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Short Communication

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Analysis of pollen in honey samples in the district of Prayagraj, India

Jafar Mehdi[1]*, Ravi Kumar Yadav[2], Anil Kumar Gupta[1]

[1]Division of Plant Breeding and Genetic Resource Conservation, CSIR Central Institute of Medicinal and Aromatic Plant, Lucknow-226022, Uttar Pradesh, India, [2]Department of Botany, Kashi Naresh Government Post Graduate College, Gyanpur, Bhadohi-221304, Uttar Pradesh, India

ABSTRACT

The analysis of pollen grains found in honey, known as melissopalynology, reveals pertinent details about the pollen and nectar sources in a location where bees produce honey, allowing researchers to identify the honey's geographical and botanical origins. To determine the significant source plants in the area, the present analysis is conducted on five samples of winter honey that were collected from Prayagraj's urban localities (Dahi, Baksi, Sirsa, Soraon, and Phoolpur). The methodology recommended by the International Commission of Bee Botany (ICBB) was followed in this study. Analysis of 5 honey samples recorded a diversity of 31 pollen types and one fungal spore type. The majority of pollen grains recovered from honey samples belong to entomophilous taxa (66%), 25% of the pollen is from anemophilous taxa, and 9% from amphiphilous taxa. Four honey samples (S1, S2, S3, and S5) were found to be unifloral while the remaining samples (S4) were multi-floral. The field mustard i.e. *Brassica campestris* L. was the predominant pollen type. The secondary frequency class contained three different pollen types, while the minor and significant minor frequency classes contained 14 and 30, respectively. With respect to the frequency of occurrence of pollen types in honey samples, field mustard was found to be a very common pollen type as they were recovered from more than 50% of the collected honey samples. The various type of pollen and spores were also observed in collected honey samples.

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***Corresponding Author:**
Jafar Mehdi
E-mail: jafarmehdi075@gmail.com

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INTRODUCTION

Bees and nectariferous plants interact with one another to make honey. Honey bees gather pollen grains in addition to nectar while foraging. Determine the geographic and botanical origin of honey by conducting a pollen study on a honey sample to learn more about the plants that the bees visited, which provides pertinent information about the nectar and pollen sources of an area (Louveaux *et al.*, 1978; Barth, 2004). To stabilize an apiary sector for commercial honey production, beekeepers must have a working knowledge of the botanical sources of honey.

Research on pollen analysis of honey samples has been conducted in India in a number of different states, including Maharashtra (Deodiker *et al.*, 1958), Andhra Pradesh (Devender & Ramakrishna, 2015), Bihar (Sahney & Seth, 2013), Haryana (Chaudhary, 2003), West Bengal (Kamble *et al.*, 2015). Melissopalynological reports, however, are only accessible from Prayagraj (Sahney & Rahi, 2015), Shahjahanpur (Chandra & Sharma, 2011), and Lucknow (Sharma & Nair, 1965; Chaturvedi, 1973) in Uttar Pradesh. In order to determine the source plants of the area for honey production, the current study is conducted on the honey samples gathered from the Prayagraj district of Uttar Pradesh. Present work is a detailed finding of melissopalynological studies in the urban localities of Prayagraj district.

MATERIALS AND METHODS

Collection of materials

The study area was Prayagraj (previously known as Allahabad) situated at the bank Ganga and Yamuna rivers in Uttar Pradesh. The meadows vegetation there makes up the Gangetic Plains, a floristic division of India that is primarily made up of wastelands and trees that are mainly grown along roadsides and in gardens. The climate in Prayagraj is similar to that of other cities in north-central India, which is a humid subtropical climate. A hot, dry summer, a cool, dry winter, and a warm, humid monsoon are the three seasons that Prayagraj experiences. A maximum temperature of 40 to 45°C (104 to 113°F) is typical throughout the summer months of April to June. The monsoon season starts in early July and lasts through September. From December to February is the winter season. During winter, the daily average

maximum temperature is about 23°C and the minimum is about 8.9°C.

