Biological Forum – An International Journal **14(4): 1175-1181(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Melissopalynological Analysis of Honey for Assessment of Nectariferous Resources of European Honey Bees (*Apis mellifera* L.) in Mid Hills of Himachal Pradesh

Kiran Rana, Abhivivek, Rohit Kumar Nayak*, Monika, Mangla Ram Bajiya and Narinderjeet Singh
Department of Entomology,
Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh), India.

(Corresponding author: Rohit Kumar Nayak)*
(Received 28 September 2022, Accepted 29 October, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The experiment was carried out to determine the nectar resources of the European honey bees (*Apis mellifera* L.) through melissopalynological analysis of honey samples at Dr. YS Parmar University of Horticulture and Forestry, Nauni (Solan) Himachal Pradesh. Three colonies of *A. mellifera* were chosen from the existing stock in the university apiary with high honey hoarding to collect honey samples throughout the year at a 15 days interval. Melissopalynological analysis of honey samples from *A. mellifera* throughout the year revealed the presence of 47 nectar resources from 23 different families, including the following: Asteraceae, Rosaceae, Fabaceae, Malvaceae, Violaceae, Caprifoliaceae, Myrtaceae, Lythraceae, Apiaceae, Proteaceae, Bignoniaceae, Meliaceae, Apocynaceae, Euphorbiaceae, Mimosaceae, Chenopodiaceae, Oxalidaceae, Aizoaceae, Cannabaceae, Sapindaceae, Lamiaceae, Acanthaceae and Cruciferae. The Asteraceae family (9) had the most species that produced nectar, followed by the Rosaceae family (8) and the Fabaceae family (7). The identified nectar flora includes weeds, ornamentals, horticultural plants, food plants, oil seed plants, and wild plants. This study contributes knowledge about nectariferous foraging plants that is crucial for the growth of *A. mellifera* colonies in mid hills of Himachal Pradesh.

Keywords: Melissopalynological analysis, honey samples, *Apis mellifera*, nectar resources.

INTRODUCTION

Honey bees (*Apis mellifera* L.) are beneficial eusocial insects belonging to order Hymenoptera (Family: Apidae). Honey bees pollinate a variety of agricultural, horticultural and wild plantations because of their flower-visiting habits and hairy bodies. Hairy body of honeybees easily picks up pollen grains and visits numerous flowers of the same species in a single trip, which has an impact on pollination (Bhalchandra *et al.*, 2014). Honey is produced by honeybees from flower nectar that is collected and stored in the hive. Honeybees also collect pollen, which supplies protein and fat for the bee brood to grow. Honeybees have benefited man by providing food and boosting crop yields through pollination. Bees and flowering plants are mutually dependent as bees need flowering plants for food in form of pollen and nectar, whereas plants need bees for pollination (Shubharani *et al*., 2012). In India, the annual production of honey was recorded 125000 MTs (Anonymous 2021). The number of plant species used by bees as a source of nectar and pollen for survival and honey production is known as the bee flora (Sakuragui *et al*., 2011). Honeybees gather pollen while visiting the flowers of various entomophilous plants to gather nectar. This pollen is retained in ripened honey which is subsequently stored in the honey combs. These pollen grains in honey can be examined under a microscope to learn more about the local nectar sources available to honey bees (Atanassova *et al.*, 2012). The phenotypic characteristics of plants are used by many taxonomists to identify the species of plants. But now, plant species can be identified using the results of pollen morphological studies. There is growing interest in pollen morphology, and it has applications in systematics, paleobotany, and allergy (Noor *et al*., 2004). Beekeeping not only depends on the better strain of honey bees but also on the abundance and occurrence of pollen and nectar sources within the surrounding area of an apiary (Free, 1993). The identification of bee plants can be greatly aided by pollen study. The information on bee flora on the basis of pollen morphology and types of pollen in Himachal Pradesh is meager. The purpose of this research work was to identify the nectar yielding plant resources attractive to *A. mellifera* and determine their abundance and distribution in Nauni, Himachal Pradesh.

MATERIAL AND METHODS

From January to December of 2017, the melissopalynological studies were carried out to examine the honey bee nectar plants in the university apiary and apiculture laboratory of the Department of

Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh). Geographically, Nauni is situated at latitudes 33.3°N and 70.70°E and at an altitude of 1256 m above mean sea level (amsl).

