollen grain frequency (Louveaux *et al*., 1978)

Sr.No.	Pollen Types	(%) of Pollen Grains
1.	Predominant pollen types	> 45% of the total pollen counted
2.	Secondary pollen types	16-45% of the total pollen counted
3.	Important minor pollen types	03-15% of the total pollen counted
4.	Minor pollen types	< 03% of the total pollen counted

Upon the classification of pollen grain frequency, honey samples were classified.

Scanning electron microscopy:
The detailed structure of pollens was observed by their SEM study. We mounted pollen samples on the SEM stubs and coated them with golden-palladium particles using Quorum SC7620 Sputter Coater. Pollen specimens were examined and captured their photographs using the ZEISS EVO/18 SEM instrument in the Gujarat Ecological Education and Research (GEER) Foundation laboratory, Gandhinagar.

RESULTS

It was discovered that 17 different plant pollen grains were present in this honey analysis. The recognized taxa are from several genera of grasses, shrubs, and herbs. These pollens differ in size, shape, and morphological characteristics. A total two honey samples were collected during the month of December from Kheralu taluka. Details regarding sample sites are illustrated in **Table (2)**.

Table 2. Details regarding sample sites/locations

Sample Details		Sample-1 (Locality 1)	Sample-2 (Locality 2)	Sample Details		Sample-1 (Locality 1)	Sample-2 (Locality 2)
1.	**District**	Mehsana	Mehsana	**7.**	**Temperature (°C)**	16˚C	20˚C
2.	**Taluka**	Kheralu	Kheralu	**8.**	**% Humidity**	89%	67%
3.	**Village**	Dabhad	Vaghavaadi	**9.**	**Wind speed (km/h)**	9 (Km/h)	8 (Km/h)
4.	**Date of Collection**	20- Dec- 2019	24- Dec- 2019	**10.**	**Volume of the sample (g)**	126.40	89.01
5.	**Collection site**	Farm area, 23°57'24.9"N 72°36'58.3"E	Road side area, 23°53'26.2"N 72°43'53.1"E	**11.**	**Honey comb height from the ground (cm)**	70	356
6.	**Host plant**	*Euphorbia nerifolia* L.	*Morus alba* L.	**12.**	**Colour of the honey**	Amber	Light Amber

From these two localities, a total seventeen different pollen types belonging to nine families were recorded. As shown in **Fig. 3**, All pollens were identified to species level except one. Among them, pollen of *Sonchus oleraceus* L., *Solanum virginianum* L., *Achyranthes aspera* L., *Tridax procumbens* (L.), *Eclipta prostrata* (L.) and unkwon pollen were found (total 6 pollen types) in Dabhad locality. Whereas, *Peristrophe bicalyculata* (Retz) Nees, *Parthenium hysterophorus* L., *Cyanthillium cinereum* (L.) H.Rob., *Digeria muricata* (L.) Mart., *Tagetes erecta* L. and the *Setaria glauca* (L.) Beauv. pollens were found in the Vaghavaadi locality. A total, eleven pollen types were recorded in both sample collection sites. Among them, pollens of *Brassica juncea* (L.) Czern., *Morus alba* L., *Foeniculum vulgare* Mill., *Ricinus communis* L., and *Amaranthus viridis* L. were found same as same in both locations, and the pollen of *Brassica juncea* (L.) Czern. was most frequently visited by bees. The detailed pollen spectrum of both samples is presented in **Table (3)**.

