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Diversity of polleniferous plants of *Apis mellifera* L. in mid hill region of Himachal Pradesh

Abhivivek, Kiran Rana, Harish Kumar Sharma and Meena Thakur

Abstract
This study was carried out in Nauni, mid hill region of Himachal Pradesh, in 2017 to determine the pollen resources of the honey bee, *Apis mellifera* L. through pollen load analysis. The pollen loads were collected from returning foragers to hives maintained at the university apiary. Pollen analysis of pollen loads of *A. mellifera* done throughout the year showed the presence of 66 pollen types belonged to 29 botanical families. Major polleniferous plant species that provided homogenous (unifloral) pollen loads were *Actinidia deliciosa, Allium cepa, Bidens pilosa, Bombax cieba, Brassica campestris, Calendula officinalis, Centaurea cyanus, Cosmos sulphureus, Cucumis sativus, Dahlia pinnata, Erigeron annuus, Eschscholzia californica, Eucalyptus hybrida, Grewia optiva, Helianthus annuus, Hypericum oblongifolium, Lagerstroemia indica, Malus domestica, Opuntia dillenii, Ornithogalum thyrsoides, Parthenium hysterophorus, Peltophorum ferrugineum, Prunus armeniaca, P. domestica, P. persica, P. puddum, Punica granatum, Pyrus communis, P. pashia, Robinia pseudoacacia, Rosa moschata, Toona ciliata, Trifolium repens, Trigonella foenum-graecum,Venidium fastuosum, Zea mays* and *Zinnia elegans.* The plant family that provided the highest pollen plant diversity was *Asteraceae* (14pollen types) followed by *Rosaceae* (10) and *Fabaceae* (8). The identified bee flora comprises ornamentals, horticultural plants, vegetables, condiments, oil seed plants, cereal, wild plants and weeds. These data provide a piece of information on bee pollen foraging plants of primary importance in the development of colonies in mid hill regions of Himachal.

Keywords: *Apis mellifera*, pollen resources, pollen loads, palynology

Introduction
Honeybees (*Apis mellifera* L.) are beneficial insects belonging to Hymenoptera (Family: Apidae). Honeybees are one of the most important pollinators because of their flower visiting habits, hairy bodies that readily pick up pollen grains and visit many flowers of the same species during a single trip thus affecting pollination (Bhalchandra *et al*., 2014) [1]. Honeybees collect pollen, which supplies protein and fat for the bee brood to grow. Honeybees have benefited man both for food and their ability to increase crop yield via pollination. Bees and flowering plants are mutually dependent as bees need flowering plants for food, whereas plants need bees for pollination (Shubharani *et al*., 2012) [2]. Bee flora is defined by the number of plant species that bees use as a source of nectar and pollen for their survival and production of honey (Sakuragui *et al*., 2011) [3]. Palynology is the study of pollen grains produced by seed plants. Pollens can be used to determine foraging resources, pollination mechanisms, migration routes and source zones of insects and pollinators (Jones and Jones, 2001)[4]. The palynological analysis of pollen loads allows the identification of a greater number of visited plant species with lower labour inputs involved (Teper, 2005) [5].
Himachal Pradesh is the principal apple growing state of the country. Every year more land is diverted from field crops to temperate fruit crops in the state with such a drastic increase in the area under fruit cultivation, a major problem related to the bee forage has arisen. Once the bloom of the crop of interest is over, the bees are left with considerably reduced floral resources. This can be overcome either by providing alternate bee forage or moving bees to locations where floral resources are plentiful. In both cases knowledge of pollen and nectar plants in the beekeeping potential areas, is essential and this can be achieved only by field observations and then confirmed by honey/pollen analysis (Sharma, 1990) [6].
Several taxonomists identify the plant species based on phenotypic character of the plant. Now the pollen morphological studies can provide a basis for the identification of plant species. An interest in pollen morphology has increased its application in systematic, paleobotany and allergy has been recognized (Noor *et al*., 2004) [7].

Beekeeping not only depends on the better strain of honeybees but also the abundance and occurrence of pollen and nectar sources within the surrounding area of an apiary (Free, 1970) [8]. Pollen study is very useful in recognition of bee plants. The information on honeybee plants on the basis of pollen morphology and types of pollen in Nauni, Solan, mid hills of Himachal Pradesh is not available. This study aimed to provide the reference information on pollen sources for *Apis mellifera* L.

