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Pollen analysis of honey samples from Allahabad District, Uttar Pradesh, India

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ABSTRACT

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Pollen analysis of 14 honey samples of *Apis dorsata*, collected from rural localities of Allahabad district, Uttar Pradesh, has been carried out. Altogether, 42 pollen morphotypes have been identified. Eight honey samples were unifloral and six were multifloral in nature, of which three were bifloral and three were polyfloral. Unifloral honeys were represented by *Brassica campestris* (in four samples), *Eucalyptus citriodora, Psidium guajava, Holoptelea integrifolia* and Poaceae (in one sample each). In multifloral honeys, *Brassica campestris* was the common secondary pollen type found in combination with other secondary pollen type/ types. In total honey samples, pollen of *Brassica campestris, Coriandrum sativum, Eucalyptus citriodora* and Poaceae were very frequent in occurrence. Bulk of honey pollen originated from entomophilous taxa (54.14%) and amphiphilous taxa (33.33%) while 9.52% pollen grains originated from anemophilous taxa. Occurrence of anemophilous pollen types, viz. *Holoptelea integrifolia* and Poaceae, in predominant frequency class has been discussed. The present study suggests that *Brassica campestris, Eucalyptus citriodora, Coriandrum sativum* and Poaceae can be regarded as the major bee forage plants of the region.

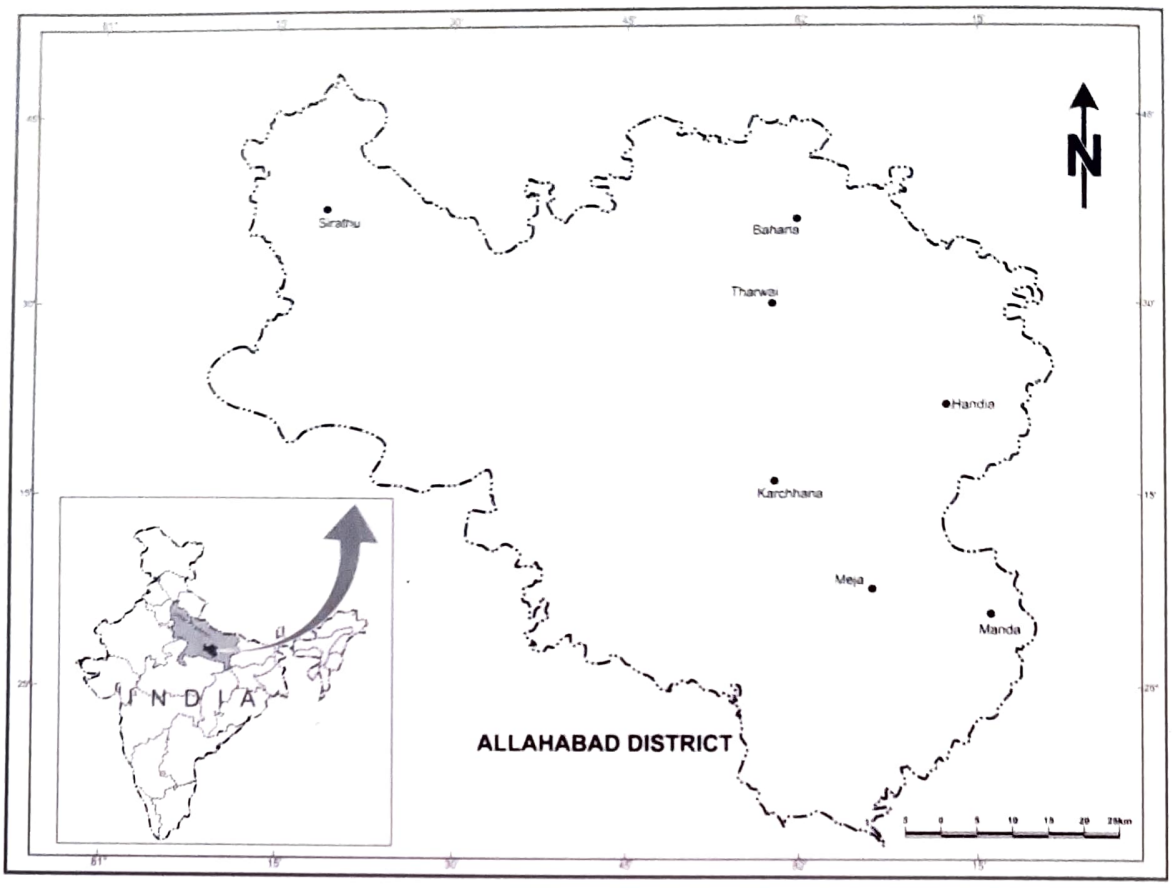
Key-words: Pollen analysis, unifloral honey, multifloral honey, bee forage plants, Allahabad District, Uttar Pradesh, India.