Collection of honey samples. At the university apiary, honey samples were taken from three randomly chosen *A. mellifera* colonies. Carefully removing the caps from the honey combs allowed the honey to be pipetted out. Each colony had a 20g sample of honey taken every two weeks. Samples were kept chilled until pollen spectrum testing could be done on them (Plate 1).

Preparation of pollen slides from honey samples. The 20g of honey sample was dissolved in 10 ml of distilled water (40ºC), then homogenised with gentle stirring. Once the sediment had settled, this solution was centrifuged at 2500 rpm for another 20 minutes. The supernatant was dispersed again and centrifuged for another five minutes until sediment separated. Entire sediment was taken on a slide and was spread on slide. Then slide was prepared by acetolysis method given by Avetisjan (1950). The pollen slides were mounted using D.P.X.

Photomicrographs of pollen grains found in honey samples. A camera attached to a trinocular microscope was used to take photomicrographs of pollen grains discovered on slides of honey samples. The measurement of pollen grains found in slides of honey samples was taken with "Magnus pro" software. According to Sawyer's (1981) classification, the pollen grains were further divided into five categories based on their size.

Identification of honeybee flora from honey sample slides. According to Singh (2017), the shape and size of the pollen grains were used to match photomicrographs of pollen grains from honey sample slides with photomicrographs of reference pollen slides from flowering plants in the Nauni area.

RESULTS AND DISCUSSION

The melissopalynological analysis of honey samples of European honey bees (*A. mellifera* L.) throughout the year showed the presence of 47 pollen types in honey samples belonging to 23 different families *viz.*, Acanthaceae, Aizoaceae, Apiaceae, Apocynacaeae, Asteraceae, Bignoniaceae, Cannabaceae, Caprifoliaceae, Chenopodiaceae, Cruciferae, Euphorbiaceae, Fabaceae, Laminaceae, Lythraceae, Malvaceae, Meliaceae, Mimosaceae, Myrtaceae, Oxalidaceae, Proteaceae, Rosaceae, Sapindaceae and Violaceae (Table 1). The maximum number of pollen types belonged to family Asteraceae (9) followed by Rosaceae (8) and Fabaceae (7). These plant species were recorded as major nectar yielding plants of honey bee *A. mellifera* in nauni (Solan). The result of our experiment support the views expressed by Shubharani *et al.* (2012) who reported that the highest contribution for nectar and pollen source for honeybee belonged to the Fabaceae and Asteraceae. Nectariferous floras are the number of plant species that bees use as a source of nectar for their survival and production of honey. The knowledge on nectar types, their flowering duration, main blooming time, density and quality in a region are prerequisites for enhancing the honey yield and successful beekeeping. Table 1 includes information on *A. mellifera* nectariferous plants, including scientific name, family, flowering period, photomicrographs of pollen grains, pollen shape, and size of pollen grains, habit and nature of pollen grains. The studies on morphometry of pollen grains showed that pollen grains varied among different nectar plant species and occurred in varying shapes and sizes. They also showed variation in symmetry, exine structure and sculpture.

The pollen grains of plant species in the Asteraceae family are small, spinolous, and round, according to studies on pollen morphology. Plants belonging to Rosaceae family had medium sized, tri-lobed and rounded triangular shape pollen grains. The pollen grains were oval and rounded triangular shaped having small to medium sized in family Fabaceae. *Grevillearobusta* A. Cunn., *Melaleuca linariifolia* Sm. and *Koelreuteria paniculata* Laxm. pollens were triangular shaped and have bilateral symmetry. Sahney *et al.* (2018) carried out melissopalynological studies on winter honeys of *A. dorsata* from Allahabad, Uttar Pradesh, India. They found pollen grains of *A. conyzoides, B. ceiba, B. campestris, C. citrinus, C. sativa, C. sativum, P. hysterophorus, Tageteserecta, T. procumbens* and *Chenopodium* sp. in the honey samples. These plants were also observed useful for *A. mellifera* in the present studies. Sodre *et al.* (2007) carried out pollen analysis on honey samples of *A. mellifera* in the Brazilian northeast and observed the presence of pollen grains of *Bidens* sp., *Chenopodium* sp., *Acacia* spp., *Leucaena leucocephala* and *Eucalyptus* sp. in the honey samples. Similar nectar yielding plants were also observed in this investigation. Gencay *et al.* (2018) found the pollens of the plant species belonging to the families Apiaceae, Asteraceae, Berberidaceae, Brassicaceae, Chenopodiaceae, Fabaceae, Lamiaceae, Malvaceae, Onagraceae, Papaveraceae, and Rosaceae at different rates in the honey samples of the Kars region of Turkey. Ikegbunam *et al.* (2022) also found that the samples were dominated by pollen grains from the families of Arecaceae, Euphorbiaceae, Myrtaceae, Irvigiaceae, Fabaceae, Combretaceae/Melastomataceae, and phyllanthaceae.