Loc 1 Dabhad (Honey sample):

A total of eleven species belonging to 7 families were observed in the honey sample collected from Loc 1. In this sample, pollen grains from *Brassica juncea* (L.) Czern. (53.03%) is a predominant type. As shown in **Fig. 2,** the Pollen grain of *Morus alba* L. (33.00%) was recorded as a secondary pollen type. Both belong to Group – V. Followed by pollen grains of *Sonchus oleraceus* L. contained 6.32% as comparatively low (%) frequency and are included as an important minor pollen type with Group IV. Whereas, pollen of *Eclipta prostrata* (L.) (2.12%), Pollen from unknown plant (1.32%), and *Ricinus communis* L. (1.05%) were found in Group-III with minor pollen type. Pollen morphotype of *Foeniculum vulgare* Mill. (1.08%), *Achyranthes aspera* L. (1.05%), *Tridax procumbens* (L.) L. (0.22%) and S*olanum virginianum* L. (0.64%) are in Group – II including the minor pollen type. The remaining species with the lowest frequency (0.12%) of *Amaranthus viridis* L. contain minor pollen types with APC Group – I.

Loc 2 Vaghavaadi (Honey sample):

Majority of pollens found from *Brassica juncea* (L.) Czern. (41.71%) from the Brassicaceae family contained a secondary pollen type with APC Group – II. Whereas, pollens of *Morus alba* L. and *Parthenium hysterophorus* L. contain 22.81% and 19.07% with the second highest frequency. They both are found as a secondary pollen type with Group – II. Pollens found with comparatively low (%) frequency are *Ricinus communis* L. (6.09%), *Cyanthillium cinereum* (L.) H.Rob. (4.25%), *Setaria glauca* (L.) Beauv. (2.40%), *Amaranthus viridis* L. (1.80%), *Foeniculum vulgare* Mill. (0.74%), *Digera muricate* (L.) Mart. (0.53%), *Tagetes erecta* L. (0.32%) and the lowest frequency of *Peristophe bicalyculata* (Retz) Nees (0.23%). Among these, first two of pollens are included in as important minor type and remaining are as a minor type of pollen. The classification recommended by Louveaux *et al*., (1978) for expressing frequencies of pollen grain has been adopted: if pollens are > 45% of the total pollen count then they are included in Predominant pollen types, if the frequency of pollens are in between 16-45% of the total pollen count then, they having Secondary pollen types, if in between 03-15% of the total pollen count refers as Important minor pollen types and having < 3% frequency were included in Minor pollen types.

The large amounts of pollen in this sample of honey have provided the hint to defining the purity of the honey sample according to the outcomes of these examinations. It also indicates a flower which contain predominant pollen type are visited by bees for their pollen. Among this vegetation there is a potential to produce

considerable quality of honey from these areas. As shown in **Fig. 3**, the different pollen structures called palynodiversity observed here, which clearly signifies the source plants for the honey production.