Materials and Methods

The palynological studies were carried out to examine the honeybee floral plants from January to December, 2017 in an apiary at the experimental farm and apiculture laboratory of the Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni- Solan (Himachal Pradesh). Geographically, Nauni is located at 33.3°N latitude, 70.70°E longitude and 1256 m above mean sea level (amsl).

1. Collection of pollen loads

Foraging honeybees with pollen loads returning to hives were captured with an insect net on different sampling dates at 15 days interval from January to December, 2017.

Pollen loads were carefully removed from the pollen basket of *A. mellifera* with a dissecting needle and bees were released. The microscopic preparation of pollen loads was

continues at the same time or were put in well labeled bottles/vials under refrigeration for preparation of slides later on.

2. Preparation of pollen load slides

Microscopic slide preparations of pollens from pollen loads were prepared using acetolysis method given by Avetisjan (1950) [9]. A small part of pollen load was placed on a microscopic slide and few drops of ethyl alcohol (96%) were dropped on the slide. The fat substances which appeared on the slide after dropping/pouring alcohol were cleaned with help of blotting paper. Then microscopic slides were treated with 1-2 drops of acetolysis mixture. This mixture was prepared fresh at every time by mixing 9 parts of acetic anhydride with 1 part of sulfuric acid. Then the content on microscopic slides was lightly warmed on an alcoholic lamp so that it could not get darker. The content was washed with ethyl alcohol (70%) and fixed with D.P.X. mountant by placing a cover slip over it.

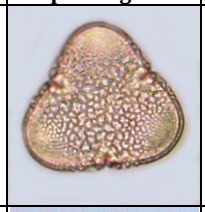
3. Photomicrographs and measurements of pollen grains

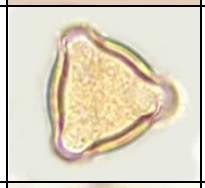
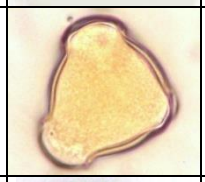
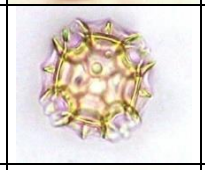
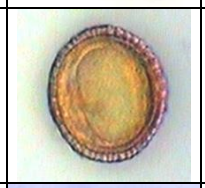
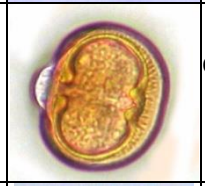
The photomicrographs of pollen grains found in pollen load slides were taken by a camera attached with trinocular microscope. The measurement of pollen grains found in pollen load slides was taken with “Magnus pro” software. The pollen grains were further divided into five categories on basis of the size of pollen grains as per classification given by Sawyer (1981) [10].

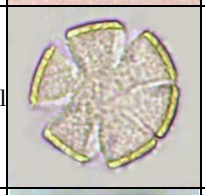
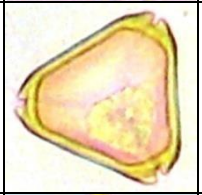
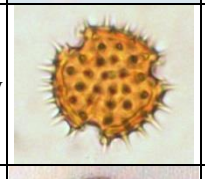
4. Identification of honeybee flora from corbicular pollen slides

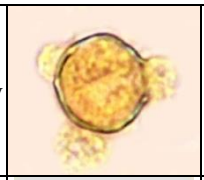
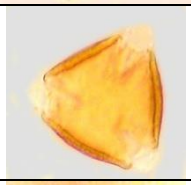
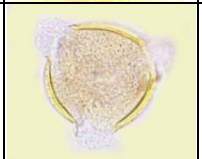
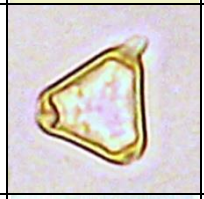
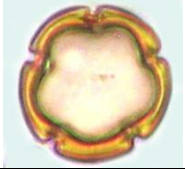
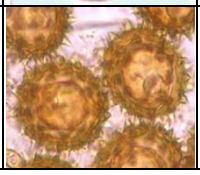
For the identification of floral plants, photomicrographs of pollen load slides were matched with photomicrographs of reference pollen slides of flowering plants of Nauni area (Singh, 2017) [11] and inferences were made on basis of the shape and size of pollen grains. These floral plants were further categorized into species, family, flowering time and plant use.