Plant species belonging to these families were recorded in honey samples of *A. mellifera* in Nauni. In the mid-hills of Himachal Pradesh, the flowering plants used as nectar sources by *A. mellifera* revealed that trees were dominated (47%) followed by herbs (40%) and shrubs (13%) (Fig. 1). According to their economic significance, the identified nectar forage plants were divided into six groups; wild plants (18), ornamental plants (13), weeds (8), horticultural plants (6), condiments (1), and oil seed crops (1) (Fig. 2). These results are close agreement with Nadaf *et al*. (2017) who identified the species that belong to varying genera of native herbs, shrubs, grasses and trees. Based on melissopalynological analysis of honey samples of *A. mellifera* colonies, it was observed that maximum honey bee nectariferous plants were recorded during early summer season (April). The nectar flora was less during autumn season (Sept-Nov), while dearth of flora was observed during winter season (Dec-Jan) in Nauni area located in the mid-hills of Himachal Pradesh. Similarly, Brar *et al*. (2018) found that availability of maximum flora was recorded during summer months while scarcity of flora was in winter months.

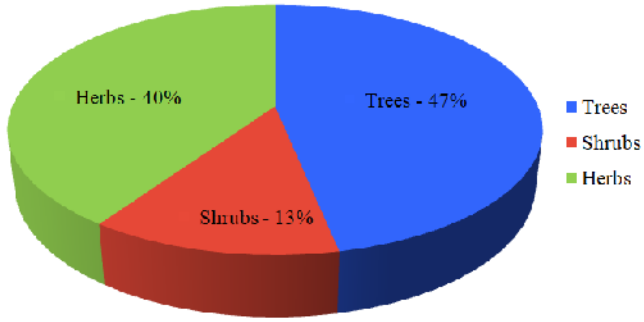


Fig. 1. Various types of vegetation for European honey bees (*Apis mellifera*) in mid hills.

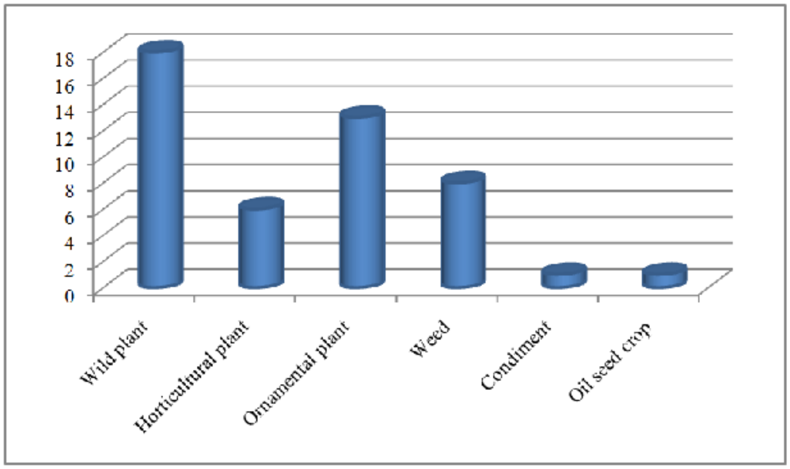


Fig. 2. Quantification of pollen plants useful for forage of *Apis mellifera* (European honey bees) in mid hills.

