Palynological Studies to Determine Pollen Resources of Bombus haemorrhoidalis Smith

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ABSTRACT

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.

Keywords: Bombus haemorrhoidalis, Pollen plants, Pollen loads, Pollen analysis.

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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 acetylolysis 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 corbicular 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 acetyolysis 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 acetylolysis 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 | Photomicrograph of pollen grain | Pollen Description                   | Type of Pollen Loads | Habit and Nature              |
|---------|-------------|--------------------------------|-------------|------------------|---------------------------------|--------------------------------------|----------------------|-----------------------------|
| 1       | Field pea   | *Pisum sativum* L.             | Fabaceae    | Jan-March        | ![Image](sample)                 | Long, medium*, bilateral symmetry    | +                    | Climber, vegetable          |
| 2       | Basuti      | *Justicia adhatoda* L.         | Acanthaceae | Feb-April        | ![Image](sample)                 | Long, large, radial symmetry         | ++                   | Shrub, Wild plant           |
| 3       | Mustard     | *Brassica campestris* L.       | Brassicaceae| Feb-March        | ![Image](sample)                 | Round, small, radial symmetry        | +                    | Herb, oilseed               |
| 4       | Pot marigold| *Calendula officinalis* L.     | Asteraceae  | Feb-April        | ![Image](sample)                 | Round, small, spinolous, radial symmetry | +                   | Herb, ornamental            |
| 5       | Blue thistle| *Echium vulgare* L.            | Boraginaceae| Feb-April        | ![Image](sample)                 | Oval, very small, bilateral symmetry | +                    | Herb, ornamental            |
| 6       | Annual chrysanthemum| *Glebionis coronaria* (L.) Cass. ex Spach | Asteraceae | Feb-April        | ![Image](sample)                 | Round, small, spinolous, radial symmetry | +                   | Herb, ornamental            |
| No. | Name            | Genus                  | Family      | Season   | Shape, Symmetry                    | Growth Form, Purpose       |
|-----|-----------------|------------------------|-------------|----------|------------------------------------|----------------------------|
| 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            | *Pyrus communis* 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         |
| No. | Common Name | Scientific Name | Family | Flowering Period | Petal Description | Petal Symmetry | Plant Type       |
|-----|-------------|-----------------|--------|------------------|-------------------|---------------|-----------------|
| 15. | Rubus       | *Rubus ellipticus* Sm. | Rosaceae | Feb-March | Trilobed, small, bilateral symmetry | + | Shrub, Wild plant |
| 16. | Scutellaria | *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 | *Trifolium repens* 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 |
| No. | Species                                      | Family             | Season   | Flower Description                                                                 | Growth Form        |
|-----|---------------------------------------------|--------------------|----------|-----------------------------------------------------------------------------------|--------------------|
| 23  | Snapdragon/Dog flower                       | Antirrhinum majus L. | March-May| Rounded, triangular, small, bilateral symmetry                                     | ++ Herb, ornamental|
| 24  | Kiwifruit                                   | Actinidia deliciosa Liang and Ferguson | April-May| Round, small                                                                      | ++ Climber, Fruit  |
| 25  | Himalayan horse chestnut                    | Aesculus indica (Wall. ex Camb.) Hook. | April-June| Oval, small, bilateral symmetry                                                   | + Tree, ornamental |
| 26  | Field thistle                               | Cirsium sp         | April-June| Round, medium, spinolous,                                                          | + Herb, Weed       |
| 27  | Woolly/Grecian foxglove                     | Digitalis lanata Ehrh. | April-July| Round, very small,                                                                | ++ Herb, Medicinal |
| 28  | Common foxglove                             | Digitalis purpurea L. | April-June| Round, small                                                                     | ++ Herb, Medicinal |
| 29  | Duranta                                     | Duranta primuli L.  | April-Oct| Triangular, small, bilateral symmetry                                             | + Shrub, ornamental|
| 30  | Jacaranda                                  | Jacaranda mimosifolia D.Don | April-June| Round, medium, radial symmetry                                                    | + Tree, ornamental |
| 31  | Bells of Ireland                           | Moluccella laevis L. | April-June| Round, small, radial symmetry                                                     | ++ Herb, ornamental|
| No. | Species                          | Family          | Flower Period | Flower Description                                      | Height | Use          |
|-----|---------------------------------|-----------------|---------------|--------------------------------------------------------|--------|--------------|
| 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 lacinatum 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 annuum var. grossum (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 latifolia Kunth* | Oxalidaceae    | May-July      | Round, small, radial symmetry                           | +      | Herb, Weed   |
| No. | Plant            | Scientific Name                          | Family      | Flowering Period | Flower Description                                      | Growth Form     | Type          |
|-----|------------------|------------------------------------------|-------------|------------------|----------------------------------------------------------|-----------------|---------------|
| 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 annuum* var*annuum* 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 |
| No. | Plant Name               | Scientific Name                                | Family       | Flowering Period | Flower Description                          | Growth Form | Use              |
|-----|--------------------------|-----------------------------------------------|--------------|-----------------|---------------------------------------------|-------------|------------------|
| 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 coccinea* Buc’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 |
| No. | Species                        | Family           | blooming | Pollen Characteristics | Pollination Type | Insect Type |
|-----|--------------------------------|------------------|----------|------------------------|------------------|-------------|
| 63  | Roxburgh'sfoal                 | *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, Clitoria ternatea, Actinidia delicosa, Peltophorum ferrugineum, Lupinus harrowii, 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 Cucurbitaceae are 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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