INTRODUCTION

Honey is a natural sweet substance produced by mutual interaction between honey bees and nectar yielding plants. Honey bees also collect pollen grains along with nectar which constitute bee forage. Melissopalynology, dealing with the microscopic analysis of pollen grains present in honey, allows identification of main nectar sources used by the bees for the production of honey in a region, classifying the honey botanically and geographically according to its origin (Louveaux et al. 1978, Von der Ohe et al. 2004, Barth 2004).

Pollen analysis of honey samples enables to understand distribution and abundance of nectar and pollen sources in a region and allows characterization of honey of different regions, in terms of flora and vegetation. Melissopalynological studies are thus important in hive management and in development of beekeeping (Tiwari et al. 2012).

In India, melissopalynological researches were initiated by Deodikar and co-workers (Deodikar & Thakar 1953, Deodikar et al. 1958) in Maharashtra. Subsequently, significant work on melissopalynology has been reported from Andhra Pradesh (Ramanujam & Kalpana 1991, Ramanujam & Khatija 1992, Ramanujam et al. 1992, Jhansi et al. 1994, Lakshmi & Suryanarayna 2004, Chaya & Verma 2004, 2008, Ramakrishna & Swathi 2013), Bihar (Suryanarayna et



Text-Figure 1. Map showing rural localities of Allahabad District from where honey samples were collected.

al. 1992), Himachal Pradesh (Sharma 1970, Sharma & Raj 1985), West Bengal (Bhattacharya et al. 1983, Jana et al. 2002, Chakrabarti & Bhattacharya 2011, Pal & Karmarkar 2013), Uttarakhand (Garg & Nair 1974), Karnataka (Agashe & Rangaswamy 1997, Seethalakshmi 1980, Bhargava et al. 2009, Chauhan & Murthy 2010, Shubharani et al. 2012) Orissa (Upadhyay & Bera 2008, 2012) and Madhya Pradesh (Sahney & Seth 2007, 2010, Chauhan & Quamar 2010).

However, melissopalynological studies from Uttar Pradesh are limited and are largely confined to Lucknow (Sharma & Nair 1965, Chaturvedi 1977, Chaturvedi & Sharma 1973, Chauhan & Trivedi 2011) apart from Unnao (Chauhan & Singh 2010) and Shahjahanpur (Chandra & Sharma 2011).

The present melissopalynological investigation is undertaken on the honey samples collected from Allahabad district to identify the bee forage plants in order to assess the potential of the region for apiculture. The paper presents first report on quantitative and qualitative pollen analysis of 14 squeezed honey samples of *Apis dorsata* combs collected from seven rural localities of Allahabad district, Uttar Pradesh.

MATERIAL AND METHOD

Fourteen squeezed honey samples of *Apis dorsata* combs were collected from seven rural localities, viz. Bahariya (Bah-03-13), Handiya (Han-03-13), Karchhana (Kar^{A}-03-13 & Kar^{B}-03-13), Manda (Man^{A}- 11-12 & Man^{B}- 11-12), Meja (Mej^{A}- 03-13, Mej^{B}- 03-13, Mej^{C} 03-13, Mej^{D}- 03-13, Mej^{E}- 03-13 & Mej^{K}- 03-13), Sirathu (Sir- 03-13) and Therwai (The-03-13) of Allahabad district (Text-figure 1) during November 2012 to March 2013. Field surveys were made around the sites of bee hives to collect the floral material for the preparation of reference pollen slides. Identification of pollen grains recovered from honey samples was largely made with the help of reference slides and relevant literature. (Erdtman 1952, Bhattacharya et al. 2006).

The methods recommended by International Commission for Bee Botany (Louveaux et al. 1978) were followed for the recovery, analysis and quantification of pollen contents. 1200 pollen grains

were counted at random (400 pollen grain from each slide) for determining the pollen frequency classes, viz. Predominant (>45%), Secondary (16-45%) Important minor (3-15%) and Minor (< 3%). Sample with one predominant pollen type was categorised under unifloral while the sample in which no predominant pollen was found was categorised under multifloral honeys. Multifloral honey samples have been divided into 'bifloral' when two pollen types were registered in secondary frequency class and 'polyfloral' when three or more pollen types were registered in secondary frequency class (Ramírez-Arriaga et al. 2011). The frequency distribution of pollen types was determined from the number of honey samples in which various pollen types were recognized, viz. very frequent (pollen types present in > 50% of the samples), frequent (20-50%), infrequent (10-20%) and rare (< 10%) (Feller-Demalsy et al. 1987). The absolute pollen count of honey samples was determined using a haemocytometer as suggested by Suryanarayna et al. (1981).

