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Melissopalynological investigations of *Apis mellifera* L. corbicular pollen and honey from Hisar, Haryana

Hasansab A. Nadaf*, G. S. Yadav, H. D. Kaushik and S. K. Sharma

College of Agriculture, CCS Haryana Agricultural University, Hisar, Haryana, India
Email: hnadaf@gmail.com

ABSTRACT: The melissopalynological analysis of corbicular pollen and honey samples collected from a single apiary of *Apis mellifera* L**.** revealed a total of 28 pollen types representing 25 genera and 19 families and a total of 26 pollen types representing 23 genera and 18 families, respectively. Only one pollen type failed to be identified while two were identified to the level of family. The observed pollen types were classified as predominant, secondary, important minor and minor pollen types. When one floral type represented is 45%, it was classified as unifloral. Of the total honey samples analyzed, January, February, March, April, June, July, October and December month samples were found to be unifloral.

KEYWORDS: *Apis mellifera,* pollen loads, unifloral

INTRODUCTION

Honeybee *Apis mellifera* L. readily gather pollen from the flowers of many entomophilous species providing both nectar and pollen (Percival 1947; Rashad and Parkar, 1958; Vaissiere and Vinson, 1994). Nectar is a carbohydrate source while pollen supplies bees with protein, lipids, vitamins and minerals needed to rear larvae (Manning, 2001). Exogenous pollen may be introduced into a beehive in numerous ways: bees carry pollen to the hive in their pollen baskets (corbicula), pollen grains may fall from bees body parts into the nectar filled combs, airborne pollen may enter the hive via air currents, used wax combs are added to hives or imported pollen is fed to bees (Rouff and Bogdanov, 2004; Erdogon and Erdogon, 2014). Pollen grains in the honey not onlyreflect regional agriculture and forest vegetation but also the floral diversity and species of plants foraged by honeybees available in the vicinity of the apiary (Louveaux *et al*., 1978). Melissopalynology (study of pollens in relation to honeybees), therefore, is useful in determining and controlling the honeys geographical and botanical origin. This information has commercial value because honey of dominant floral type can bring higher price than honey of mixed or unknown floral sources as the unifloral honeys maintain always the same physico-chemical and organoleptic characteristics and are well appreciated for commerce (Barth, 2004). Though pollen analysis studies have been carried out from different parts of India (Bharghava *et al.,* 2009; Attri, 2010; Timande and Tembhare, 2010; Cherian *et al,* 2011; shubharani *et al*., 2012, Tiwari *et al*., 2012) but no such efforts has ever been made in Haryana. Hence the aim of the present work is to identify the pollen sources availed by *A. mellifera* during different season through melissoplaynological investigations of corbicular pollen and honey from Hisar, Haryana, India.

* Author for correspondence

MATERIAL AND METHODS

The materials required and methods followed in the present studies are described hereunder in detail. Ten samples of both pollen loads and honeys were collected from ten different hives of Research Apiary of Project Coordinating unit, All India Coordinated Research Project (AICRP) on Honey Bees and Pollinators, Haryana Agricultural University (HAU), Hisar (29.1605N 75.7204E) twice in a month from January, 2012 to December, 2012 except second fortnight of July and entire month of August due to sharp decline in its population upon foraging by bees in the garden nearby which was sprayed with insecticides. The honey bees reaching the hives after foraging were caught, the corbicular pollen from such bees were collected and observed for their floral identity. In some cases pollens were also collected from the hive for the same purpose. Honey bees were anesthetized with ethyl acetate. The pollen loads were then pushed off the hind legs into an individual specimen tubes.

Method given by Bilisik *et al*. (2008) was followed in which a piece of each pollen load was mixed with glycerine-jelly and stained using basic fuschin (Wodehouse, 1935). The honey samples for microscopic pollen analysis were prepared according to Salonen *et al*. (2009) by slight modified method recommended by International Commission for Bee Botany (Louveaux *et al*., 1978) in the laboratory as follows: ten grams of honey were dissolved in 20 ml of distilled water and centrifuged (10 min, 3500 rpm). The supernatant was disposed off and the sediment washed again with 20 ml of water. The supernatant was again removed after the second centrifugation (5 min, 3500 rpm) and the residue transferred using a Pasteur pipette onto a microscope slide, which was left to dry, covered by a piece of paper, until the next day. The sample area was subsequently covered with Kaiser's glycerol-gelatin and a coverslip, and again left to dry under paper for 24 hours.

