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Palynological Studies to Determine Pollen Resources of *Bombus haemorrhoidalis* Smith

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A B S T R A C T

Palynological studies of pollen loads were carried out to determine the pollen sources for bumble bees under mid-hill conditions of Himachal Pradesh during 2016. The pollen loads were collected from queens and workers of *B. haemorrhoidalis* captured with the help of an insect net and then were released. A total of 73 pollen loads were collected. Microscopic preparations were made and palynological analysis was carried out. The analysis showed the occurrence of 68 pollen types served as pollen sources for bumble bee from amongst the fruit trees, medicinal plants, ornamentals, vegetable crops, weeds and wild trees. Out of these, 21 pollen types which formed homogenous pollen loads were considered as principal forage plants namely *Solanum melongena, S. lycopersicum, Capsicum annuum, Digitalis purpurea, Digitalis lanata, Oenothera biennis, Martynia annua, Hibiscus syriacus, Agapanthus umbellatus, Salvia moorcroftiana, Moluccella laevis, Cassia sophera, C. fistula, Clitoria ternatea, Actinidia deliciosa, Peltophorum ferrugineum, Lupinus hartwegii, Dahlia imperialis, D. pinnata, Zinnia elegans* and *Justicia adhatoda*.

Introduction

Bumble bees (*Bombus* spp.) are beneficial insects belonging to order Hymenoptera (Family: Apidae). They have important advantages in their ability to visit flowers in closed spaces (Biliński, 1973, 1976), buzz mechanism while collecting pollen from anthers (Buchmann, 1983), and especially long proboscis in some species (Sladen, 1912). These are important pollinators of crops especially where honey bees (*Apis* spp.) are ineffective or their activity is limited by adverse climatic conditions. They are particularly important pollen vectors for many entomophilous crops and wild flowers which require cross pollination, and can also improve success rates in partly self-fertile or wind pollinated species (Corbet *et al.*, 1991). These are ecologically as well as economically important pollinators in cool and temperate crops and also act as model organisms in specific research (Ayasse and Jarau, 2014). Bumble bees are known for providing a service of significant pollination as their pollen loads carry a greater dry mass than those of honey bees (Broussard *et al.*, 2011).Bumblebees require pollen for their reproduction as it is the sole protein source for developing larvae, and recent evidence suggests that adult workers have an ongoing need for pollen throughout their lives (Smeets

and Duchateau, 2003). The composition of social bee's corbicular pollen loads contains information about both the bee's foraging behavior and the surrounding floral landscape (Marchand *et al.,* 2015).

Palynology is the study of pollen grains produced by seed plants and spores. Pollens can be used to determine foraging resources, pollination mechanisms, migration routes and source zones of insects and pollinators (Jones and Jones, 2001). The palynological analysis of pollen loads allows the identification of plant species visited by bees for pollens and this method is more efficient than visual observation based methods because it allows the identification of a greater number of visited plant species with lower labour inputs involved (Teper, 2005). The palynological studies of pollen loads of bumble bees in different parts of the world have been carried out but no such type of work has been done in our country. Keeping all this in view, the present investigation was undertaken to determine the pollen sources of bumble bee (*Bombus haemorrhoidalis*) under mid-hill conditions of Himachal Pradesh.

Materials and Methods

Preparation of standard pollen slides

The standard pollen slides were prepared by using the fresh and mature flowers of Nauni area. Pollen grains from fresh flowers were placed on microscopic slide and few drops of ethyl alcohol (96%) were dropped on slide. The fat substances appeared on the slide after dropping/pouring alcohol, were cleaned with the 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 alcoholic lamp so that it could not get darker. The content was washed up with ethyl alcohol (70%) and fixed with D.P.X mountant by placing a cover slip over it.