RESULT

Altogether, 42 pollen morphotypes belonging to 27 families were identified from the microscopic examination of 14 honey samples collected from seven rural localities of Allahabad district (Plate 1).

Out of 14 honey samples, 8 were unifloral and 6 were multifloral in nature. *Brassica campestris, Eucalyptus citriodora, Psidium guajava, Holoptelea integrifolia* and Poaceae were recorded as predominant types. Pollen types registered as secondary pollen types include *Callistemon citrinus, Coriandrum sativum, Brassica campestris, Eucalyptus citriodora, Psidium guajava, Holoptelea integrifolia, Rorippa dubia, Syzygium cumini, Ageratum conyzoides* and *Phoenix sylvestris* while *Eucalyptus citriodora, Callistemon citrinus, Psidium guajava, Coriandrum sativum, Spathodea campanulata, Phyllanthus emblica, Luffa acutangula,* Poaceae, *Parthenium hysterophorus, Holoptelea integrifolia, Moringa oleifera, Mangifera indica, Brassica campestris, Spilanthes paniculata, Rorippa dubia, Citrus* sp., *Ageratum conyzoides, Cicer arietinum* were recorded as important minor pollen types and those of *Lathyrus sativus, Acalypha indica, Bombax ceiba, Cassia* sp., *Helianthus annuus, Zea mays, Azadirachta indica,* Amaranthaceae, *Rungia repens, Coriandrum sativum, Jatropha gossypiifolia, Pinus roxburghii, Moringa oleifera, Holoptelea integrifolia, Rorippa dubia, Madhuca indica, Pisum sativum, Cycas* sp., *Phyllanthus emblica, Cajanus cajan, Luffa acutangula, Melia azedarach, Calendula officinalis, Convolvulus arvensis, Hygrophila auriculata, Salvia dorrii, Morus alba, Schleichera oleosa, and* Poaceae were present in minor frequency class.

Four samples registered presence of 13-14 pollen types (Kar^{A}-03-13, Kar^{B}-03-13, Mej^{A}-03-13 & Mej^{D}-03-13), four samples showed 10-12 pollen types (Man^{A}-11-12, Man^{B}-11-12, Mej^{K}-03-13 & Sir-03-13) while six samples recorded 7-9 pollen types (Mej^{E}-03-13, Han-03-13, Mej^{B}-03-13, Mej^{C}-03-13, The-03-13 & Bah-03-13).

With regard to absolute pollen counts, one sample (Bah-03-13) belonged to Gp- IV, two samples (Kar^{A}-03-13 & Kar^{B}-03-13) to Gp–III, ten samples to Gp-II and one sample to Gp- I.

Pollen types, their frequency classes, absolute pollen count and pollen diversity in the honey samples are presented in Table 1.

With regard to frequency of occurrence of pollen types in total honey samples, *Brassica campestris, Coriandrum sativum, Eucalyptus citriodora* and Poaceae were very frequent in occurrence as they were recovered from more than 50% of the honey samples. It may be mentioned here that *Lathyrus sativus* was also very frequent in occurrence but its pollen grains were recovered in minor frequency class only. 11 pollen types were the frequent types as they were present in 20-50% of the honeys. 9 pollen types were infrequent types present in 10- 20% of the honeys and 17 pollen types occurring in < 10% of the honeys were the rare types (Text-figure 2).

DISCUSSION

The present study provides first melissopalynological report on honey samples collected from Allahabad district. Results of the present investigation reveal that the pollen spectra of honey samples collected from various rural localities of