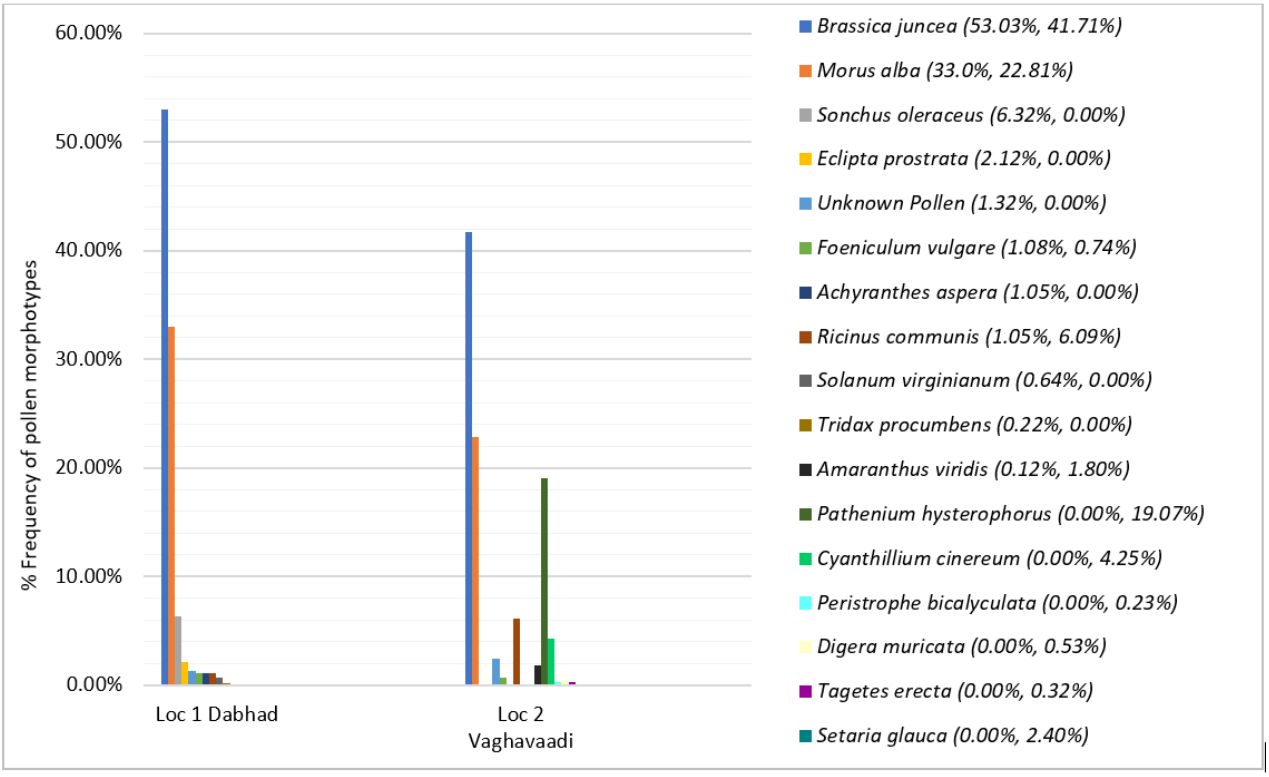

Fig. 2. Frequency (%) of plant pollens showing in both localities

Table 3. Frequency class and APC group of various pollen type

Sr. No.	Name of the plant species / Family	No. of pollens per 10 g sample		% Frequency		Frequency class pollen type		APC group	
		Loc 1	Loc 2	Loc 1	Loc 2	Loc 1	Loc 2	Loc 1	Loc 2
1.	*Brassica juncea* (L.) Czern. / Brassicaceae	52,17,100	17,76,250	**53.03%**	**41.71%**	**Predominant**	**Secondary**	**Group-V**	**Group-V**
2.	*Morus alba* L. / Moraceae	31,76,950	9,43,950	33.00%	22.81%	Secondary	Secondary	Group-V	Group-IV
3.	*Parthenium hysterophorus* L. / Asteraceae	-	7,71,400	-	19.07%	-	Secondary	-	Group-IV
4.	*Sonchus oleraceus* L. / Asteraceae	6,19,150	-	6.32%	-	Important minor	-	Group-IV	-
5.	*Ricinus communis* L. / Euphorbiaceae	1,01,500	2,43,600	1.05%	6.09%	Minor	Important minor	Group-III	Group-III
6.	*Eclipta prostrata* (L.) / Asteraceae	2,03,000	-	2.12%	-	Minor	-	Group-III	-
7.	*Cyanthillium cinereum* (L.) H.Rob. / Asteraceae	-	1,62,400	-	4.25%	-	Important minor	-	Group-III
8.	Unknown Pollen	1,42,100	-	1.32%	-	Minor	-	Group-III	-
9.	*Setaria glauca* (L.) Beauv. / Poaceae	-	1,01,500	-	2.40%	-	Minor	-	Group-III
10.	*Achyranthes aspera* L. / Amaranthaceae	91,350	-	1.05%	-	Minor	-	Group-II	-
11.	*Foeniculum vulgare* Mill. / Apiaceae	91,350	30,450	1.08%	0.74%	Minor	Minor	Group-II	Group-II
12.	*Amaranthus viridis* L. / Amaranthaceae	10,150	71,050	0.12%	1.80%	Minor	Minor	Group-I	Group-II
13.	*Solanum virginianum* L. / Solanaceae	60,900	-	0.64%	-	Minor	-	Group-II	-
14.	*Digera muricata* (L.) Mart. / Amaranthaceae	-	20,300	-	0.53%	-	Minor	-	Group-II
15.	*Tridax procumbens* (L.) L. / Asteraceae	20,300	-	0.22%	-	Minor	-	Group-II	-
16.	*Tagetes erecta* L. / Asteraceae	-	10,150	-	0.32%	-	Minor	-	Group-I
17.	*Peristrophe bicalyculata* (Retz) Nees / Acanthaceae	-	10,150	-	0.23%	-	Minor	-	Group-I