Collection of pollen loads from corbicula of bumble bee while visiting flowers

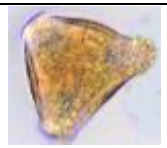
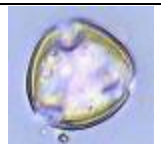
Foraging bumble bee queens and workers of *B. haemorrhoidalis* with pollen loads were captured with the help of an insect net on different sampling dates at 15 days interval from February onwards throughout the year. Pollen loads were carefully removed with a dissecting needle into an individual specimen tubes and the bees were released unharmed. The microscopic preparations of pollen loads were made on the same day or were put in well labeled vials under refrigeration for preparation of slides later on.

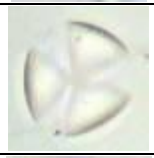
Preparation of pollen slides

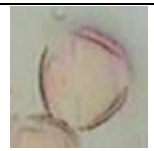
Microscopic preparations of pollens from pollen loads were made by using acetolysis method given by Avetisjan (1950). Pollen load was placed on microscopic slide and few drops of 96%ethyl alcohol were dropped on it. The fat substances appeared on the slide after pouring alcohol was cleaned gently with blotting paper. Few drops of freshly prepared acetolysis mixture were added on to the slides which comprised of acetic anhydride mixed with concentrated sulphuric acid in the ratio 9:1. Then the content on microscopic slides was lightly warmed on alcohol lamp so that it could not get darker. The content was washed up with 70%ethyl alcohol and mounted with D.P.X. The prepared slides were studied under light microscope for morphological studies and photomicrograph of pollen grains was taken. The measurement of pollen grains was taken with "Magnus Pro" software. The pollen grains were divided into five categories on basis of size of pollen grains as per classification given by Sawyer (1981).

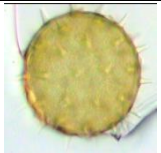
Table.1 Description of pollen grains found in bumble bee pollen loads and their morphology

Sr. No.	Common name	Scientific Name	Family	Flowering Period	Photo-micrograph of pollen grain	Pollen Description	Type of Pollen Loads	Habit and Nature
1.	Field pea	*Pisum sativum* L.	Fabaceae	Jan-March		Long, medium*, bilateral symmetry	+	Climber, vegetable
2.	Basuti	*Justicia adhatoda* L.	Acanthaceae	Feb-April		Long, Large, radial symmetry	++	Shrub, Wild plant
3.	Mustard	*Brassica campestris* L.	Brassicaceae	Feb-March		Round, small, radial symmetry	+	Herb, oilseed
4.	Pot marigold	*Calendula officinalis* L.	Asteraceae	Feb-April		Round, small, spinolous, radial symmetry	+	Herb, ornamental
5.	Blue thistle	*Echium vulgare* L.	Boraginaceae	Feb-April		Oval, very small, bilateral symmetry	+	Herb, ornamental
6.	Annual chrysanthemum	*Glebionis coronaria* (L.) Cass. ex Spach	Asteraceae	Feb-April		Round, small,spinolous, radial symmetry	+	Herb, ornamental

7.	Paperflower	*Helichrysum bracteatum* (Vent.) Andrews	Asteraceae	Feb-April		Round, small, spinolous,radial symmetry	+	Herb, ornamental
8.	Lupin	*Lupinus hartwegii* Lindl.	Fabaceae	Feb-April		Rounded triangular, medium	++	Herb, ornamental
9.	Common poppy	*Papaver rhoeas* L.	Papaveraceae	Feb-April		Rounded triangular, small, radial symmetry	+	Herb, ornamental
10.	Peach	*Prunus persica* (L.) Batsch	Rosaceae	Feb-March		Triangular, medium, bilateral symmetry	+	Tree, Fruit
11.	Kainth	*Pyrus pashia* L.	Rosaceae	Feb-March		Rounded triangular, small, radial symmetry	+	Tree, Fruit
12.	Pear	*Pyruscommunis* L.	Rosaceae	Feb-March		Rounded triangular, medium,	+	Tree, Fruit
13.	Radish	*Raphanus sativus* (L.) Domin	Brassicaceae	Feb-March		Round, small, radial symmetry	+	Herb, Vegetable
14.	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Feb-March		Round, medium, radial symmetry	+	Shrub, Medicinal