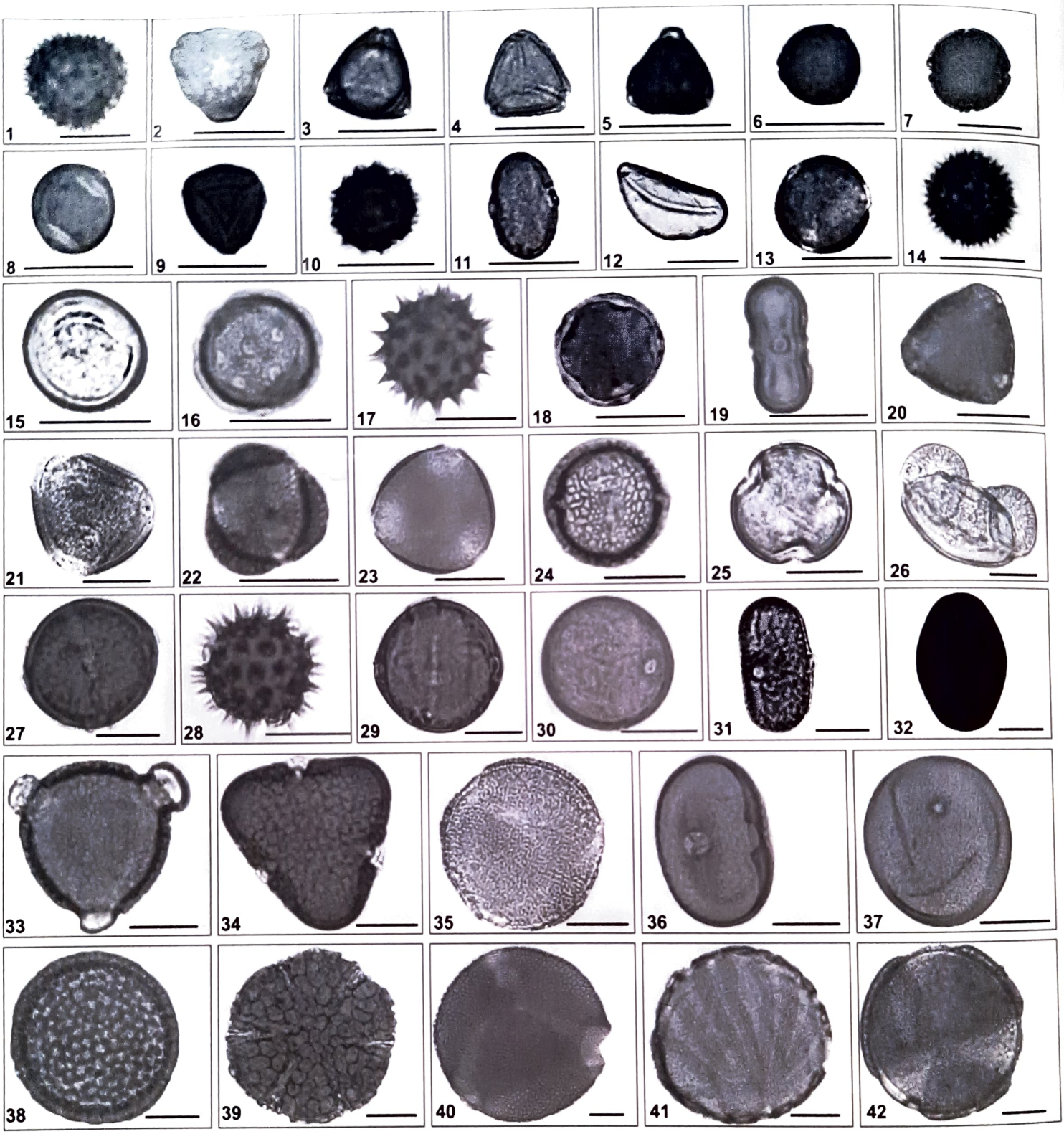
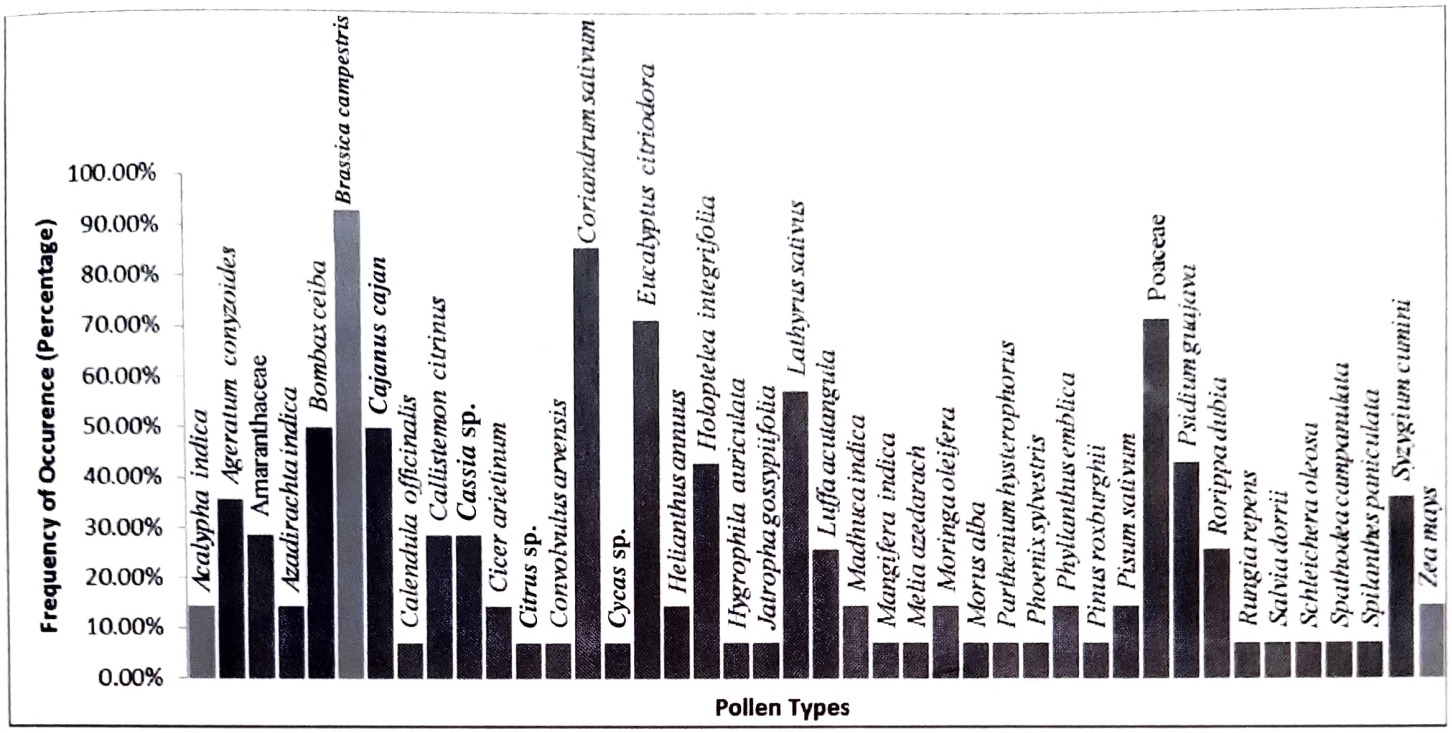


