# ECO-BIOLOGICAL STUDIES ON BUMBLEBEE (*BOMBUS HAEMORRHOIDALIS* SMITH) FROM NORTHERN PAKISTAN IN RELATION TO CROP POLLINATION


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by

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**Department of Entomology**
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**Pir Mehr Ali Shah**
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# CERTIFICATION

I hereby undertake that this research is an original one and no part of this thesis falls under plagiarism. If found otherwise at any stage I will be responsible for the consequences.

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بسم الله الرحمن الرحيم

*IN THE NAME OF ALLAH,*

*THE MOST GRACIOUS,*

*THE MERCIFUL*

*I dedicate This Humble Task,*

*Fruit of My Thoughts and Study*

*To My*

***Mother***

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# ABSTRACT

Bumblebees are important for their pollination services to different plant species providing a major source of variability and survival for cross pollinated plant species. They belong to temperate regions due to their need of hibernation needs in winter. Their importance as buzz pollination makes them unique for pollination of different commercial crops like tomatoes, pepper, strawberries etc under glasshouse farming systems. Different bumblebee species has been identified in northern areas of Pakistan including Azad Jammu and Kashmir, Gilgit Baltistan, Khyber Pakhtoonkhwa and some parts of northern Punjab. *Bombus haemorrhoidalis* has been identified as important and common bumblebee species in lower northern Pakistan. Different experiments were conducted for ecological and biological studies to monitor the indigenous bumblebee, *B. haemorrhoidalis* abundance and species richness in the northern Pakistan including Rawalpindi-Islamabad, Rawalakot and Naran-Kaghan valley It will be helpful for conservation of our local bumblebee fauna and to identify the possible plant species for their long term survival and stability of environmental balance of species.

According to the results regarding abundance of native bumblebee in relation to other pollinators *Bombus haemorrhoidalis* was the most abundant pollinator in comparison with other pollinators in all sub-localities. In 2012, maximum Shannon index, Simson index and Eveness were recorded in Naran Kaghan and minimum in Rawalpindi/Islambad. In 2013, maximum Shannon index and Eveness were recorded in Naran Kaghan while Simson index was maximum in Rwalpindi/Islambad and minimum Shannon index in Rawalakot. The foraging

source of native bumble comprised of 42, 43 and 48 plant species in Naran Kaghan, Rawalkot and Rawalpindi/Islamabad, respectively. Maximum species (11) belonged to Family, Asteraceae from all three study areas.

The nest seeking queens emerged after spending winter diapause in nature preferred relatively open field landscape followed by open fields, forest boundaries proved more populated habitats followed by field boundaries for nesting sites. Withered grasses remained most favored patches and stone and moss the least ones for nesting sites of *B. haemorrhoidalis*. at all locations. Rearing of *B. haemorrhoidalis* was successfully done and biological parameter of like, preoviposition period, egg hatching period, larval and pupal duration, period of first workers emerged, numbers of workers and sexual and mother queen life span were observed under controlled laboratory conditions. Seasonal fluctuation of sexual morphs indicated first emergence of winter diapausing queens in March - April, maximum population of workers in September, males and daughter queens in October, in field conditions.

Indigenous bumblebee *B. haemorrhoidalis* was used for pollination of tomato crop in comparison with European bumblebee *Bombus terrestris* at Hydroponic Research Farm. Non significant differences were obtained in the means comparison of number of seeds per fruit, fruit weight, fruit height and fruit diameter for both pollinators.

*Chapter 1*

# INTRODUCTION

Insects have been serving mankind from centuries not only as the source of food, feed but also working for maintenance of plant to animals balance. Different organisms including vertebrates and invertebrates help to keep this balance, however, insects particularly bees are important for their role in crop pollination (Richards, 1993; Daly, 1997). Bees include a variety of hymenopterans insects with major groups including *Apis* and non-*Apis* bees where the latter ones consists of great variety having alkali bees, *Osmia* bees, carpenter bees, sweat bees, bumblebees etc as important group of insects for crop pollination (Gardner and Ascher, 2006). Ecosystem services to help reproduce and establish fruit set in agricultural and wild flowers are fostered by these insects (Accorti, 2000).

The population of pollinators is depended on availability of floral host plants (Goulson, 1999) and their composition patterns (Chittka *et al*., 2004). Their increase in species richness could raise the chances of plant pollination (Corbet, 1997) with plant species exclusively depending on small number of pollinators are in more danger (Kwak *et al*., 1998). Non *Apis* bee like the bumblebees are important due to their utilization of diverse floral plants and belong to the richest and most conspicuous flower visitors in alpine, temperate and arctic environments for pollination in both natural and managed cropping systems (Corbet *et al*., 1991). They are crucial from agriculture and conservation perspective for cultivated crops, fruits and wild plantations (Fridden, 1967). These bumblebees are the generalist foragers of floral resources available in their access for good nectar and pollen sources. They are also capable to work at low temperatures as compared to honeybees accounting for their richness in cool environments with season of adverse conditions and evenly dispersed flowers (Newsholme *et al.,* 1972).