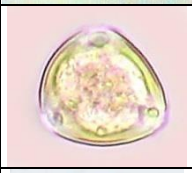
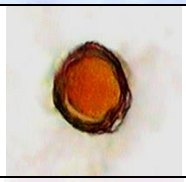
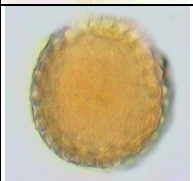
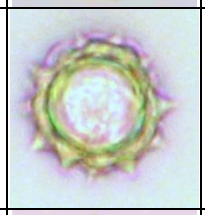
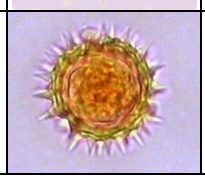
Table 1: Description of pollen grains found in pollen loads of *A. mellifera* and their morphology

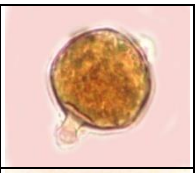
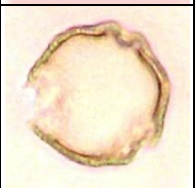
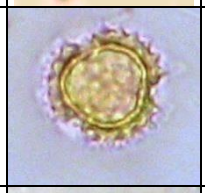
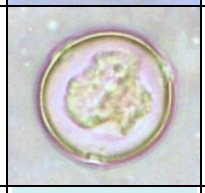
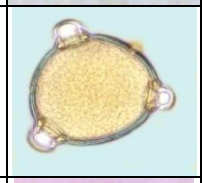
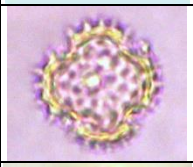
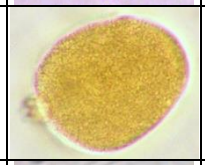
Sr. No.	Common name	Scientific name	Family	Flowering Period	Photomicrograph of pollen grains	Pollen shape	Size of pollen grains (in micron)	Habit and Nature
1.	Simbal	*Bombax ceiba* L.	Malvaceae	Feb-March		Tri-lobed, medium, bilateral symmetry	45.63±0.51	Tree, wild plant
2.	Apricot	*Prunus armeniaca* L.	Rosaceae	Feb-March		Tri-lobed, medium, bilateral symmetry	39.58±0.41	Tree, horticultural plant
3.	Peach	*Prunus persica* (L.) Batsch	Rosaceae	Feb-March		Rounded triangular, medium	42.98±0.64	Tree, horticultural plant
4.	Pear	*Pyrus communis* L.	Rosaceae	Feb-March		Rounded triangular, medium	34.02±0.55	Tree, horticultural plant