Plate 1

1. *Ageratum conyzoides*. 2. *Eucalyptus citriodora*. 3. *Callistemon citrinus*. 4. *Psidium guajava*. 5. *Syzygium cumini*. 6. *Acalypha indica*. 7. *Phyllanthus emblica*. 8. *Morus alba*. 9. *Schleichera oleosa*. 10. *Parthenium hysterophorus*. 11. *Rungia repens*. 12. *Phoenix sylvestris*. 13. *Rorippa dubia*. 14. *Spilanthes paniculata*. 15. *Cycas* sp. 16. Amaranthaceae. 17. *Helianthus annuus*. 18. *Holoptelea integrifolia*. 19. *Coriandrum sativum*. 20. *Cicer arietinum*. 21. *Mangifera indica*. 22. *Brassica campestris*. 23. *Cassia* sp. 24. *Citrus* sp. 25. *Moringa oleifera*. 26. *Pinus roxburghii*. 27. *Melia azedarach*. 28. *Calendula officinalis*. 29. *Madhuca indica*. 30. Poaceae. 31. *Pisum sativum*. 32. *Azadirachta indica*. 33. *Cajanus cajan*. 34. *Bombax ceiba*. 35. *Spathodea campanulata*. 36. *Lathyrus sativus*. 37. *Zea mays*. 38. *Jatropha gossypiifolia*. 39. *Salvia dorrii*. 40. *Luffa acutangula*. 41. *Hygrophila auriculata*. 42. *Convolvulus arvensis*. Scale bars - 20 µm.



Text-figure 2. Frequency of occurrence of pollen types in the honey samples

Allahabad district show variation in the pollen composition, qualitatively as well as quantitatively. (Text-figure 3)

Out of 14 honey samples, 8 were unifloral and 6 were multifloral in nature. In unifloral honeys *Brassica campestris, Eucalyptus citriodora, Psidium guajava, Holoptelea integrifolia* and Poaceae were the predominant types. Among the multifloral honeys, three samples were bifloral and rest three were polyfloral in nature. *Brassica campestris* was the common secondary pollen type in all the mulifloral samples, in combination with other secondary types viz. *Coriandrum sativum, Holoptelea integrifolia* or *Psidium guajava* in bifloral honey samples and with *Syzygium cumini* & *Ageratum conyzoides, Phoenix sylvestris* & *Ageratum conyzoides* and *Psidium guajava* & *Eucalyptus citriodora* in polyfloral honeys.

Among the above mentioned predominant and secondary pollen types, *Brassica campestris, Eucalyptus citriodora, Coriandrum sativum* and Poaceae were also very frequent in occurrence in the honey samples. This indicates that *Brassica campestris, Eucalyptus citriodora, Coriandrum sativum* and Poaceae are the important source plants playing significant role in the honey production and can be regarded as major bee forage plant of the Allahabad district.

Among these, *Brassica campestris* was registered as the chief source plant as it was recovered from thirteen honey samples, as predominant in four samples, as secondary pollen type in seven samples and as important minor pollen type in one sample.

Coriandrum sativum was present in twelve honey samples but registered as secondary pollen type in only two honey samples while in rest of the samples as important minor (five samples) or minor pollen type (five samples).

Eucalyptus citriodora was present in ten honey samples, as predominant in one sample, secondary in one sample and as important minor pollen type in eight honey samples.