DISCUSSION

The plant and animal territories have collaborated to produce honey, which has long been revered for its purported medicinal and nutritional properties (Rana *et al*., 2018). Analysis of honey indicates a good potential for the development of bee colonies in this locality. Bees used pollen for brood rearing, growth in colony strength, and nectar for their carbohydrate requirement. The identification of pollen and nectar sources in honey would help beekeepers maintain their colonies (Rakesh *et al*., 1988). For honey beekeepers, it is vital to note that the restricted supply of floral nutrients might impact the bees' productivity and growth (Tulu *et al*., 2020). The selected region for the present study has good potential for sustaining beekeeping ventures because of predominantly plantation and agricultural crops.

Beekeeping is also a sustainable activity that helps local economies flourish in water-scarce areas and supports pollination services in ecosystems that are extremely valuable. This abundant rich vegetation makes the area one of the hotspots of biodiversity. Samples were found to be rich in both pollen concentration and pollen diversity. Each species of plant has a unique structural pattern and genetic coding that allow its pollen grains to be distinguished from those of other species (Addi *et al*., 2021). Melliferous plants play a significant role in this great diversity of plant life, enabling the production of a large range of honey types (Homrani *et al*., 2020). On the basis of the results obtained in these examinations, the quantities of pollen in a given sample of honey have furnished the cue to determine the purity and genuineness of the honey samples. As is the case in many regions of the area, people prefer local beekeeping, and they trust beekeepers to source their honey directly from plants and ensure that it is of high quality (Chauhan *et al*., 2017).

The majority of local honey is incorrectly labeled, and the qualities of regional productions are not well-researched. Increasing consumer awareness of local honeys in this context helps to increase their value and protect them from fraud. Verifying the predominant botanical origin and quality is one of the primary responsibilities. Within this framework, sensory qualities are what customers identify most quickly and, combined with melissopalynology, they help to uncover the honey's botanical and geographic provenance (Marcazzan *et al*., 2018).

Moreover, proper understanding and mutualism between bees and available plant taxa in the region & and particular season plays a very important role to improve beekeeping industry (Bhusari *et al.,* 2005; Chauhan *et al.,* 2017). However, the creation of flowering calendars across the nation is complicated by these topographical variances and the disparities in temperature zones (Gratzer *et al.,* 2021). Human activity is a threat to bee populations. Bee populations are negatively impacted by pesticide use, invasive species, monocultures with limited flower resources, and climate change. Given that mammal feeding behavior is impacted by climate change, these changes can impact insects more (Salmanpour *et al.,* 2023). These data reflects the floral situation of the place were particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area.