Bee pollination is considered a vital source of crop pollination resulting in about 30% of human's food crops pollinated in North America (Henrich, 1979a) with bumblebees as major pollinators (Faegri and Van der Pijl, 1979). These bees prefer to forage a diverse climatic region from open sunny meadows to dense forests. However, these are restricted to high altitudes and known as buzz pollinators due to their characteristic sound generation character with vibrating body. Their abundance correlates with the vegetation type and intensity (Sabir *et al*, 2011). However, recent studies showed their trend of decline in abundance throughout the world (Picken, 1984). Increase in farming (Heard *et al*., 2007), utilization of chemicals as pesticides and fertilizers, large scale agro-ecosystem establishment and destruction of forage resources are considered their major causes of decline (Radeghieri *et al.,* 1998). Other factors like habitat devastation and crumbling may also possibly be involved in their decline (Opdam *et al.,* 1993).

These generally construct underground nests which are inconspicuous and difficult to find (Kells and Goulson, 2003). Nest seeking queens have almost always been observed close to forest and field boundaries (Sevensson *et al*., 2000). Bumblebees normally explore the man made landmarks in the open areas and perform same flying pattern when leaving the nest for first time (Goulson *et al*., 2004). Restoration and conservation of suitable habitats is certainly very critical for their survival in natural and farmlands ecosystems (Kraus *et al*., 2009) for suitable sites of colony initiation, winter diapuse and constant food requirements (Steffan-Dewenter and Tscharntke, 1999). Bumblebees have competition for nest site among the different species and sometime within species but it depend upon time of the year and habitat type (Ranta, 1982: Pyke, 1982). These bees prefer to construct their nest in burrows of small rodents and mammals along field boundaries with tussocky places (Svensson *et al*., 2000). Most bumblebee species build their nest undergrounds but fewer species build nests on the surface of ground or some time above the ground, on tree hole and in birds' nests (Dramstad *et al*., 1996).

Highly commercial crops like tomatoes, peppers, strawberry, cucumber, brinjal, peaches and apple grown on large scale in greenhouses and tunnels need buzz pollinators like bumblebees unlike honeybees (Aytekin *et al*., 2002; Goulson, 2003). These are of great interest to farmers enhancing their production with high economic returns, reduced labor for pollination and increased quality of harvest (Picken, 1984; Kevan *et al*., 1991). Different bumblebee species have been commercialized and used in many developed countries like the USA, most Europe, New Zealand, Japan, Tasmania, Chile and Argentina to enhance crop pollination (Goulson, 2004). However, the main crop pollinated in greenhouses is tomato involving about 95% of all bumblebee sales worldwide with over 40,000 hectares of greenhouse cultivation. The increased economic returns due to their pollination are anticipated to be 12,000 million Euro per year (Velthuis and Van Doorn, 2006). In Pakistan, these bumblebees are imported from Biobest, Belgium and Koppert, Netherland for greenhouse crops in hydroponic research farm of Pir Mehr Ali Shah

Arid Agriculture University, Rawalpindi. These bees are used as crop pollinator in different varieties of tomato crops and sweet pepper crops.

Northern Pakistan has very distinctive and rich fauna and flora especially the bumblebees that help in ecosystem conservation (Pittoni, 1939; Banaszak, 1992; Barbattini, 1994). It is the regions with the most diverse and rich earth's biological wealth (Malcolm *et al*., 2002). About 5700 species of floral resources with 400 widespread species and 1000 of vascular plants occur in these regions (Khan *et al*., 2009). Major work done on bumblebees dates back to early twentieth century and needs more diverse studies about relationship of bumblebees with flora of this area. Some plants are pollinated only by the single species of bumblebees (Rathcke and Jules, 1993) whose reduction may reflect the plant communities (Corbet *et al*., 1991). In Northern Pakistan, ten bumblebee species, including five *Bombus semenovianus, B. tunicatus, B. rufofasciatus, B. asiaticus* and *B. melanurus* dominant species were found from non-agricultural habitat and thirteen species including *Bombus haemorrhoidalis* Smith found from agricultural landscape (Sabir *et al.*, 2008). Five bumblebee species i.e., *B. haemorrhoidalis B. rufofasciatus B. trifasciatus B. kashmirensis B. subtypicus* are most common in Naran Kaghan Valley of Pakistan and which used to forage twenty four floral host plants of ten different plant families (Sheikh *et al.*, 2015).

Local bumblebee (*B. haemorrhoidalis* Smith) species belongs to Himalayan and South East Asian countries (Williams, 1991) and also reported from Pakistan (Richerds, 1929). In India, it has been recorded as sole pollinator of large cardamom and other crops (Deka *et al*., 2011). It is a dominant bumblebee species of agricultural habitat in northern Pakistan (Sabir *et al.*, 2008) and the sole species of lower northern Pakistan including, Rawalpindi, Islamabad and Murree hills ranging from 542-1986 m altitude which pollinate twenty four plant species of thirteen plant families in wild and managed crops in this region (Sheikh *et al.*, 2014).