15.	Rubus	*Rubus ellipticus Sm.*	Rosaceae	Feb-March		Trilobed, small, bilateral symmetry	+	Shrub, Wild plant
16.	Scutellarium	*Scutellaria albida L.*	Lamiaceae	Feb-March		Round, small, bilateral symmetry	+	Herb, Weed
17.	Rocket larkspur	*Delphinium ajacis L.*	Ranunculaceae	March-May		Oval, small, radial symmetry	+	Herb, ornamental
18.	Apple	*Malus domestica Borkh*	Rosaceae	March-April		Rounded triangular, small, radial symmetry	+	Tree, Fruit
19.	Yellow bells	*Teco mastans (L.) Juss. ex Kunth*	Bignoniaceae	March-April, June-July, Sept-Oct		Round, medium, bilateral symmetry	+	Shrub, ornamental
20.	White clover	*Trifoliumrepens L.*	Fabaceae	March-June		Oval, small, bilateral symmetry	+	Herb, Weed
21.	Wild tobacco	*Nicotia natabacum L.*	Solanaceae	March- May		Rounded triangular, small	+	Herb, Wild plant
22.	Kachnar	*Bauhinia variegate L.*	Fabaceae	March-April		Triangular, medium, bilateral symmetry	+	Tree, ornamental

23.	Snapdragon/ Dog flower	*Antirrhinum majus L.*	Veronicaceae	March-May		Rounded triangular, small, bilateral symmetry	++	Herb, ornamental
24.	Kiwifruit	*Actinidia deliciosa Liang and Ferguson*	Actinidiaceae	April-May		Round, small	++	Climber, Fruit
25.	Himalayan horse chestnut	*Aesculus indica (Wall. ex Camb.) Hook.*	Sapindaceae	April-June		Oval, small, bilateral symmetry	+	Tree, ornamental
26.	Field thistle	*Cirsium sp*	Asteraceae	April-June		Round, medium, spinolous,	+	Herb, Weed
27.	Woolly/ Grecian foxglove	*Digitalis lanata Ehrh.*	Plantaginaceae	April-July		Round, very small,	++	Herb, Medicinal
28.	Common foxglove	*Digitalis purpurea L.*	Plantaginaceae	April-June		Round, small	++	Herb, Medicinal
29.	Duranta	*Duranta primuli L.*	Verbenaceae	April-Oct		Triangular, small, bilateral symmetry	+	Shrub, ornamental
30.	Jacaranda	*Jacaranda mimosifolia D.Don*	Bignoniaceae	April-June		Round, medium, radial symmetry	+	Tree, ornamental
31.	Bells of Ireland	*Moluccella laevis L.*	Lamiaceae	April-June		Round, small, radial symmetry	++	Herb, ornamental

32.	Pomegranate	*Punica granatum L.*	Lythraceae	April-June		Round, small, radial symmetry	+	Tree, Fruit
33.	Sage	*Salvia moorcroftiana Wall. exBenth.*	Lamiaceae	April-May		Round, large, radial symmetry	++	Herb, Medicinal
34.	Kangaroo apple	*Solanum laciniatum Ait.*	Solanaceae	April-Aug		Oval, small, radial symmetry	+	Shrub, Medicinal
35.	Chicory	*Cichorium intybus L.*	Asteraceae	May-June		Trilobed,spinolous, medium, bilateral symmetry	+	Herb, Medicinal
36.	Blue African lily	*Agapanthus umbellatus L.*	Amaryllidaceae	May-July		Boat shaped, large, bilateral symmetry	++	Herb, ornamental
37.	Capsicum	*Capsicum annuumvargrossum (L.) Sendt.*	Solanaceae	May-July		Trilobed, small, bilateral symmetry	++	Shrub, Vegetable
38.	Rose of Sharon	*Hibiscus syriacus L.*	Malvaceae	May-Aug		Round, very large, echinateradial symmetry	++	Shrub, ornamental
39.	Garden pink-sorrel	*Oxalis latifoliaKunth*	Oxalidaceae	May-July		Round, small, radial symmetry	+	Herb, Weed