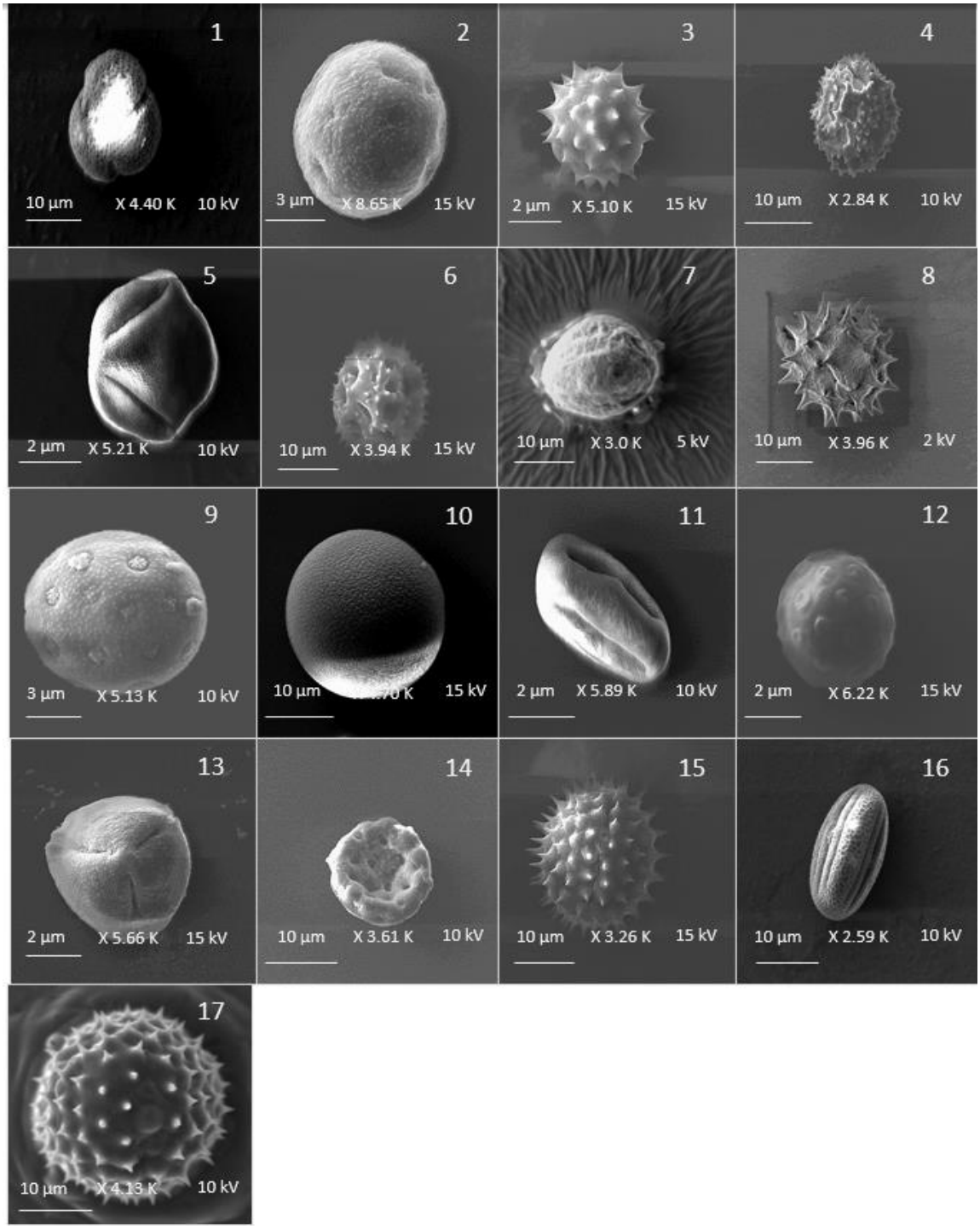

Fig. 3. Scanning Electron Microscopic structure of pollen grains, collected by honey bee during the study. Where number of pollens listed with their plants name, each image has (X) = Times magnification, Scale bar range in (µm) = Micrometer (In between 2 µm - 10 µm), Beam voltage range in (kV) = kilo Volt (which is in between 2 kV - 15 kV) & pollen structures were mainly captured in their polar view and equatorial view - (1. *Brassica juncea* (L.) Czern./ (Brassicaceae), 2. *Morus alba* L./ (Moraceae), 3. *Parthenium hysterophorus* L./(Asteraceae), 4. *Sonchus oleraceus* L./ (Asteraceae), 5. *Ricinus communis* L./ (Euphorbiaceae), 6. *Cyanthillium cinereum* (L.) H.Rob./ (Asteraceae), 7. Unknown pollen, 8. *Eclipta prostrata* (L.)/ (Asteraceae), 9. *Amaranthus viridis* L./ (Amaranthaceae), 10. *Setaria glauca* (L.) Beauv./ (Poaceae), 11. *Foeniculum vulgare* Mill./ (Apiaceae), 12. *Achyranthes aspera* L./ (Amaranthaceae), 13. *Solanum virginianum* L./ (Solanaceae), 14. *Digera muricata* (L.) Mart./ (Amaranthaceae), 15. *Tagetes erecta* L./ (Asteraceae), 16. *Peristrophe bicalyculata* (Retz) Nees/ (Acanthaceae), 17. *Tridax procumbens* (L.) L./ (Asteraceae).

CONCLUSION

The pollen analysis of honey samples from the two different localities of kheralu taluka indicates that honey are constituted of the pollens of various taxa. Both samples of honey are very rich in the pollens. From this study, we can conclude that the type of honey from Loc 1 (**Dabhad village honey sample**) is **unifloral** with the highest (53.03%) frequency and the sample from Loc 2 (**Vaghavaadi village honey sample**) is having **multifloral** type. Bees have used maximum number of plant species from Asteraceae family for their forage. Both honey samples showed a diversity with a total seventeen different types of pollen. This, melissopalynological analyses showed that the most dominating pollens in honey samples are from Brassicaceae, Moraceae and Asteraceae family. This implies that the bees travelled a long distance in search of suitable food materials, such as nectar, to meet their nutritional needs as well as honey production. This is an important indicator of ecological interaction between bees and plants in the area of production.