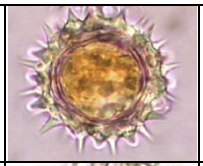
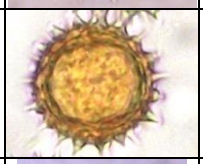
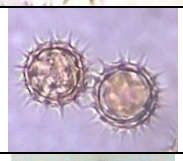
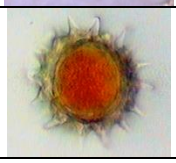
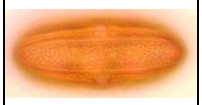
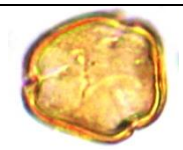
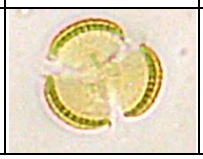
5.	Basuti	*Adhatoda vasica* L.	Acanthaceae	Feb-March		Long, large, radial symmetry	57.38±0.38	Shrub, wild plant
6.	Kainth	*Pyrus pashia* L.	Rosaceae	Feb-March		Rounded triangular, small, radial symmetry	29.56±0.42	Tree, horticultural plant
7.	Bramble	*Rubus ellipticus Sm.*	Rosaceae	Feb-March		Tri-lobed, small, bilateral symmetry	21.35±0.86	Shrub, wild plant
8.	Cape Daisy	*Venidium fastuosum Jacq.*	Asteraceae	Feb-March		Round, small, spinolous	24.74±0.57	Herb, ornamental plant
9.	Plum	*Prunus domestica* L.	Rosaceae	Feb-March		Rounded triangular, medium, radial symmetry	36.47±0.23	Tree, horticultural plant
10.	Citrus	*Citrus aurantifolia(Christm.) Swingle*	Rutaceae	Feb-March		Round, radial symmetry	26.08±0.94	Tree, horticultural plant
11.	Wonder flower	*Ornithogalum thyrsoides Jacq.*	Asparagaceae	Feb-March		Boat shaped, large, bilateral symmetry	55.46±.63* 36.04±0.54**	Herb, ornamental plant
12.	Cornflower	*Centaurea cyanus* L.	Asteraceae	Feb-April		Oval, medium, bilateral symmetry	36.82±0.24 29.54±0.57	Herb, ornamental plant
13.	Kashmal	*Berberis lycium Royle*	Berberidaceae	March-April		Round, medium, radial symmetry	31.64±0.16	Shrub, wild plant
14.	Phiunli	*Hypericum oblongifolium Choisy*	Hypericaceae	March-April		Round triangular, small, radial symmetry	18.49±0.98	Shrub, wild plant
15.	Honey suckle	*Lonicera angustifolia Wall.*	Caprifoliaceae	March-April		Rounded triangular, large, bilateral symmetry	51.52±0.31	Shrub, wild plant

16.	Apple	*Malus domestica Borkh*	Rosaceae	March-April		Tri-lobed, medium, bilateral symmetry	32.26±0.85	Tree, horticultural plant
17.	Yellow clover	*Medicago denticulata Willd.*	Fabaceae	March-April		Oval, very small, bilateral symmetry	17.62±0.64 14.38±0.27	Herb, weed
18.	California poppy	*Eschscholzia californica Cham.*	Paparveraceae	March-April		Round, medium, radial symmetry	31.95±0.71	Herb, condiment
19.	Methi	*Trigonella foenum-graecum* L.	Fabaceae	March-April		Oval, small, bilateral symmetry	22.49±0.56 16.81±0.38	Herb, ornamental plant
20.	White clover	*Trifolium repens* L.	Fabaceae	March-April		Oval, small, bilateral symmetry	26.37±0.49 22.69±0.63	Herb, wild plant
21.	Blue beared	*Caryopteris bicolor Roxb.*	Lamiaceae	March-April		Round,, very small, radial symmetry	19.68±0.43	Shrub, wild plant
22.	Bottle brush	*Callistemon citrinus (Curtis) Skeel*	Myrtaceae	March-May		Triangular, Very small, bilateral symmetry	17.87±0.18	Tree, ornamental plant
23.	Coriander	*Coriandrum sativum* L.	Apiaceae	March-May		Long, small, bilateral symmetry	27.59±0.36 13.26±0.24	Herb, condiment
24.	Calendula	*Calendula officinalis* L.	Asteraceae	March-May		Round, small, spinolous, radial symmetry	27.65±0.79	Herb, ornamental plant
25.	Cauliflower	*Brassica oleracea var botrytis* L.	Cruciferae	March-May		Round, very small, radial symmetry	19.52±0.59	Herb, vegetable
26.	Eucalyptus	*Eucalyptus hybrida L'Hér.*	Myrtaceae	March-May		Triangular, very small, bilateral symmetry	18.97±0.14	Tree, wild plant