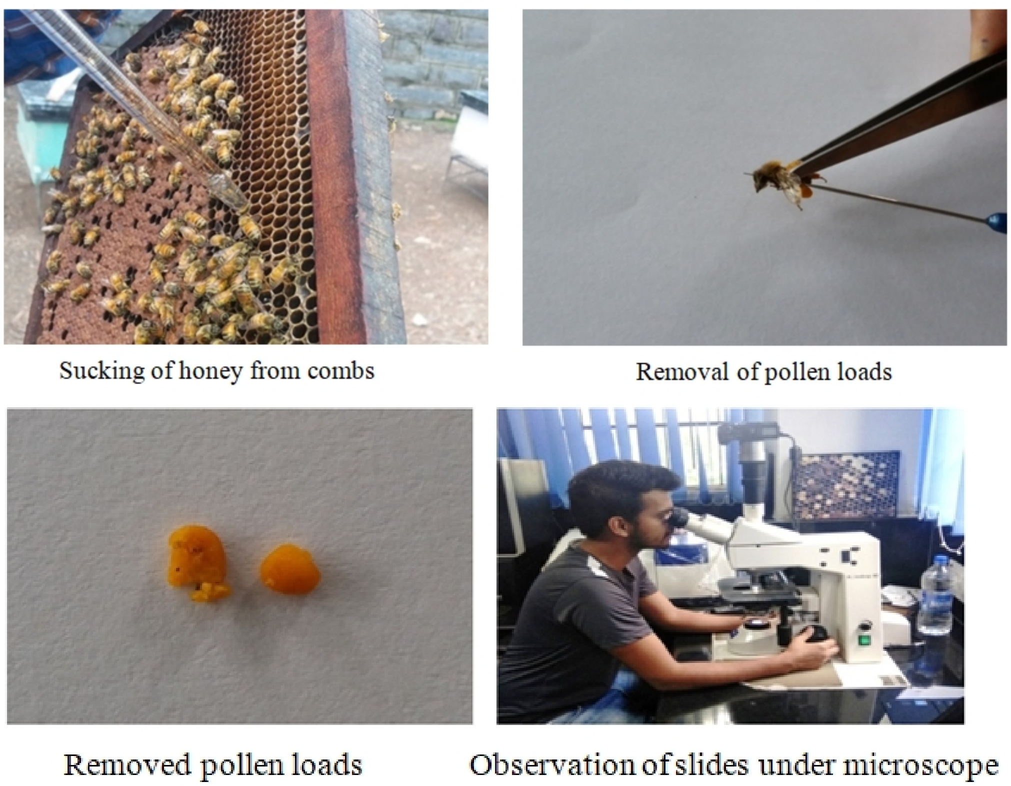
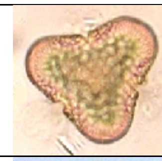
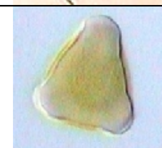
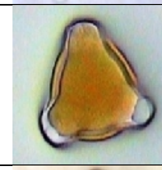
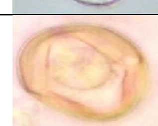
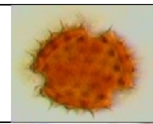
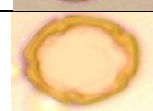
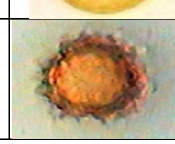
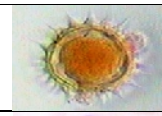
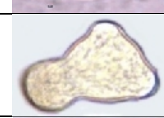
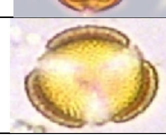


Plate 1. Collection of *Apis mellifera* pollen loads and honey samples.

Table 1: Description of pollen grains found in honey samples of *Apis mellifera* and their morphology.