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Conflict of Interest: The authors declare no conflict of interest

REFERENCES

Abdel-Rasheed, K. G., Moustafa, Y. M., Hassan, E. A., Abdel-Ati, Y. Y., & Gadel-Hak, S. E. H. (2017). Honeybee collected pollen as antibiotics to control american foul brood disease, at Giza Governorate. *Egyptian Journal of Agricultural Research*, *95*(4), 1551-1563.

Addi, A., & Bareke, T. (2021). Botanical origin and characterization of monofloral honeys in Southwestern forest of Ethiopia. *Food Science and Nutrition*, *9*(9), 4998-5005.

Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Bertoli, E., & Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterranean Journal of Nutrition and Metabolism*, *3*, 15-23.

Babarinde, G. O., Babarinde, S. A., Adegbola, D. O., & Ajayeoba, S. I. (2011). Effects of harvesting methods on physicochemical and microbial qualities of honey. *Journal of Food Science and Technology*, *48*, 628-634.

Bhattacharya, K., Majumdar, M. R., & Bhattacharya, S. G. (2006). A Textbook of Palynology (Basic and Applied), New Central Book Agency (P) Ltd.

Bhusari, N. V., Mate, D. M., & Makde, K. H. (2005). Pollen of Apis honey from Maharashtra. *Grana*, *44*(3), 216-224.

Chauhan, M. S., Farooqui, A., & Trivedi, A. N. J. A. L. I. (2017). Plants foraged by bees for honey production in northern India: The diverse flora of India and its implications for apiculture. *Acta palaeobotanica*, *57*(1), 119-132.

Chefrour, C., Draiaia, R., Tahar, A., Kaki, Y. A., Bennadja, S., & Battesti, M. J. (2009). Physicochemical characteristics and pollen spectrum of some north-east Algerian honeys. *African Journal of Food, Agriculture, Nutrition and Development*, *9*(5).

Devender, R., Ramakrishna, H., Reddy, C. S., & Ammani, K. (2015). Palynodiversity of mangroves in Gilakaladindi from Krishna district, Andhra Pradesh. *Phytomorphology*, *65*(3/4), 139-144.

Gratzer, K., Wakjira, K., Fiedler, S., & Brodschneider, R. (2021). Challenges and perspectives for beekeeping in Ethiopia. A review. *Agronomy for Sustainable Development*, *41*(4), 1-15.