The pollen grains were examined using ocular microscope assisted with computer and Videology USB Viewer Software Version 2.1.100 and compared with the reference pollen slides of the vegetation surrounding the hives.The reference pollen slides were prepared following the methodology of Schweitzer (2009). Minimum of 1000 pollen grains were counted per sample as recommended by Behm *et al*. (1996). From this data, the percentage of the each taxon of pollen grains was calculated and the average was worked out for each month. The unknown pollens were recorded as unidentified. Pollen types were classified into four types: predominant pollen (>45%), secondary pollen (16-45%), important minor pollen (3-15%) and minor (<3%) pollen. When one pollen type represented 45% of the total number of pollen grains, the sample was classified as unifloral honey (Louveaux *et al.,* 1978).

RESULTS AND DISCUSSION

The observed pollen types in the pollen loads are to be considered typical of the habitats and vegetation forms of the study area, allowing the identification of the floral origin of the pollen supply during the analysed period. The pollen types harvested by *A. mellifera* at Hisar included *Acacia katechu* (Mimosaceae), *Aegle marmelos* (Myrtaceae), *Azadirachta indica* (Meliaceae), *Brassica juncea* (Brassicaceae), *Callistemon lanceolatus* (Myrtaceae), *Cicer arietinum* (Papillionaceae), *Citrus sinensis* (Rutaceae), *Coriandrum sativum* (Apiaceae), Cucurbitaceae type (Cucurbitaceae), *Eucalyptus* sp. (Myrtaceae), *Gossypium hirsutum* (Malvaceae), *Helianthus annus* (Compositae), *Parthenium hysterophorus* (Asteraceae), *Peucedanum graveolens* (Apiaceae), *Phoenix dactylifera* (Palmae), *Pisum sativum* (Leguminosae), Poaceae type (Poaceae), *Pongamia glabra* (Leguminosae), *Psidium guajava* (Myrtaceae), *Raphanus sativus* (Brassicaeae), *Sesamum indicum* (Pedaliaceae), *Tagetes errecta* (Compositae), *Tamarindus indica* (Cesalpiniaceae), *Trianthema portulacastrum* (Aizoaceae), *Trifolium alexandrinum* (Papilionaceae), *Trigonella foenum-graecum*, *Ziziphus jujube* (Rhamnaceae) and unidentified. An analysis of pollen loads and honey samples collected from a single apiary revealed a total of 28 pollen types identified representing 25 genera and 19 families and a total of 26 pollen types representing 23 genera

and 18 families, respectively. Only one pollen type failed to be identified and two were identified to level of family. The pollen types varied both in time, frequency and species richness. The identified species belong to varying genera of native herbs, shrubs, grasses and trees.

The analysis of pollen loads of *A. mellifera* revealed mean per cent frequency of greater than 45 per cent in most of the loads with 100 per cent for *B. juncea* in January and 91.2 per cent for *Z. jujube* in July while, pollen loads of May, September and November were found to be multifloral with no predominant pollen types recorded. The maximum number of secondary pollen types (three) was recorded in pollen loads of September with maximum mean per cent frequency of 43.6 per cent for cucurbitaceae type followed by two pollen types in March with 25.3 per cent maximum mean frequency for *Eucalyptus* sp.. The pollen loads collected in the months of January and October recorded no important minor pollen types while the maximum number of important minor pollen types were recorded in November *viz.,P. sativum* (15.3%), cucurbitaceae type (7.7%), *B. juncea* (5.1%) and unidentified (11.9%) followed by May, *T. portulacastrum* (8.0%), *R. sativus* (6.9%) and *P. glabra* (3.0%) and *H. annus* (9.7%), *A. katechu* (9.5%) and *G. hirsutum* (8.9%) in June. The highest mean per cent frequency of minor pollen types was recorded at 2.4 per cent for *C. arietinum* in February followed by 2.1 per cent for poaceae in March. The pollen loads of January, July, September and November recorded no minor pollen types (Table 1).