40.	Tomato	*Solanum lycopersicum L.*	Solanaceae	May-Oct		Round, very small, radial symmetry	++	Herb, Vegetable
41.	Brinjal	*Solanum melongena L.*	Solanaceae	May-Oct		Oval, small, bilateral symmetry	++	Shrub, Vegetable
42.	Chinese trumpet vine	*Tecoma grandiflora (Thunb.) Loisel.*	Bignoniaceae	May-June		Round, small, bilateral symmetry	+	Climber, ornamental
43.	Green chilly	*Capsicum annuumvarannuum L.*	Solanaceae	June-Sept		Oval, small, bilateral symmetry	+	Shrub, Vegetable
44.	Cucumber	*Cucumis sativus L.*	Cucurbitaceae	June-Sept		Rounded triangular, large	+	Climber, Vegetable
45.	Purple coneflower	*Echinacea purpurea (L.) Moench*	Asteraceae	June-July		Round, small, spinolous,radial symmetry	+	Herb, Medicinal
46.	Gladiolus	*Gladiolus hybrida L.*	Iridaceae	June-Aug		Long, large, bilateral symmetry	+	Herb, ornamental
47.	Bitter gourd	*Momordica charantia L.*	Cucurbitaceae	June-Sept		Round, large, radial symmetry	+	Climber, Vegetable

48.	Evening-primrose	*Oenothera biennis L.*	Onagraceae	June-Aug		Trilobed, large, bilateral symmetry	++	Herb, Medicinal
49.	Yellow gulmohar	*Peltophorum ferrugineum Benth.*	Fabaceae	June-Aug		Round, medium, bilateral symmetry	++	Tree, ornamental
50.	French beans	*Phaseolus vulgaris L.*	Fabaceae	June-Sept		Triangular, medium	+	Climber, Vegetable
51.	Clary sage	*Salvia sclarea L.*	Lamiaceae	June-July		Oval, medium, radial symmetry	+	Herb, Medicinal
52.	Krishna neel	*Anagallis arvensis L.*	Primulaceae	June-July		Oval, small, bilateral symmetry	+	Herb, Weed
53.	Okra	*Abelmoschus esculentus (L.) Moench*	Malvaceae	June-Sept		Round, very large, echinate,radial symmetry	+	Shrub, Vegetable
54.	Zinnia	*Zinnia elegans Jacq.*	Asteraceae	July-Oct		Round, small, spinolous, radial symmetry	++	Herb, ornamental

55.	Aparajita	*Clitoria ternatea L.*	Fabaceae	July-Sept		Triangular, large, bilateral symmetry	++	Climber, Medicinal
56.	Dahlia	*Dahlia pinnata Cav.*	Asteraceae	Aug-Nov		Round, small, spinolous,radial symmetry	++	Herb, ornamental
57.	White datura	*Daturas tramonium L.*	Solanaceae	July-Aug		Round, medium, radial symmetry	+	Shrub, Medicinal
58.	Basant	*Hypericum perforatum L.*	Hypericaceae	July-Sept		Oval, very small, bilateral symmetry	+	Herb, Medicinal
59.	Amaltas	*Cassia fistula L.*	Fabaceae	July-Aug		Round, small, radial symmetry	++	Tree, ornamental
60.	Wild salvia	*Salvia coccineaBuc'hoz ex Etl.*	Lamiaceae	July-Sept		Oval, large, bilateral symmetry	+	Herb, Wild
61.	Van bhindi	*Solanum khasianum C.B. Clarke*	Solanaceae	July-Aug		Oval, small, bilateral symmetry	+	Herb, Medicinal
62.	Kasunda	*Cassia sophera (L.)Roxb*	Fabaceae	July-Sept		Rounded triangular, small	++	Shrub, Wild plant