Homrani, M., Escuredo, O., Rodríguez-Flores, M. S., Fatiha, D., Mohammed, B., Homrani, A., & Seijo, M. C. (2020). Botanical origin, pollen profile, and physicochemical properties of Algerian honey from different bioclimatic areas. *Foods*, *9*(7), 938.

Kevan, P. G., & Baker, H. G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology*, *28*(1), 407-453.

Kuldasheva, F., Madartov, B., Nurmurodova, N., & Eshdavlatov, O. (2022). Study of Organoleptic, Physical and Chemical Indicators of honey in tashkent region. *Agro Inform*, (1), 43-51.

Lau, P., Bryant, V., Ellis, J. D., Huang, Z. Y., Sullivan, J., Schmehl, D. R., ... & Rangel, J. (2019). Seasonal variation of pollen collected by honey bees (Apis mellifera) in developed areas across four regions in the United States. *Plos one*, *14*(6), e0217294.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee world*, *59*(4), 139-157.

Mander, L., & Punyasena, S. W. (2014). On the taxonomic resolution of pollen and spore records of Earth's vegetation. *International Journal of Plant Sciences*, *175*(8), 931-945.

Marcazzan, G. L., Mucignat-Caretta, C., Marina Marchese, C., & Piana, M. L. (2018). A review of methods for honey sensory analysis. *Journal of Apicultural Research*, *57*(1), 75-87.

Molan, P. C. (2001). The potential of honey to promote oral wellness. *General dentistry*, *49*(6), 584-590.

Moore, P. D., Webb, J. A., & Collinson, M. E. (1991). Pollen Analysis, 2nd edn Blackwell. *Search in*.

Nair, S., Meddah, B., & Aoues, A. (2013). Melissopalynological characterization of north Algerian honeys. *Foods*, *2*(1), 83-89.

Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Oxidative Medicine and Cellular Longevity*, *2017*.

Pimentel, T. C., Rosset, M., de Sousa, J. M. B., de Oliveira, L. I. G., Mafaldo, I. M., Pintado, M. M. E., ... & Magnani, M. (2022). Stingless bee honey: An overview of health benefits and main market challenges. *Journal of Food Biochemistry*, *46*(3), e13883.

Proestos, C., Chorianopoulos, N., Nychas, G. J., & Komaitis, M. (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *53*(4), 1190-1195.

Rakesh, K., & Jagtap, S. D. (1988). Melittopalynological studies on Rock bee honey samples during Winter season in Purander area of Pune district in Maharashtra. *Journal of Palynol*, *34*, 157-162.

Rana, S., Mishra, M., Yadav, D., Subramani, S. K., Katare, C., & Prasad, G. B. K. S. (2018). Medicinal uses of honey: a review on its benefits to human health. *Progress in Nutrition*, *20*, 5-14.

Salmanpour, F., Shakoori, Z., Kia, M., Eshaghi, R., Ghaderi, M., Ghomi, S., ... & Farhadinia, M. S. (2023). Mineral lick use by a community of large herbivores in northern Iran. *Ecology and Evolution*, *13*(1), e9731.

Shivanna, K. R., Rangaswamy, N. S., Shivanna, K. R., & Rangaswamy, N. S. (1992). Self-incompatibility.

Terzo, S., Mulè, F., & Amato, A. (2020). Honey and obesity-related dysfunctions: A summary on health benefits. *The Journal of Nutritional Biochemistry*, *82*, 108401.

Tulu, D., Aleme, M., Mengistu, G., Bogale, A., Bezabeh, A., & Mendesil, E. (2020). Improved beekeeping technology in Southwestern Ethiopia: Focus on beekeepers' perception, adoption rate, and adoption determinants. *Cogent Food and Agriculture*, *6*(1), 1814070.

Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honey bees. *Annual Review of Entomology*, *63*, 327-344.