Five squeezed honey samples of giant honey bees (*Apis dorsata*) were collected during December 2015 from five different localities viz. Bakshi, Dahi, Phoolpur, Sirsa, and Soraon of Prayagraj district. These samples of honey were collected with the help of honey hunters. The collected samples were used for further studies.

Pollen Analysis

To prepare honey slides for the recovery, analysis, and quantification of pollen grains, Louveaux *et al.* (1978) recommendations for the International Commission of Bee Botany (ICBB) were followed. Five slides were made from each sample of honey and carefully examined with a compound microscope. From each slide, approximate 300 pollen grains were counted at random for determining frequency classes and divided into four classes. First predominant (>45% of the counted pollen grains), secondary (16-45% pollen), third important minor (3-15% pollen), and last minor (<3% pollen).

Reference slides and pertinent literature were used to identify the pollen grains found in honey samples (Bhattacharya *et al.*, 2006). The classification of a honey sample with a single dominant pollen type is "Unifloral", while the classification of a sample with no dominant pollen type is "Multifloral". Pollen types have been categorized as extremely frequent (pollen type present in more than 50 percent of samples), frequent (20-50 percent), infrequent (10-20 percent), and rare (10 percent) based on the frequency of occurrence in all honey samples (Feller-Demalsy *et al.*, 1987). According to Suryanarayana *et al.* (1981), the absolute pollen count (APC) of a honey sample was calculated using a hemocytometer.

RESULT AND DISCUSSION

The microscopic analysis of five samples of honey collected from five different locations in the Prayagraj district. The identified 31 different types of pollens and one fungal spore belong to 21 different families (Table 1 & Figure 1).

Table 2 lists the pollen types, their frequency classes, APCs, and pollen diversity found in the honey sample. Four of the five honey samples were discovered to be unifloral in nature, and one to be multifloral. Predominant kinds were identified as *Brassica campestris*. *Ageratum conyzoides*, *Psidium*, and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (*Eucalyptus citriodora*) produced secondary pollen types. Table 2 lists the types of pollen, their frequency classes, their APCs, and their diversity in honey samples taken from the Prayagraj district. While pollen grains of *Coriandrum sativum* L., *Solanum nigrum Acerbi ex Dunal*, *Phlox* sp., *Citrus* sp., (Grass) *Poaceae*, *Ageratum conyzoides* (L.) L., *Leucaena* sp., *Laythyrus odoratus* L., *Phyllanthes* sp., *Polygonum* sp. and *Alternaria*

Table 1: Plant habit and mode of pollination of source for honey production collected from different localities of Prayagraj district