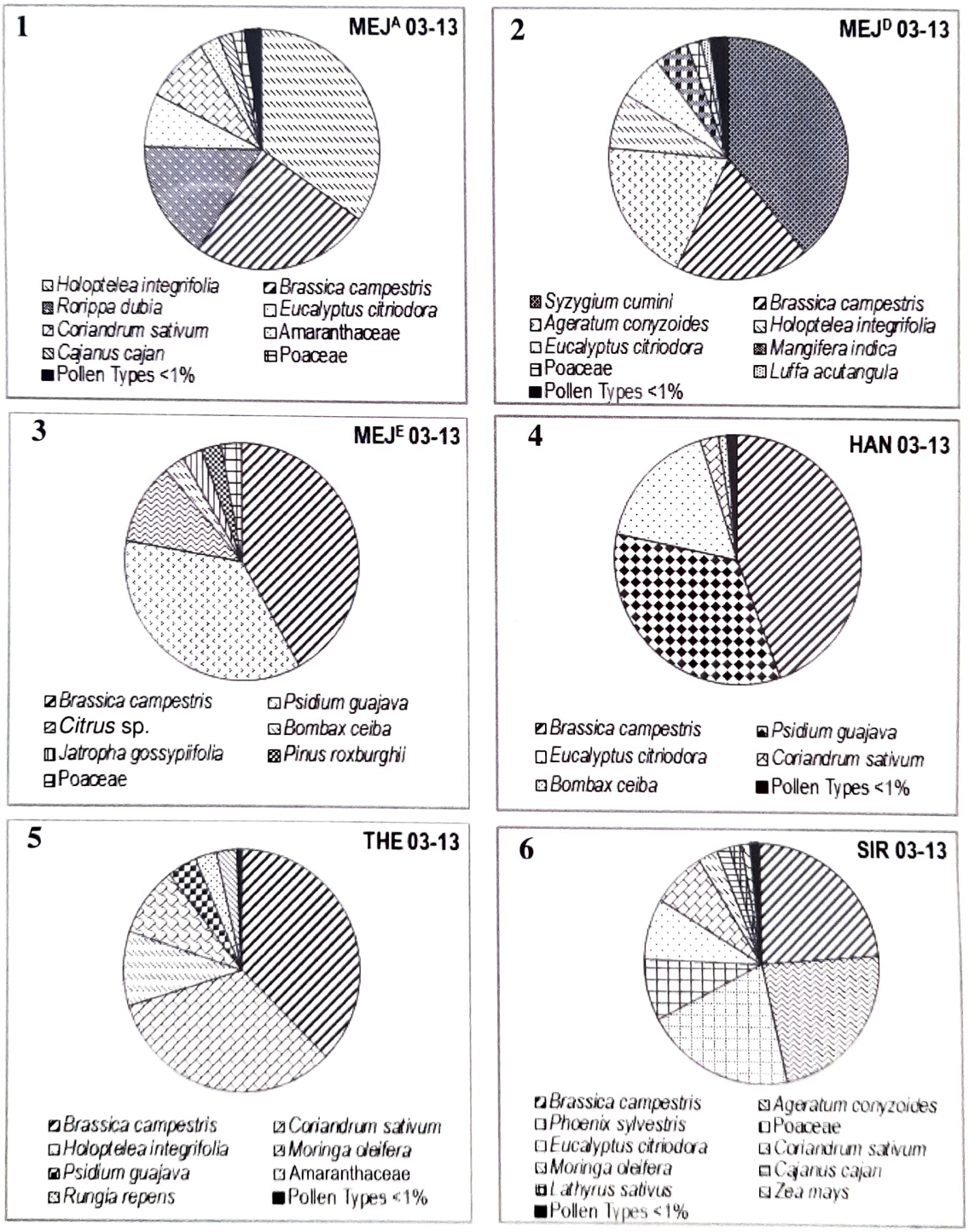
Pollen grains of Poaceae were also recovered from ten honey samples, as predominant in one sample, as important minor in two samples and as minor pollen type in rest of the seven honey samples.