27.	Dhain	*Woodfordia fruiticosa Kurz.*	Lythraceae	March-May		Round, very small, radial symmetry	16.02±0.35	Shrub, wild plant
28.	Kachnar	*Bauhinia variegata Benth.*	Fabaceae	April		Triangular, Large, bilateral symmetry	50.31±0.76	Tree, wild plant
29.	Kiwifruit	*Actinidia deliciosa Liang and Ferguson*	Actinidiaceae	April-May		Round, small	23.24±0.16	Climber, horticultural plant
30.	Fasle acacia	*Robinia pseudoacacia* L.	Fabaceae	April-May		Rounded triangular, medium, radial symmetry	33.42±0.39	Tree, wild plant
31.	Copper bottle brush	*Melaleuca linariifolia Sm.*	Myrtaceae	April-May		Triangular, very small, bilateral symmetry	14.92±0.85	Tree, ornamental
32.	Jacaranda	*Jacaranda mimosifolia D. Don*	Bignoniaceae	April-May		Round, medium, bilateral symmetry	38.36±0.43	Tree, ornamental
33.	Duranta	*Duranata repens* L.	Verbenaceae	April-May		Triangular, small	27.62±0.32	Shrub, ornamental
34.	Daru	*Punica granatum* L.	Punicaceae	April-May		Oval, small	23.02±0.27 20.31±0.48	Tree, wild plant
35.	Onion	*Allium cepa* L.	Amaryllidaceae	April-May		Boat shaped, medium, bilateral symmetry	31.56±0.19 22.39±0.34	Herb, vegetable
36.	Toon	*Toona ciliata M. Roem.*	Meliaceae	April-May		Round, small, medium, radial symmetry	22.12±0.43	Tree, wild plant
37.	Sunflower	*Helianthus annuus* L.	Asteraceae	April-July		Round, small, spinolous, radial symmetry	26.35±0.72	Herb, ornamental plant

38.	Wild rose	*Rosa moschata Herrm.*	Rosaceae	May-June		Round, small	24.62±0.21	Shrub, ornamental
39.	Shisham	*Dalbergia sissoo Roxb.*	Fabaceae	May-June		Rounded triangular, small	22.51±0.26	Tree, wild plant
40.	Cactus	*Opuntia dillenii Haw.*	Cactaceae	May-June		Round, very large, radial symmetry	118.62±0.44	Shrub, ornamental
41.	Cosmos	*Cosmos sulphureus Cav.*	Asteraceae	May-June		Round, small, spinolous, Radial symmetry	28.73±0.35	Herb, ornamental plant
42.	Biul	*Grewia optiva Drumm.*	Malvaceae	June		Oval, medium	57.38±0.92 43.12±0.76	Tree, wild plant
43.	Thorn apple	*Datura stramonium* L.	Solanaceae	June-July		Round, medium, radial symmetry	42.03±0.56	Herb, wild plant
44.	Bathu	*Chenopodium album* L.	Chenopodiaceae	June-Sept		Round, small, radial symmetry	22.38±0.13	Herb, weed
45.	Malvastrum	*Malvastrum tricuspidatum A. Gray.*	Malvaceae	June-Sept		Round, small, echinated, radial symmetry	28.96±0.82	Herb, wild plant
46.	Parthenium	*Parthenium hysterophorus* L.	Asteraceae	June-Oct		Round, very small, radial symmetry	16.27±0.44	Herb, weed
47.	Coat button	*Tridax procumbens* L.	Asteraceae	June-Oct		Round, small, spinolous, radial symmetry	23.75±0.60	Herb, weed
48.	Spanish needle	*Bidens pilosa* L.	Asteraceae	June-Oct		Round, medium, spinolous, radial symmetry	22.18±0.46	Herb, weed