S. No.	Name of Plants species	Common name/Local name	Family	Growth Form	Mode of pollination
1	*Achyranthes* sp.	-	Amaranthaceae	Herb	EN
2	*Adhatoda vesica*	Malabar Nut	Acanthaceae	Shrub	EN
3	*Ageratum conyzoides* (L.) L.	Goat weed	Asteraceae	Herb	EN
4	*Alternaria* sp. (Fungus)	-	Pleosporaceae	_	-
5	*Bombax ceiba* L.	-	Malvaceae	Tree	EN
6	*Brassica campestris* L.	Field mustard	Brassicaceae	Herb	EN
7	*Cajanus cajan* (L.) Mill.sp.	Pigeon pea	Fabaceae	Herb	EN
8	*Callistemon citrinus (Curtis)* Skeels	Bottlebrush Tree	Myrtaceae	Tree	EN
9	*Chenopodium* sp.	-	Amaranthaceae	Herb	AN
10	*Citrus* sp.	-	Rutaceae	Shrub	EN
11	*Convolvulus* sp.	-	Convolvulaceae	Herb	EN
12	*Coriandrum sativum* L.	Coriander	Apiaceae	Herb	EN
13	*Cucurbita maxima* Duchesne	Giant Pumpkin	Cucurbitaceae	Climber	EN
14	*Datura* sp.	-	Solanaceae	Herb	EN
15	*Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson	Lemon-Scented Gum	Myrtaceae	Tree	AM
16	*Holoptelea integrifolia* (Roxb.) Planch.	Indian Elm	Ulmaceae	Tree	AN
17	*Laythyrus odoratus* L.	Sweet pea	Fabaceae	Shrub	AM
18	*Leucaena* sp.	-	Fabaceae	Shrub	AN
19	Monocot plant sp.	-	-	-	-
20	*Morus alba* L.	White Mulberry	Moraceae	Tree	AM
21	*Phlox* sp.	-	Polemoniaceae	Herb	AN
22	*Phyllanthus* sp.	-	Phyllanthaceae	Herb	EN
23	*Pisum sativum* L.	Garden pea	Fabaceae	Herb	EN
24	Grass	-	Poaceae	Herb	AN
25	*Polygonum* sp.	-	Polygonaceae	Herb	EN
26	*Prosopis* sp.	-	Fabaceae	Tree	EN
27	*Psidium guajava* L.	Common guava	Myrtaceae	Tree	EN
28	*Ricinus communis* L.	Castor bean	Euphorbiaceae	Shrub	AN
29	*Solanum nigrum* Acerbi ex Dunal	Makoi or black nightshade	Solanaceae	Herb	EN
30	*Sonchus arvensis* L.	Field sowthistle	Asteraceae	Herb	EN
31	*Thuja occidentalis* L.	Northern white-cedar	Cupressaceae	Tree	AN
32	*Triticum aestivum* L.	Bread wheat	Poaceae	Herb	AN

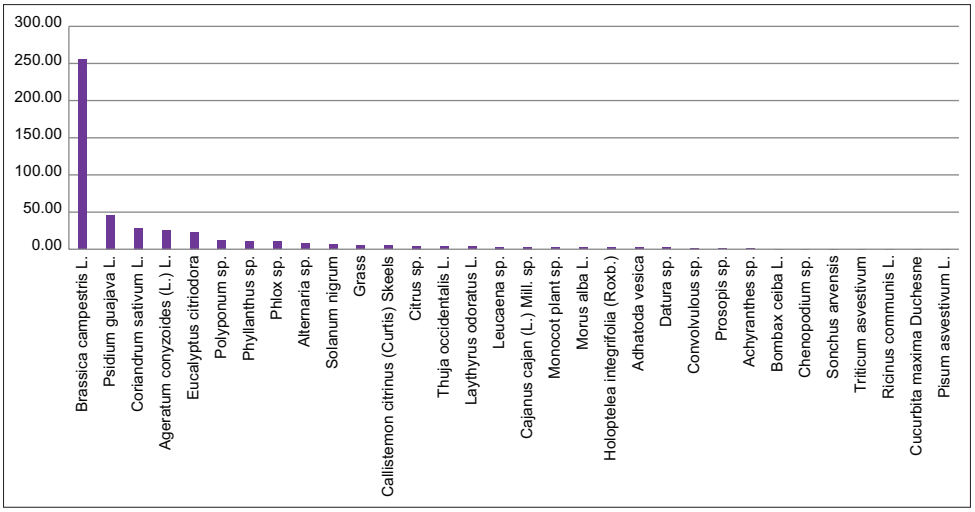

Figure 1: Frequency of pollen types present in the honey samples

Table 2: Pollen types, frequency class, APC and pollen diversity in honey samples collected different localities of Prayagraj district