63.	Roxburgh'sfol dwing	*Dicliptera bupleuroides L.*	Acanthaceae	Aug-Nov		Long, medium, bilateral symmetry	+	Herb, Weed
64.	Common morning-glory	*Ipomoea purpurea (L.) Roth*	Convolvulaceae	Aug-Sept		Round, very large, radial symmetry	+	Climber, Weed
65.	Cat's claw	*Martynia annua L.*	Martyniaceae	Aug-Oct		Round, large, radial symmetry	++	Herb, Medicinal
66.	Cotton rosemallow	*Hibiscus mutabilis L.*	Malvaceae	Sept-Oct		Round, very large,echinate radial symmetry	+	Tree, ornamental
67.	Dronpushpi	*Leucas cephalotes (Roth) Spreng.*	Lamiaceae	Sept-Oct		Round, small, radial symmetry	+	Herb, Medicinal
68.	Tree dahlia	*Dahlia imperialis Roezl ex Ortgies*	Asteraceae	Nov-Dec		Round, small, radial symmetry	++	Tree, ornamental

* Classification of pollen grains based on size (Sawyer, 1981), <20µm (Very small), 20-30µm (Small), 30-50µm (Medium), 50-100 µm(Large), >100 µm (Very large), + Multifloral pollen loads, ++ Unifloral pollen loads

Results and Discussion

Pollen analysis of pollen loads recorded 68 plant species belonging to 27 botanical families as pollen source to bumblebees throughout their active season during 2016 under mid hill conditions of Himachal Pradesh (Table 1). The most dominant pollen types belonged to family Asteraceae (9), Fabaceae (9), Solanaceae (8) and Lamiaceae (7). These are distributed to 51% herbs, 19% shrubs, 18% tree species and 12% climbers. Twenty one pollen loads were found to be homogeneous (one-species). These plant species were considered as principle forage plants of *B. haemorrhoidalis* namely *Solanum melongena, S. lycopersicum, Capsicum annuum, Digitalis purpurea, D. lanata, Oenothera biennis, Martynia annua, Hibiscus syriacus, Agapanthus umbellatus, Salvia moorcroftiana, Moluccella laevis, Cassia sophera, C. fistula, Clitoriaternatea, Actinidia deliciosa, Peltophorum ferrugi-neum, Lupinushar twegii, Dahlia imperialis, D. pinnata, Zinnia elegans* and *Justicia adhatoda*.

The pollen morphology varies among different plant species; occur in varying shapes and sizes. They also show variation in symmetry, exine structure and sculpture. A great variation was observed in pollen types of the plant species belongs to family Fabaceae. The plant species belonging to family Asteraceae have spinolous and small pollen grains whereas in family Malvaceae pollen grain types are echinate and large in size. *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. Pollen grains of plants of Rosaceae family were triangular and trilobed having small to medium size. The pollen grains of plant species of Cucurbitaceaeare large, round and triangular. Pollen grains of *Agapanthus umbellatus* L. (Amaryllidaceae) are boat shaped and bilateral. The pollen morphology is useful to identify various species and taxa in their respective families (Shubharani *et al.*, 2013).Pollen study have significant application in recognition of bee plants (Noor *et al.*, 2009).

Analysis of pollen loads reveals that this region is rich in bee pollen plants. The flowering plants of an area having good value as bee pasture are necessary for development of bee colonies. Bumble bees visited these plants extensively for development and colony multiplication. It is a known fact that, due to bee activity farmers are benefitted tremendously because of the ample presence of bee foraging plants in the vicinity of their farms as bee pollination increase the crop yield in a kind of mutualistic relationships (Sahli and Conner, 2007). According to Thakur (2012) in India, about 80 percent or more of the crop plants are dependent on insect pollination. The identification and propagation of bumble bee flora will help in improving the development of bombiculture. University of Horticulture and Forestry, Nauni Solan is the pioneer research institute for developing technology for laboratory rearing of bumble bee in the country. This study will also be useful for conservation and multiplication of economically important multipurpose plants.

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