49.	Pride of India	*Lagerstroemia indica* (L.) *Pers.*	Lythraceae	July-Aug		Round, medium, radial symmetry	32.06±0.39	Tree, Ornamental plant
50.	Heartleaf ice plant	*Aptenia cordifolia (L.f.) N. E. Br.*	Aizoaceae	July-Aug		Round, very small, radial symmetry	15.73±0.53	Herb, ornamental plant
51.	Yellow gulmohar	*Peltophorum ferrugineum Benth.*	Fabaceae	July-Sept		Round, medium, radial symmetry	42.82±0.27	Tree, ornamental
52.	Goat weed	*Ageratum conyzoides* L.	Asteraceae	July-Sept		Round, very small, spinolous, radial symmetry	14.75±0.69	Herb, weed
53.	Bhang	*Cannabis sativa* L.	Cannabaceae	July-Sept		Round, small, radial symmetry	28.15±0.26	Herb, weed
54.	Cucumber	*Cucumis sativus* L.	Cucurbitaceae	July-Sept		Rounded triangular, large	56.38±0.94	Herb, vegetable
55.	Daisy Fleabane	*Erigeron annuus* (L.) *Pers.*	Asteraceae	July-Sept		Round, very small, spinolous, radial symmetry	16.43±0.45	Herb, wild plant
56.	Maize	*Zea mays* L.	Poaceae	Aug-Sep		Round, very large, radial symmetry	109.27±0.59	Herb, cereal crop
57.	Ipil-Ipil	*Leucaena leucocephala (Lam.) de Wit.*	Fabaceae	Aug-Oct		Oval, medium, bilateral symmetry	36.56±0.68	Tree, wild plant
58.	Chichri	*Plectranthus rugosus Wall.*	Lamiaceae	Aug-Oct		Round, large, radial symmetry	56.83±0.54	Shrub, wild plant
59	Pajja	*Prunus puddum Franch.*	Rosaceae	Nov-Dec		Rounded triangular, medium, radial symmetry	32.18±0.86	Tree, horticultural plant

60.	Dahlia	*Dahlia pinnata Cav.*	Asteraceae	Aug-Nov		Round, small, spinolous, radial symmetry	27.36±0.24	Herb, ornamental plant
61.	Zinnia	*Zinnia elegans Jacq.*	Asteraceae	Sept-Oct		Round, small, spinolous, radial symmetry	23.08±0.78	Herb, ornamental plant
62.	Marigold	*Tagetes erecta* L.	Asteraceae	Sept-Nov		Round, small, spinolous, radial symmetry	21.69±0.62	Herb, ornamental plant
63.	Tree Dahlia	*Dahlia imperialis Roezl.*	Asteraceae	Sept-Nov		Round, small, spinolous, radial symmetry	23.67±0.49	Herb, ornamental plant
64.	Dicliptera	*Dicliptera bupleuroides* L.	Acanthaceae	Sept-Nov		Long, medium, bilateral symmetry	38.43±0.12 23.56±0.45	Herb, wild plant
65.	Bhekhal	*Prinsepia utilis Royle*	Rosaceae	Nov-Jan		Rounded triangular, medium, radial symmetry	42.29±0.73	Shrub, wild plant
66.	Sarson	*Brassica campestris* L.	Cruciferae	Nov-March		Round, small, radial symmetry	25.34±0.31	Herb, oil seed crop