The analysis of honey samples recorded mean per cent frequency of predominant pollen types at greater than 45 per cent in all samples except May, September and November which recorded no predominant pollen types (Table 2).The January, June and July samples recorded no secondary pollen types. The maximum number of secondary pollen type were recorded in March, *T. alexandrinum* (19.7%) and *Eucalyptus* sp. (19.9%), in May, *P. guajava* (34.5%) and cucurbitaceae type (38.2%) and in September cucurbitaceae type (44.3%) and poaceae type (24.6%). The each of April, July, September, October and December samples recorded two important minor pollen types with maximum mean per cent frequency of *P. dactylifera* (5.4%), *G. hirsutum* (7.8%), *S. indicum* (14.1%), unidentified (8.7%) and cucurbitaceae type (8.4%), respectively. The January, February and March recorded *Eucalyptus* sp. (9.8%), *C. sinensis* (8.1%) and *P. dactylifera* (7.4%), respectively. November month samples contained pollen grains of poaceae type (2.7%), unidentified (2.1%) and *P. hysterophorus* (1.4%). In the analysed honeys the most frequent pollen types (nine times) included cucurbitaceae type (January, March, May, June, July, September, October, November and December) and *Eucalyptus* sp. type (seven times) in January, February, March, April, May, November and December. The highest number of pollen types (nine) was recorded in May followed by eight each in April and November month samples while, the lowest number (three) was recorded in January (Table 3).Of the total honey samples analyzed, January, February, March, April, June, July, October and December month samples were found to be unifloral and the rest were multifloral. In the unifloral samples those with pollen type frequency greater than 45 per cent, the predominant pollen types were *B. juncea, C. sinensis, T. alexandrinum, P. guajava, Z. jujube, cucurbitaceae* type and *Eucalyptus* sp.

The pollen analysis of *A. mellifera* collected pollen loads and honeys from Hisar generated significant information pertaining to botanical origin of honeys, whether unifloral or multifloral and documentation of bee foraging plants, as well. The unifloral pollen from *B. juncea, C. sinensis, T. alexandrinum, P. guajava, Z. jujube,* cucurbitaceae and *Eucalyptus* sp. appear as the majority in the months from January and February, March, April, June, July, October and December, respectively representing the plants commonly found in the area. Some of the observed species and genera considered melittophilous had already been indicated as potential bee plants in Hisar region of Haryana (Sihag 1990a; Sihag 1990b; Mishra and Kaushik, 1992; Kaur and Sihag, 1994). The uniflorality in most of samples in the present investigations indicates selective behaviour and preferences of

Table 1. Mean per cent frequency of pollen types recorded in the pollen loads of *A. mellifera* at Hisar

Month	Pollen types			
	Predominant (>45%)	**Secondary (16-45%)**	**Important minor (3-16%)**	**Minor (<3%)**
January	*B. juncea* (100%)	-	-	
February	*B. juncea* (59.8%)	*T. foenum-graecum* (30.2%)	*C. sinensis* (6.6%)	*C. arietinum* (2.4%)
March	*C. sinensis* (51.2%)	*Eucalyptus* sp. (25.3%), *T. alexandrinum* (13.8%)	*P. dactylifera* (7.8%)	Poaceae (2.1%)
April	*T. alexandrinum* (64.0%)	*C. sinensis* (19.1%)	*A. indica* (9.2%), *C. sativum* (3.7%)	*P. glabra* (1.9%), *P. graveolens* (1.0%), *A. marmelos* (1.1%)
May	-	Cucurbitaceae (41.4%), *P. guajava* (38.5%)	*T. portulacastrum (8.0%), R. sativus* (6.9%) *P. glabra* (3.0%)	*C. lanceolatus* (2.0%)
June	*P. guajava* (70.0%)	-	*H. annus* (9.7%), *A. katechu* (9.5%), *G. hirsutum* (8.9%)	*T. indica* (1.9%)
July*	*Z. jujube* (91.2%)	-	*G. hirsutum* (8.8%)	-
August**	-	-	-	-
September	-	Cucurbitaceae (43.6%), Poaceae (25.4%), *S. indicum* (17.0%)	*T. errecta* (7.9%), *Unidentified (6.9%)*	-
October	Cucurbitaceae (55.6%)	Poaceae (24.4%), Unidentified (18.2%)	-	*S. indicum* (1.8%)
November	-	*Eucalyptus* sp. (39.0%), Poaceae (20.9%)	*P. sativum* (15.3%), Cucurbitaceae (7.7%), *B. juncea* (5.1%), Unidentified (1.9%)	-
December	*B. juncea* (32.9%)	Cucurbitaceae (7.5%), *P. hysterophorus* (3.8%)	*Eucalyptus* sp. (54.6%)	Unidentified (1.2%)