Sr. No.	Common Name	Scientific Name	Family	Flowering Period	Photomicrograph of pollen grains	Pollen shape	Size of pollen grains in micron	Habit and Nature
1.	Simbal	*Bombax ceiba* L.	Malvaceae	Feb-March		Tri-lobed, medium, bilateral symmetry	46.26±0.38	Tree, wild plant
2.	Apricot	*Prunus armeniaca* L.	Rosaceae	Feb-March		Tri-lobed, medium, bilateral symmetry	37.96±0.92	Tree, horticultural plant
3.	Peach	*Prunus persica* (L.) Batsch	Rosaceae	Feb-March		Rounded triangular, medium	43.65±0.59	Tree, horticultural plant
4.	Kainth	*Pyrus pashia* L.	Rosaceae	Feb-March		Rounded triangular, small, radial symmetry	29.56±0.42	Tree, horticultural plant
5.	Panzy	*Viola tricolor* L.	Violaceae	Feb-March		Pentagonal, small	65.04±0.35	Herb, ornamental plant
6.	Bramble	*Rubus ellipticus* Sm.	Rosaceae	Feb-March		Tri-lobed, small, bilateral symmetry	22.49±0.61	Shrub, wild plant
7.	Pear	*Pyrus communis* L.	Rosaceae	Feb-March		Rounded triangular, medium	36.42±0.15	Tree, horticultural plant
8.	Cornflower	*Centaurea cyanus* L.	Asteraceae	Feb-April		Oval, medium, bilateral symmetry	35.37±0.76 26.61±0.34	Herb, ornamental plant
9.	Honey suckle	*Lonicera angustifolia* Wall.	Caprifoliaceae	March-April		Rounded triangular, large, bilateral symmetry	52.13±0.25	Shrub, wild plant
10.	Apple	*Malus domestica* Borkh	Rosaceae	March-April		Tri-lobed, medium, bilateral symmetry	33.64±0.83	Tree, horticultural plant
11.	White clover	*Trifolium repens* L.	Fabaceae	March-April		Oval, small, bilateral symmetry	27.45±0.28 23.95±0.56	Herb, wild plant
12.	Dandelion	*Taraxacum officinale*	Asteraceae	March-April		Round, medium, spinolous	37.46±0.71	Herb, weed
13.	Eucalyptus	*Eucalyptus hybrid* L'Hér.	Myrtaceae	March-May		Triangular, very small, bilateral symmetry	19.04±0.86	Tree, wild plant
14.	Dhain	*Woodfordia fruticosa* (L.) Kurz.	Lythraceae	March-May		Round, very small, radial symmetry	17.58±0.29	Shrub, wild plant

15.	Calendula	*Calendula officinalis* L.	Asteraceae	March-May		Round, small, spinolous, radial symmetry	29.73±0.45	Herb, ornamental plant
16.	Bottle brush	*Callistemon citrinus* (Curtis) Skeel	Myrtaceae	March-May		Triangular, Very small, bilateral symmetry	18.24±0.76	Tree, ornamental
17.	Coriander	*Coriandrum sativum* L.	Apiaceae	March-May		Long, small, bilateral symmetry	26.85±0.80 14.33±0.25	Herb, condiment
18.	Fasle acacia	*Robinia pseudoacacia* L.	Fabaceae	April-May		Rounded triangular, medium, radial symmetry	34.65±0.43	Tree, wild plant
19.	Silky oak	*Grevillea robusta* A. Cunn.	Proteaceae	April-May		Triangular, large, bilateral symmetry	51.93±0.26	Tree, ornamental plant
20.	Jacaranda	*Jacaranda mimosifolia* D. Don	Bignoniaceae	April-May		Round, medium, bilateral symmetry	39.63±0.51	Tree, ornamental plant
21.	Copper bottle brush	*Melaleuca linariifolia* Sm.	Myrtaceae	April-May		Triangular, very small, bilateral symmetry	15.35±0.74	Tree, ornamental plant
22.	Bird's foot Treefoil	*Lotus corniculatus* L.	Fabaceae	April-May		Oval, very small, bilateral symmetry	14.62±0.27 11.74±0.63	Herb, wild plant
23.	Toon	*Toona ciliata* M. Roem.	Meliaceae	April-May		Round, small, medium, radial symmetry	23.62±0.45	Tree, wild plant
24.	Ohi	*Albizia chinensis* (Osbeck) Merr.	Fabaceae	May-June		Round, large, radial symmetry	61.53±0.36	Tree, wild plant
25.	Shisham	*Dalbergia sissoo* Roxb.	Fabaceae	May-June		Rounded triangular, small	21.75±0.24	Tree, wild plant
26.	Garna	*Carissa opaca* Stapf.	Apocynacaeae	May-June		Round, medium	35.69±0.86	Shrub, wild plant
27.	Tarcharbi	*Sapium sebiferum* Roxb.	Euphorbiaceae	May-June		Round, medium, bilateral symmetry	31.49±0.98	Tree, ornamental plant
28.	The field thistle	*Cirsium verutum* (D. Don) Spreng.	Asteraceae	May-June		Round, medium, spinolous	32.53±0.49	Herb, weed
29.	khair	*Acacia catechu* Willd.	Mimosaceae	May-July		Round, medium, radial symmetry	37.69±0.56	Tree, wild plant
30.	Bathu	*Chenopodium album* L.	Chenopodiaceae	June-Sept		Round, small, radial symmetry	24.41±0.63	Herb, weed
31.	Congress grass	*Parthenium hysterophorus* L.	Asteraceae	June-Oct		Round, very small, spinolous, radial symmetry	14.28±0.84	Herb, weed