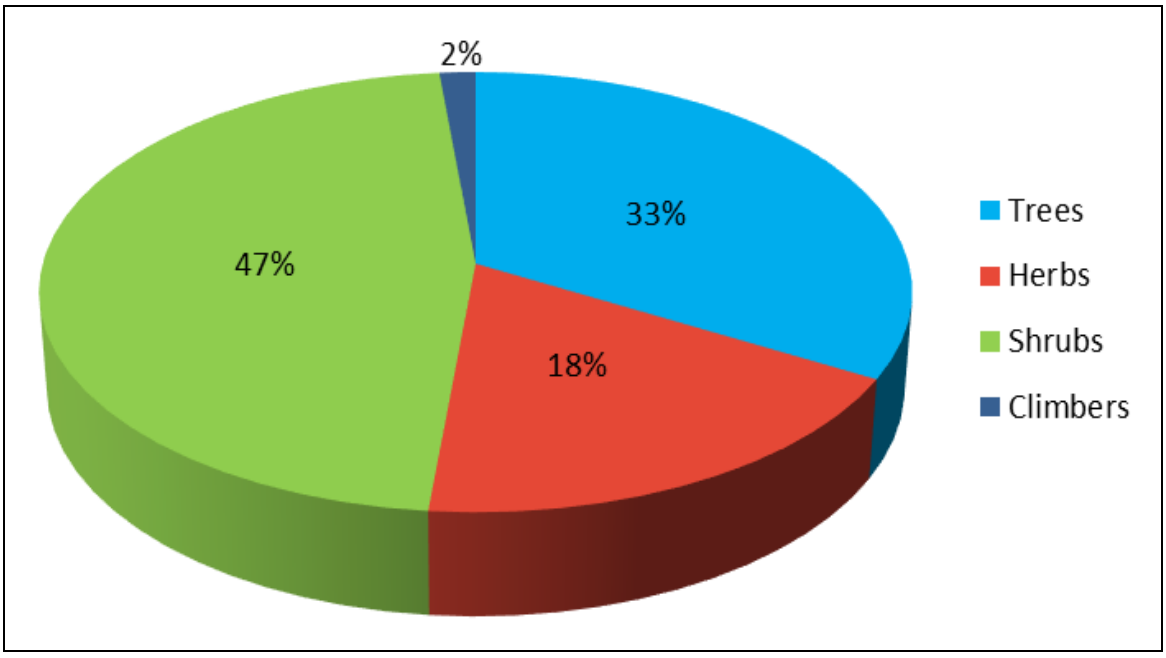


Fig 1: Different types of vegetations for *A. mellifera* in mid hills

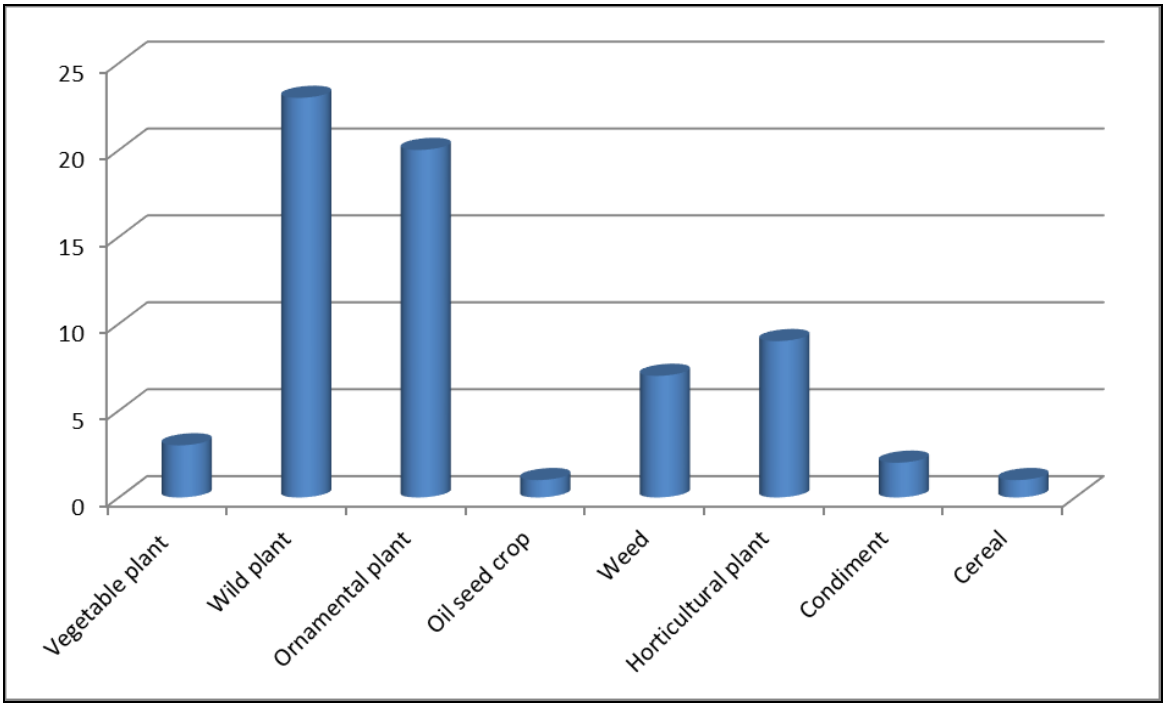


Fig 2: Quantification of pollen plants useful for forage of *A. mellifera* in mid hills

Results and Discussion

Description of pollen grains found in honeybee pollen loads and their morphology in Table 1 provides information on *A. mellifera* pollen plants along with the scientific name, family, flowering period, photomicrographs of pollen grains, pollen shape, size of pollen grains and habit and nature. The morphometry of pollen grains varies among different plant species; occurs in varying shapes and sizes. They also show variation in symmetry, exine structure and sculpture.