* Observations were not taken for second fortnight
** Observations were not taken for entire month

bees to a particular crop. This curious behaviour of bees is called floral fidelity. As a consequence of floral fidelity during certain period, almost all the pollens recorded in the present studies were unifloral, derived from a single species, corresponding to its flowering period. Also, floral fidelity could be related to the distance of the hives to floral sources, sometimes being more advantageous for bees to forage on single flowering species that occur in proximity rather than search for different flowers (Krebs and Davis, 1996). Similarly, the plant species which are recorded as predominant pollen types in the present studies were found to be in close proximity to the Research Apiary. The uniflorality and multiflorality have been reported from different parts of the country (Attri, 2010; Timande and Tembhare, 2010; Cherian *et al.,* 2011; Shubharani *et al*., 2012).

A colony of *A. mellifera* harvests many plant species, thus receiving good nutritional balance (Schmidt and Buchmann, 1993) that is reflected in the present studies were the plant species variability is greatest in the important minor pollen followed by the minor pollen group, secondary and predominant pollen groups. This seems to confirm

Table 2. Mean per cent frequency of pollen types from the analysis of honey samplescollected from hives at Hisar

Month	Pollen types			
	Predominant (>45%)	**Secondary (16-45%)**	**Important minor (3-16%)**	**Minor (<3%)**
January	*B. juncea* (88.3%)	-	*Eucalyptus* sp. (9.8%)	Cucurbitaceae (1.9%)
February	*B. juncea* (67.5%)	*T. foenum-graecum* (20.4%)	*C. sinensis* (8.1%)	*Eucalyptus* sp. (2.1%), *C. arietinum* (1.9%)
March	*C. sinensis* (48.3%)	*T. alexandrinum* (19.7%), *Eucalyptus* sp. (19.9%)	*P. dactylifera* (7.4%)	Cucurbitaceae (2.7%), Poaceae (2.0%)
April	*T. alexandrinum* (59.1%)	*C. sinensis* (23.9%)	*P. dactylifera* (5.4%), *Eucalyptus* sp. (3.1%)	*P. glabra* (2.6%), *C. sativum* (2.5%), *A. marmelos* (2.0%), *P. graveolens* (1.4%)
May	-	*P. guajava* (34.5%), Cucurbitaceae (38.2%)	*T. portulacastrum* (7.5%), *R. sativus* (6.3%), *C. sinensis* (6.0%)	*C. lanceolatus* (2.6%), *P. glabra* (2.4%), *Eucalyptus* sp. (1.3%), *P. dactylifera* (1.2%)
June	*P. guajava* (55.3%)	-	Cucurbitaceae (14.2%), *H. annus* (11.5%), *G. hirsutum* (6.3%), *C. sinensis* (6.0%), *A. katechu* (5.5%)	*T. indica* (1.2%)
July*	*Z. jujube* (79.1%)	-	*G. hirsutum* (7.8%), *P. guajava* (7.4%)	Culurbitaceae (2.9%), *H. annus* (2.8%)
August**	-	-	-	-
September	-	Cucurbitaceae (44.3%), Poaceae (24.6%)	*S. indicum* (14.1%), *Z. jujebe* (12.1%)	*P. guajava* (2.6%), *T. errecta* (2.3%),
October	Cucurbitaceae (54.3%)	Poaceae (27.7%)	Unidentified (8.7%), *S. indicum* (5.6%)	*Z. jujube* (2.3%), *P. guajava* (1.4%)
November	-	*Eucalyptus* sp. (39.4%)	Cucurbitaceae (23.2%), Poaceae (13.6%), *P. guajava* (12.9%), *B. juncea* (4.7%)	Poaceae (2.7%), Unidentified (2.1%), *P. hysterophorus* (1.4%)
December	*Eucalyptus* sp. (55.2%)	*B. juncea* (31.6%)	Cucurbitaceae (8.4%), *P. hysterophorus* (3.5%)	Unidentified (1.3%)