Syzygium cumini (secondary pollen type in five samples), *Psidium guajava* (predominant in one sample), *Ageratum conyzoides* (secondary in two samples), *Holoptelea integrifolia* (predominant in one sample) and *Callistemon citrinus* (secondary in one sample) were frequent in occurrence and may be

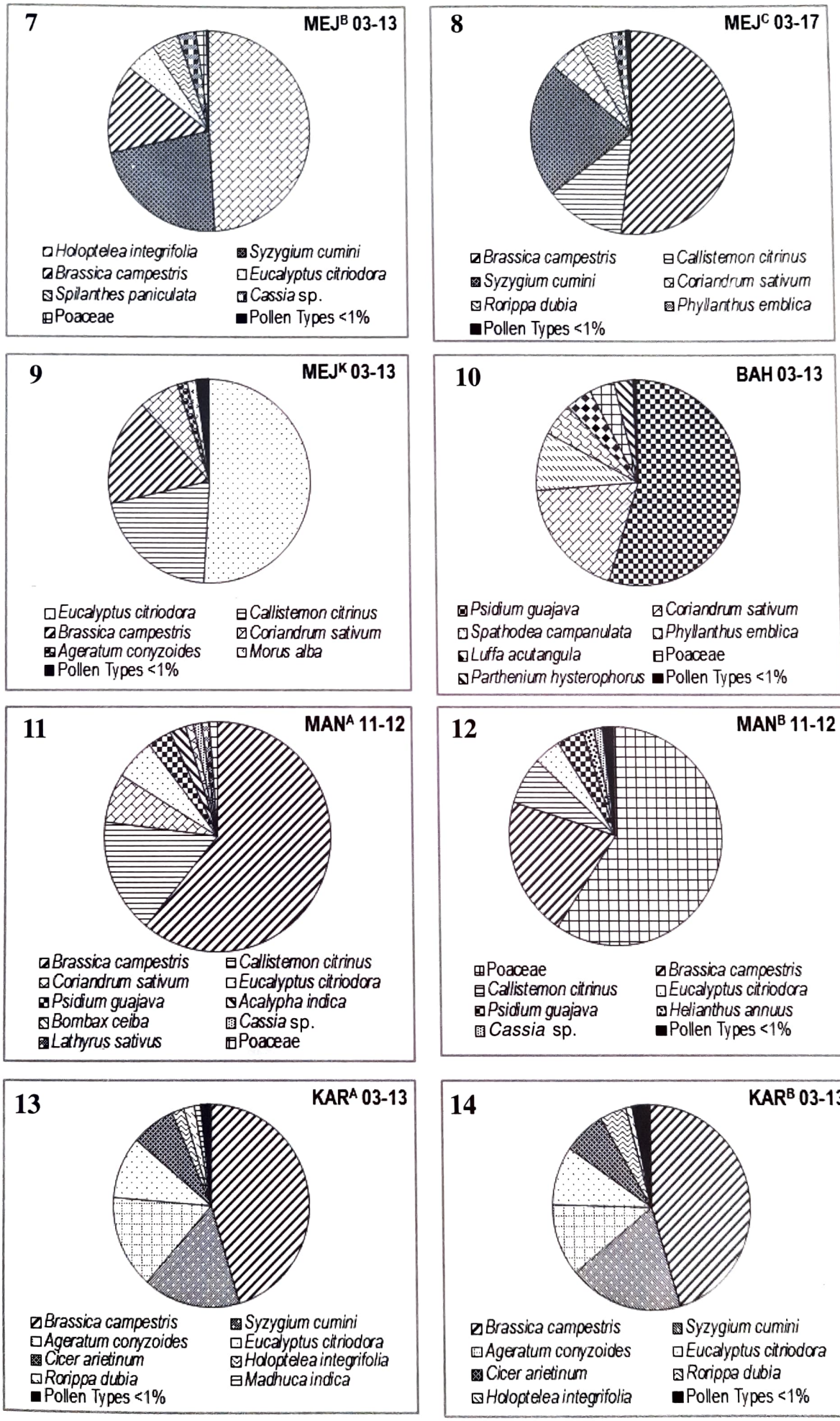
considered as other important bee forage plants of the region. It can be inferred that all the above taxa were in full bloom at the time of honey production.

It is evident from the above account that the same pollen type may be present in different frequency classes in different honey samples. A pollen type present in predominant frequency class/ secondary or important minor in one honey sample may be represented in other frequency class in other sample.

Bees frequently collect a wide variety of pollen grains but they generally concentrate on a few species (Bauma et al. 2011, Dimou & Thrasyvoulou 2007). In the present work a diverse spectrum of 42 pollen types was recovered from 14 honey samples and the pollen diversity per sample ranged from 7 to 14 pollen types (average 10.5). Maximum diversity of 14 pollen types was recorded in two samples (Mej^{A}-03-13 & Mej^{D}-03-13) while minimum diversity of 7 pollen types was registered in Mej^{E}-03-13 & Han-03-13.



Text-figure 3. Pollen spectra of honey samples (1-6 Multifloral)



Text-figure 3 (Continued). Pollen spectra of honey samples (7-14 Unifloral)

Table 1. Pollen types, frequency classes, APC and pollen diversity in honey samples collected from rural localities of Allahabad district, Uttar Pradesh.