32.	Spanish needle	*Bidens pilosa* L.	Asteraceae	June-Oct		Round, medium, spinolous, radial symmetry	23.56±0.46	Herb, weed
33.	Malori	*Oxalis corniculata* L.	Oxalidaceae	June-July		Round, medium	35.62±075	Herb, wild plant
34.	Pride of India	*Lagerstroemia indica* (L.) Pers.	Lythraceae	July-Aug		Round, medium, radial symmetry	33.52±0.39	Tree, ornamental plant
35.	Coat button	*Tridax procumbens* L.	Asteraceae	July-Aug		Round, small, spinolous, radial symmetry	22.78±0.60	Herb, weed
36.	heartleaf iceplant	*Aptenia cordifolia* (L.f.) N. E. Br.	Aizoaceae	July-Aug		Round, very small	16.02±0.53	Herb, ornamental plant
37.	Yellow gulmohar	*Peltophorum ferrugineum* Benth.	Fabaceae	July-Sept		Round, medium, radial symmetry	43.62±0.87	Tree, ornamental plant
38.	Bhang	*Cannabis sativa* L.	Cannabaceae	July-Sept		Round, small, radial symmetry	29.55±0.43	Herb, weed
39.	Goat weed	*Ageratum conyzoides* L.	Asteraceae	July-Sept		Round, very small, spinolous, radial symmetry	13.95±0.68	Herb, weed
40.	Golden Tree	*Koelreuteria paniculata* Laxm.	Sapindaceae	August		Triangular, medium, bilateral symmetry	32.62±0.36	Tree, ornamental plant
41.	Chichri	*Plectranthus rugosus* Wall.	Lamiaceae	Aug-Oct		Round, large, radial symmetry	55.33±0.74	Shrub, wild plant
42.	Ipil-Ipil	*Leucaena leucocephala*(Lam.) de Wit.	Fabaceae	Aug-Oct		Oval, medium, bilateral symmetry	34.51±0.68	Tree, wild plant
43.	Pajja	*Prunus puddum* Franch.	Rosaceae	Aug-Nov		Rounded triangular, medium	34.23±0.61	Tree, horticultural plant
44.	Marigold	*Tagetes erecta* L.	Asteraceae	Sept-Nov		Round, small, spinolous, radial symmetry	22.69±0.82	Herb, ornamental plant
45.	Dicliptera	*Dicliptera bupleuroides* L.	Acanthaceae	Sept-Dec		Long, medium, bilateral symmetry	39.63±0.65 28.43±0.89	Herb, wild plant
46.	Bhekhal	*Prinsepia utilis* Royle	Rosaceae	Nov-Jan		Rounded triangular, medium	41.59±0.23	Shrub, wild plant
47.	Sarson	*Brassica campestris* L.	Cruciferae	Nov-March		Round, small, radial symmetry	24.84±0.31	Herb, oil seed crop

CONCLUSION

The identification of nectar sources for honey bees and an improvement in plant conservation will be made possible by the studies on pollen morphology and the melissopalynological analysis of honey. Farmers can be advised to plant and conserve crops with multiple uses that can also be food for honeybees.

Acknowledgement: Authors are thankful to All India Coordinated Research Project on honey bees and pollinators, ICAR, New Delhi for providing financial and technical help.
Conflict of interest. None.