* Observations were not taken for second fortnight
** Observations were not taken for entire month

the view that variability is always small among pollen species in the dominant groups, while greater among minor pollen, important minor pollen and secondary pollen groups (Boff *et al.*, 2011; Sabo *et al*., 2011). This is expected when bees get access to flowers of different bee forage plants in the same period. The pollen types recorded in the present studies have been reported by many authors from different locations, Attri, 2010 (*Albizzia* spp., *Citrus* sp., Cucurbitaceous pollen*, D. sissoo, P. sativum, R. sativus, S. indicum, T. alexandrinum, T. foenum-graecum, Z. mays* etc.), Cherian *et al.,* 2011 (*A.*

Table 3.Types of honey and number of pollen types from analysis of hone samples collected from hives at Hisar

Month	Types of honey	Unifloral pollen type	Number of pollen types
January	Unifloral	*B. juncea*	**3**
February	Unifloral	*B. juncea*	**5**
March	Unifloral	*C. sinensis*	**6**
April	Unifloral	*T. alexandrinum*	**8**
May	Multifloral	———	**9**
June	Unifloral	*P. guajava*	**7**
July*	Unifloral	*Z. jujube*	**5**
August**	———	———	———
September	Multifloral	———	**6**
October	Unifloral	Cucurbitaceae	**6**
November	Multifloral	———	**8**
December	Unifloral	*Eucalyptus* sp.	**5**

* Observations were not taken for second fortnight
** Observations were not taken for entire month

indica, A. lebbeck, B. campestris and *P. guajava* as predominant pollens), Shubharani *et al*., 2012 (*Eucalyptus* sp., *H. annus, Mimosa* sp*., Z. mays* etc.) and Tiwari *et al*., 2012 (*C. sativum, Citrus* sp., Poaceous pollen etc.*).*

The microscopic analysis revealed that the genera represented in both pollen loads as well as in the honey were almost same. However, the higher frequency percentage is recorded in pollen loads than in honey samples. This might be due to part of the pollen used as food material for growth of brood and hence less pollen was taken into the super chamber. Though both, pollen loads and honey samples recorded same pollen types, but the timing of occurrence of these pollen types varied as some of the pollen types like *C. sinensis* (May and June) and *P. guajava* (September, October and November) were encountered in honeys during months, not coinciding with their flowering period. This might be due to sticking of these pollens inside the hives.Some resources utilized by honey bees were not observed in the cultivated field, such as *A. indica, Eucalyptus* sp., *P. dactylifera* and *T. indica*. However, they have been observed nearby on roads, bunds and in patches indicating that bees gathered resources from non-cultivated trees. However, the economically important plants constitute major part of the flora of this area. Thus, there is potential to produce considerable quantity of honey from these resources.

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REFERENCES

Attri P. K. (2010) Melissopalynological studies of *Apis cerana* summer honey from Chamba district of the Himachal Pradesh. Asian Journal of Experimental Biology and Sciences 1 (4): 930-939.

Barth O. M. (2004) Melissopalynology in Brazil: A review of pollen analysis of honeys, propolis and pollen loads of bees. Scientia Agricola (Piracicaba, Braz) 61 (3): 342-350.

Behm F., Von Der Ohe K. and Henrich W. (1996) Zuverlassigkeit der Pollenanalyse von Honig.

Bestimung der Pollenhaufigkeit. Deutsche Lebensmitte, 92: 183-187.

Bhargava H. R., Jyothi J. V. A., Bhushanam M. and Surendra N. S. (2009). Pollen analysis of *Apis* honey, Karnataka, India.Apiacta 44: 14-19.