Pollen types	Sample code.													
	Man^{A} 11-12	Man^{B} 11-12	Bah 03-13	The 03-13	Mej^{K} 03-13	Mej^{A} 03-13	Mej^{B} 03-13	Mej^{C} 03-17	Mej^{D} 03-13	Mej^{E} 03-13	Sir 03-13	Han 03-13	Kar^{A} 3-13	Kar^{B} 03-13
Acalypha indica	M	M												
Ageratum conyzoides					M				S		S		I	I
Amaranthaceae			M	M	M								M	
Azadirachta indica		M							M					
Bombax ceiba	M	M				M			M	M		M	M	
Brassica campestris	P	S		S	S	S	I	P	S	S	S	S	P	P
Cajanus cajan					M	M		M	M		M	M		M
Calendula officinalis						M								
Callistemon citrinus	I	I			S			I						
Cassia sp.	M	M					M					M		
Cicer arietinum													I	I
Citrus sp.										I				
Convolvulus arvensis						M								
Coriandrum sativum	I		S	S	I	I	M	I	M		I	M	M	M
Cycas sp.														M
Eucalyptus citriodora	I	I			P	I	I		I		I	S	I	I
Helianthus annuus		M									M			
Holoptelea integrifolia				I		S	P		I				M	M
Hygrophila auriculata					M									
Jatropha gossypiifolia						M								
Lathyrus sativus	M	M	M	M	M	M			M		M			
Luffa acutangula			I		M	M		M						
Madhuca indica													M	M
Mangifera indica									I					
Melia azedarach					M									
Moringa oleifera				I							M			
Morus alba					M									
Parthenium hysterophorus			I											
Phoenix sylvestris											S			
Phyllanthus emblica			I					M						
Pinus roxburghii										M				
Pisum sativum													M	M
Poaceae	M	P	I			M	M			M	I		M	M
Psidium guajava	I	I	P	I						S		S		
Rorippa dubia						I		I					M	I
Rungia repens				M										
Salvia dorrii						M								
Schleichera oleosa											M			
Spathodea campanulata			I											
Spilanthes paniculata							I							
Syzygium cumini							S	S	S				S	S
Zea mays									M		M			
Pollen Diversity	10	11	9	8	12	14	8	8	14	7	12	7	13	13
APC	Gp-II	Gp-II	Gp-IV	Gp-I	Gp-II	Gp-II	Gp-II	Gp-II	Gp-II	Gp-II	Gp-II	Gp-II	Gp-III	Gp-III

With regard to absolute pollen counts highest pollen richness was observed in sample Bah-03-13 as it belonged to Gp-IV while sample The-03-13 demonstrated poorness in terms of quantity as it belonged to Gp-I.

Variations in the absolute pollen counts and diversity of pollen types in honey samples as well as representation of a pollen type in various frequency classes in honey samples as mentioned above may be due to variations in the availability and abundance of the blooming vegetation within the foraging range of the honey bees.

Bulk of honey pollen originated from entomophilous taxa (54.14%) and amphiphilous taxa (33.33%) while 9.52% pollen grains originated from anemophilous taxa. Though poorly represented anemophilous pollen grains have been reported in honey samples (Reddy & Reddy 2008, Bhargava et al. 2009, Chauhan & Murthy 2010). However in the present investigation in two honey samples anemophilous pollen grains of Poaceae (Man^{B}-11-12) and *Holoptelea integrifolia* (Mej^{B}-03-13) are present in predominant frequency class. *Holoptelea integrifolia* and Poaceae are known to be high pollen producers (Agnihotri & Singh 1975, Sahney & Chaurasia 2008b) and are reported to be dominant pollen types in the airborne pollen flora of Allahabad, recording their peak occurrence in October-November (Poaceae) and February-March (*Holoptelea integrifolia*) (Nautiyal & Midha 1984, Sahney & Chaurasia 2008a, c, d). It may be mentioned here that sample Man^{B}-11-12 (predominant pollen type Poaceae) was collected during November while that of Mej^{B}-03-13 (predominant pollen type *Holoptelea integrifolia*) was collected during March. Thus it is very possible that abundant pollen grains present in the atmosphere get into honey comb through the wind current. Moreover insect pollination is also reported in some grasses eg. *Dichanthium annulatum* (Pant et al.1982).

The present investigation on the pollen analysis of honey samples of Allahabad provides data regarding the bee forage plants in the area of investigation. The study indicates a good potential for apiculture in the region because of the diversity of bee forage plants in the rural localities of Allahabad district which include *Brassica campestris, Eucalyptus citriodora, Coriandrum sativum, Syzygium cumini, Psidium guajava, Ageratum conyzoides, Holoptelea integrifolia, Callistemon citrinus and* Poaceae. Thus the cultivation of above mentioned plants with good forage value should be encouraged to promote beekeeping industry.

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