REFERENCES

Anonymous (2021). National Bee Board, Department of Agriculture and Farmers Welfare (DA&FW), Ministry of Agriculture and Farmers Welfare Government of India. www.nbb.gov.in/pdf/honey.in.

Atanassova, J., Yurukova, L. and Lazarova, M. (2012). Pollen and Inorganic Characteristics of Bulgarian Unifloral Honeys Czech. *Journal Food Science*, *30*, 520-526.

Avetisjan, B. M. (1950). Uproshennij Acetolinij Metod Obrabotniki Pilci. *Botanicheskii Zhurnal*, *35*(4), 385-386.

Bhalchandra, W., Baviskar, R. K. and Nikam, T. B. (2014). Diversity of Nectariferous and Polleniferous Bee Flora at Anjaneri and Dugarwadi Hills of Western Ghats of Nasik district (MS) India. *Journal of Entomology and Zoology Studies*, *2*(4), 244-249.

Brar, A. S., Sharma, H. K. and Rana, K. (2018). Colony Strength and Food Reserves of *Apis mellifera* L. under Stationary and Migratory Beekeeping in Himachal Pradesh, India. *Journal of Entomology and Zoology Studies*, *6*(5), 1156-1159.

Free, J. B. (1993). *Insect Pollination of Crops* (No.Ed. 2). Academic press.

Gencay, C. O., Ozenirler, C., Bayram, N. E., Zare, G. and Sorkun, K. (2018). Melissopalynological Analysis for Geographical Marking of Kars Honey. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *24*, 53-59.

Ikegbunam, N. C., Nweze, N. O., Walter, O. J., Njokuocha, R. C. and Chiori, O. C. (2022). Melissopalynological Characterization of Honey Samples from Southeastern, Nigeria. *Turkish Journal of Agriculture-Food Science and Technology*, *10*(2), 203-210.

Nadaf, H. A.,Yadav, G. S., Kaushik, H. D. and Sharma, S. K. (2017). Melissopalynological Investigations of *Apis mellifera* L. Corbicular Pollen and Honey from Hisar, Haryana. *Entomon*, *42*(1), 23-30.

Noor, M. J., Mushtaq, A., Rehana, A., Aulia, K. and Sadaf, P. (2004). Palynological Studies of Cultivated Plant Species at University of Arid Agriculture, Rawalpindi, Pakistan. *Asian Journal of Plant Sciences*, *3*(4), 476-479.

Sahney, M., Rahi, S., Kumar, A. and Jaiswal, R. (2018). Melissopalynological Studies on Winter Honeys from Allahabad, Uttar Pradesh, India. *Palynology*, *42*(4), 540-552.

Sakuragui, C. M., Alves, E. M., Lorenzetti, E. R., Janunzzi, A. M., Borges, R. A. X. and De Toledo, V. A. (2011). Bee Flora of an Insular Ecosystem in Southern Brazil. *Journal of the Botanical Research Institute of Texas*, *5*, 311-319.

Sawyer, R. W. (1981). *Pollen Identification for Beekeepers*. University College Cardiff Press.

Shubharani, R., Sivaram, V. and Roopa, P. (2012).Assessment of Honey Plant Resources through Pollen Analysis in Coorg Honeys of Karnataka State. *International Journal of Plant Reproductive Biology*, *4*, 43-51.

Singh, N. (2017). *Palynological Studies on Bumble Bee Flora Under Mid-Hill Conditions of Himachal Pradesh*. Masters dissertation, Thesis, YS Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh.

Sodre, G. D. S., Marchini, L. C., Carvalho, C. A. D. and Moreti, A. C. (2007). Pollen Analysis of Honey Samples from the Two Main Producing Regions in the Brazilian Northeast. *Anais da Academia Brasileira de Ciências*, *79*, 381-388.

How to cite this article: Kiran Rana, Abhivivek, Rohit Kumar Nayak, Monika, Mangla Ram Bajiya and Narinderjeet Singh (2022). Melissopalynological Analysis of Honey for Assessment of Nectariferous Resources of European Honey Bees (*Apis mellifera* L.) in Mid Hills of Himachal Pradesh. *Biological Forum – An International Journal*, *14*(4): 1175-1181.