S. No.	Plants species	Common name/Local name	S1	S2	S3	S4	S5
1	*Achyranthes* sp.	-					M
2	*Adhatoda vesica*	Malabar Nut	M				
3	*Ageratum conyzoides*	Goat weed	M	M	I	S	M
4	*Alternaria* sp. (Fungus)	-					I
5	*Bombax ceiba* (L.) L.	-				M	
6	*Brassica campestris L.*	Field mustard	P	P	P	M	P
7	*Cajanus cajan* (L.) Mill.sp.	Pigeon pea	M	M	M		M
8	*Callistemon citrinus (Curtis)* Skeels	Bottlebrush Tree	M	M			
9	*Chenopodium*	-				M	
10	*Citrus* sp.	-			I		
11	*Convolvulus* sp.	-			M		
12	*Coriandrum sativum* L.	Coriander	I	I		I	I
13	*Cucurbita maxima* Duchesne	Giant Pumpkin			M		
14	*Datura* sp.	-			M		
15	*Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson	Lemon-Scented Gum					S
16	*Holoptelea integrifolia (Roxb.) Planch.*	Indian Elm			M		
17	*Laythyrus odoratus* L.	Sweet pea				I	
18	*Leucaena* sp.	-			I		
19	Monocot plant sp.	-				M	
20	*Morus alba* L.	White Mulberry				M	
21	*Phlox* sp.	-			I		
22	*Phyllanthus* sp.	-				I	M
23	*Pisum sativum* L.	Garden pea		M	M		
24	Grass	-			I		M
25	*Polygonum* sp.	-				I	
26	*Prosopis* sp.	-		M			
27	*Psidium guajava* L.	Common guava				S	
28	*Ricinus communis* L.	Castor bean					M
29	*Solanum nigrum* Acerbi ex Dunal	Makoi or black nightshade	I				
30	*Sonchus arvensis* L.	Field sowthistle				M	
31	*Thuja occidentalis* L.	Northern white-cedar			M		M
32	*Triticum aestivum* L.	Bread wheat		M			
	Pollen Diversity		7	8	13	12	11
	APC		GPIV	GPIII	GPIII	GPIII	GPIII

APC = Absolute pollen count; P = predominant; S = Secondary; I = important minor; M= minor

sp. (spore) were recorded in important minor frequency class. 23 pollen types were registered in minor frequency class viz. *Callistemon citrinus (Curtis) Skeels*, *Cajanus cajan* (L.) Mill.sp., *Adhatoda*, A. *conyzoides*, *Prosopis* sp., *Triticum* L, *Pisum sativum* L., *Holoptelea integrifolia (Roxb.) Planch*, *Cucurbita maxima* Duchesne, *Psidium guajava* L., *Datura* sp., *Convolvulus* sp., *Monocot* sp., *Brassica campestris* L., *Bombax ceiba* L., *Chenopodium* sp., *Sonchus arvensis* L., *Morus alba* L., *Phyllanthus* sp., Grass, *Achyranthes* sp., *Ricinus communis* L., and *Thuja occidentalis* were relatively common pollen types in