Pollen analysis of pollen loads of *A. mellifera* L. throughout the year (2017) showed the presence of 66 pollen types. These pollen types belonged to 29 different families viz., Acanthaceae, Actinidiaceae, Aizoaceae, Amaryllidaceae, Apiaceae, Asparagaceae, Asteraceae, Berberidaceae, Bignoniaceae, Cactaceae, Cannabaceae, Caprifoliaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Fabaceae, Hypericaceae, Lamiaceae, Lythraceae, Malvaceae, Meliaceae, Myrtaceae, Paparveraceae, Poaceae, Punicaceae, Rosaceae, Rutaceae, Solanaceae and Verbenaceae. The maximum number of pollen types belonged to families Asteraceae (14) followed by Rosaceae (10) and Fabaceae (8). The homogenous (unifloral) pollen grains were found in 37 pollen loads, thus these plant species namely *Actinidia deliciosa, Allium cepa, Bidens pilosa, Bombax cieba, Brassica campestris, Calendula officinalis, Centaurea cyanus, Cosmos sulphureus, Cucumis sativus, Dahlia pinnata, Erigeron annuus, Eschscholzia californica, Eucalyptus hybrida, Grewia optiva, Helianthus annuus, Hypericum oblongifolium, Lagerstroemia indica, Malus domestica, Opuntia dillenii, Ornithogalum thyrsoides, Parthenium hysterophorus, Peltophorum ferrugineum, Prunus armeniaca, P. domestica, P. persica, P. puddum, Punica granatum, Pyrus communis, P. pashia, Robinia pseudoacacia, Rosa moschata, Toona ciliata, Trifolium repens, Trigonella foenum-graecum,Venidium fastuosum, Zea mays* and *Zinnia elegans* were considered as principle pollen plants of *A. mellifera* in mid hills.

Studies on pollen morphology revealed that pollen grains of plant species belonging to the family Asteraceae were spinolous and small. Plants belonging to Rosaceae family had pollen grains of mostly triangular and tri-lobed shape having small to medium size. The pollen grains of plant species of Cucurbitaceae were large and had a round and triangular shapes. The pollen grains are echinate and large sized in family Malvaceae. *Pisum sativum, Justicia adhatoda, Dicliptera bupleuroides and Gladiolus* hybrid pollens are long and have bilateral symmetry. The pollen grains of both species of *Tecoma* (Bignoniaceae) are tricolporate and bilateral. There was a great variability in the pollen types of plants belonging to the family Fabaceae. Similar variations in size and shape of pollen grains were reported by Singh *et al.* (2017) [12] who explored the bumble bee flora in Nauni, Solan. Shubharani *et al.* (2013) [13] have studied morphology of different pollen grains of honey bee forage plants in Western Ghats of Karnataka and reported similar types of pollen grains of plants of Asteraceae and Fabaceae families.

The flowering plants recorded as pollen resources of *A. mellifera* in mid hills of Himachal showed the dominance of Shrubs (47%). Others were distributed as trees (33%), herbs (18%) and climbers (2%) (Fig 1). Observation on pollen plant species further showed presence of rich and diversified natural flora and cultivated crops. The identified pollen forage plants were categorized according to their economic importance into ornamental plants (20), horticultural plants (9), vegetables (3), condiments (2), oil seed plants (1), cereal (1), wild plants (23) and weeds (7) (Fig 2).

Some of the pollen types identified in the mid hills of Himachal were found in few other studies through pollen load analyses of *A. mellifera* in India, such as in Banthra, Lucknow (Chaturvedi,1973) [14], Bhimtal in the Kumaon Himalaya, U.P (Garg, 1996) [15], Western Ghats of Karnataka (Shubharani *et al.* 2013) [13] and Midnapore, West Bangal (Nandi and Karmakar, 2018) [16] and even in other countries such as in Ethopia (Gebremichael,2006) [17], Islamabad, Pakistan (Noor *et al*., 2009) [18], Egypt, (Ismail *et al*., 2012) [19] Oman (Sajwani *et al*,. 2014) [20] southeastern Poland (Stawiarz *et al*., 2017) [21] and Kafrelsheikh province of northern Egypt (Taha *et al*. 2017) [22].

Conclusion

These studies on pollen morphology and honey bee pollen load analysis will help identify different floral resources used by bees and improved the conservation of economically important plants.

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