Bilisik A., Cakmak I., Saatcioglu G., Bicakci A. and Malyer H. (2008) Spectrum of pollen collected by honey bees in bursa lowland area in high season.Uludag Bee Journal 8 (4): 143-148.

Boff S., Luz C .F. P., Araujo A. C. and Pott A. (2011) Pollen analysis reveals plants foraged by Africanized honey bees in the Southern Pantanal, Brazil. Neotropical Entomology 40 (1): 47-54.

Cherian K. J., Bhowal M. and Godghate S. D. (2011) Pollen and physicochemical analysis of honey produced by *Apisceranaindica* of Nagpur, Maharashtra (India). Journal of Environmental Research and Development 5 (3): 542-550.

Erdogan Y. and Erdogan U. (2014) Pollen analysis of honeys from the Coruh valley (Turkey). International Journal of plant Animal and Environmental Sciences 4(3):5-13.

Kaur K. and Sihag R. C. (1994) Bee flora of India: A review. Indian Bee journal 56 (3-4): 105-126.

Krebs J. R. and Davies N. B. (1996) Introduce aoaecologiacomportamental. 3rd ed. Sao paulo, EditoraAtheneu, p. 420.

Louveaux J., Maurizio A. and Vorwhol G. (1978) Methods of Melissopalynology. Bee World 59: 139-157.

Manning R. (2001) Fatty acids in pollen: a review of their importance to honey bees. Bee World 82: 60-75.

Mishra R. C. and Kaushik H.D. (1992) Bee flora and bee keeping maps of India. Changing villages 11 (3): 88-108.

Percival M. (1947) Pollen collection by Apismellifera. New phytologist 46:142-165.

Rashad S. E. and Parker R. L. (1958) Major pollen sources in the Manhattan, Kansas area and the influence of weather factors upon pollen collection by honey bees.In: Proceedings of International Congress on Entomology, Montreal, August 17-25, 1956, 10:1037-1046.

Rouff K. and Bogdanovs (2004) Authenticity of honey and other bee products. Apiacta 35:162-170.

Sabo M., Potocnjak M., Banjari I. and Petrovic D. (2011)Pollen analysis of honeys from Varazdin Country, Croatia. Turkish Journal of Botany 35: 581-587.

Salonen A., Ollikka J., Gronlund E., Ruottinen L. and Tiitto, R.J. (2009) Pollen analysis of honey from Finland. Grana 48: 281-289.

Schmidt J. O., Buchmann S.L. (1993)Other products of the hive. In Grahan, J.M. (ed). The hive and the honeybee. Hamilton, Dadant & Sons, p. 1324.

Schweitzer P. (2009) Pollinic analysis. Available at: www.beekeeping.com/abeille de/france/articles/ pol linic_analysis.htm(Accessed on 15-01-2011)

Shubharani R., Sivaram V. and Roopa P. (2012) Assessment of honey plant resources through pollen analysis in Coorg honeys of Karnataka State. The International Journal of Plant Reproductive Biology 4 (1): 31-39.

Sihag R.C. (1990a) Ecology of European honeybee, *Apis mellifera* L. in semi-arid sub-tropical climates.I Melliferous flora and over seasoning of the colonies. Korean Journal of Apiculture 5 (1): 31-43.

Sihag R.C. (1990b) Seasonal management of honeybee (*Apis mellifera* L.) in Haryana (India). Indian Bee Journal 52 (1-4): 51-56.

Timande J. and Tembhare D.B. (2010) Seasonal impact on comb products of little honey bee, *Apis florae* (Hymenoptera–Apidae). The Bioscan 5 (4): 621-625.

Tiwari J. K., Gairola A., Tiari P. and Ballabha R. (2012) Pollen analysis of some honey samples from Kamad area of District Uttarakashi in Garhwal Himalaya, India. Asian Journal of Experimental Biological Sciences 3 (4): 778-784.

Vaissiere B. E. and Vinson S. B. (1994) Pollen morphology and its effect on pollen collection by honeybees, *Apismellifera* L. (Hymenoptera: Apidae) with special reference to upland cotton, *Gossypium hirsutum* L. (Malvaceae). Grana 33:128-138.

Wodehouse R. P.(1935) Pollen grains. New York Hafner Publishing Company. 574 pp.

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