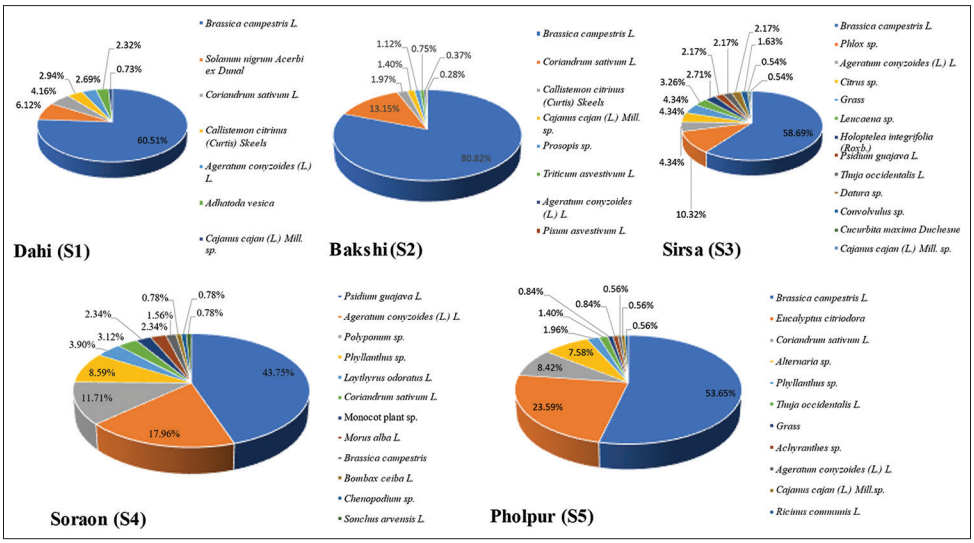

Figure 2: Pollen spectra of S1, S2, S3, S5 Uni-floral and S4 Multifloral

the total honey samples, as they were recovered from more than 50% of the samples. Eight pollen types were identified as being more common than the other 24 types of pollen (Figure 1).

One sample (Dahi) belonged to Group IV in terms of the absolute pollen count, whereas four samples (Bakshi, Sirsa, Soraon, and Phoolpur) belonged to Group III. Five honey samples were gathered from various locations in the Prayagraj district, and their pollen spectra showed a variety in the pollen makeup (Figure 2). From samples of honey, 31 different pollen type of pollen and one fungal spore were found in the samples. The diversity of pollen types ranged from 10-16. Maximum diversity of pollen types was recorded in honey samples S3 (13 pollen types) & S4 (12 pollen types). The samples S4 were multi-floral in nature. In sample S4 (Soraon) *Psidium* and *Ageratum* were present as secondary pollen types with 47.75% and 17.96% frequency respectively while in sample S5 (Phoolpur) *Eucalyptus* (23.59%) were present as secondary pollen types. Both the localities Soraon (S4) & Phoolpur (S5) are on outside of Prayagraj city. Samples S1 (7 pollen types) and S2 had the least amount of pollen variety (8 pollen types). Both samples were of a unifloral type. In samples S1, S2, S3 and S5 *B. Campestris* L. was the predominant pollen type with 60.51%, 80.82%, 58.69% & 53.65% frequency. Numerous researchers have noted *Brassica* sp. as a significant source of pollen and nectar in a number of Indian states, including West Bengal (Pal & Karmakar, 2013), Assam (Sharma & Saharia, 2011), Garhwal (Tiwari *et al.*, 2010), Karnataka (Shubharani *et al.*, 2012), Andhra Pradesh (Devender *et al.*, 2019), Madhya Pradesh (Sahney & Rahi, 2015). *B. campestris* was the predominant pollen type in the samples of honey collected from Prayagraj (Allahabad) district (Sahney *et al.*, 2018). Further in samples S1, S2 and S3 the secondary pollen type was absent. Blooming crops of *B. campestris* L. in Bakshi, Dahi, Phoolpur and Sirsa considered as rich source of pollen to the honey bees for honey production.

CONCLUSIONS

The majority of pollen grains found in honey samples were from entomophilous taxa (66%) and amphiphilic taxa (9%) while 25% of the pollen came from anemophilous taxa. *B. campestris*, A. *conyzoides*, and *P. guajava* were represented in the predominant/secondary frequency class among the entomophilous taxa, and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (*E. citriodora*) was among the amphibious species in the honey samples. *B. campestris*, *Psidium*, *E. citriodora*, and A. *conyzoides* can be considered major bee source plants in the investigated urban localities of the Prayagraj region. Based on current melissopalynological investigation their pollen has been recorded in predominant and secondary frequency classes and is also very common in occurrence in the honey samples. The honey samples also contained some fungal spores also. Citing the importance of melissopalynology, it is recommended to expand more research work in this field. Also, the work elucidates that *B. campestris* L. was present in highest frequency in the pollens. Mustard plant and other plants is a rich repository of numerous phytochemical constituents and secondary metabolites hence authors suggest expanding the cultivation of this plant in order to reform the melissopalynological field.

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CONFLICT OF INTEREST

There is no potential conflict of interest.

AUTHOR CONTRIBUTIONS

JM designed the manuscript and performed the experiments. JM and RKY analyzed the data. AKG and RKY prepared the first draft of the manuscript.

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