Bumblebees are solitary social bees living in small colonies with generally 200-300 individuals (Dramstad, 1996); Fry, 1995) always with a single mated queen (Richards, 1929). Colonies start their development in early spring and switch to production of sexual as male and daughter queens in the end of colony cycle. Foraging worker bumblebees feed on nectar and pollen during foraging floral plants and also responsible for nourishing the young larvae on compressed pollen grains and nectar in the colony (Richards, 1973). In nature queens of *B. haemorrhoidalis* emerge in early spring after spending winter diapuse and start their nests. In the start of colony workers are produced while at end of the colony cycle, sexuals are produced in September and October (Sheikh *et al.*, 2014). Bumblebees are normally non-aggressive but sting in defense of their nest or when disturbed during foraging (Newsholme et *al.*, 1972).

Different controlled environment methods had been established for year-round mass rearing of their colonies for crop pollination needs in managed systems (Alford, 1975; Pouvreau, 1976). In the start of $20^{th}$ century researchers started work to rear them and found different techniques for their mass rearing. After its success stories and commercial rearing in Europe, many laboratories in China, Korea, and Mexico have started rearing them for research, pollination and biological studies with remarkable progress (An *et al*., 2001; Peng *et al*., 2003). Now bumblebees are reared as industries and used round the world (Velthuis and Doorn, 2006). Belgium, Israel and Netherland are the main bumblebee exporting countries and China, Japan, Mexico, Italy and Spain are main importing countries. Biobest and Koppert are the dominant companies rearing bumblebees for commercial pollination services.

Five bumble bee species including *B. terrestris, B. impatiens, B. ignitus, B. lucorum* and *B. occidentalis* are reared throughout the world. Among them, *B. terrestris* and *B. impatiens* are the most commonly used species (Velthuis and Doorn, 2006). Commercialization of bumblebees, however, might cause spreading of parasites and pathogens from their native countries to non-native ones (Goka *et al.,* 2001). Recently attempt to rear *B. haemorrhoidalis* Smith, an indigenous bumblebee species of India and Pakistan, was made in India helpful for establishing the Bombiculture industry in India. In the start of colony cycle workers emerged and when number of workers reached above 50, this stage is known as foundation stage. And at the end of colony cycle sexuals including male and daughter queens are produced (Chauhan *et al*., 2013). Their breeding and rearing in the country can help increase their utilization in crop pollination but also reduce the cost of import and avoid possible problems of exotic species (Kimberly *et al.*, 2006). Tunnel farming and hydroponic farms establishment in Pakistan need them to foster the pollination process and increase crop yields with more stress on selection of indigenous bumblebees

Keeping in view of their importance in buzz pollination and conservation of indigenous bumblebee species, present study has been planned keeping in mind the following objectives:

- Monitoring of indigenous bumblebee, *B. haemorrhoidalis* Smith for its foraging floral host range and nesting preference in the Northern Pakistan

- Biological studies for laboratory rearing of *B. haemorrhoidalis* Smith under controlled conditions and

- Efficiency of *B. haemorrhoidalis* Smith as crop pollinator of tomato under greenhouse conditions

*Chapter 2*

# REVIEW OF LITERATURE

Cultivated and wild plants need different pollination sources to meet their pollination needs and keep their species breed. Air, water, vertebrate and invertebrate animals are considered important to help plants get pollinated. Most cultivated crops are cross pollinated and almost one third of human food depends on insect pollination (McGregor, 1976). The prospective value of bumblebees as pollinator of agricultural crops has been known for many decades. These are considered much better pollinators of wild and cultivated flowers due to their longer tongue, foraging different floral resources and working at low temperature (Holm, 1966). These provide central ecological service as pollinators in Northern Pakistan (Sabir *et al*., 2007).

The genus *Bombus* comprises about two hundred fifty species throughout the world (Williams *et al.*, 2008) with twenty nine species from the Indian Kashmir and Himalayan range (Williams, 1991). From northern Pakistan, 13 bumblebee species including, *Bombus semenovianus, B. tunicatus, B. rufofasciatus, B. asiaticus* and *B. melanurus, B. lucorum, B. biroi, B. subtypicus, B. kashmirensis, B. haemorrhoidalis, B. himalayanus*, *B. marussinus*, and *B. avinoviellus* has been reported with their floral and spatial distribution (Sabir *et al.,* 2008). Five bumblebee species, *B. haemorrhoidalis*, *B. rufofasciatus*, *B. trifasciatus*, *B. kashmirensis* and *B. subtypicus* were recently reported from Naran Kaghan Valley, Pakistan with their twenty four foraging floral host plants from ten different plants families (Sheikh *et al.,* 2015). *B. haemorrhoidalis* Smith belongs to subgenus

*Orientalibombus* is the species of Himalayan range and South East Asian countries (Williams, 1991) which was recorded from Pakistan during early 20$^{th}$ century with uniform distribution from Kashmir and Northern Pakistan (Richerds, 1929; Suhail *et al*., 2009; Sabir *et al.*, 2011). It has also been reported from India (Raine *et al* , 2011), Burma (Skorikov, 1938) and Nepal (Richards, 1929; Frison, 1935). It is the only *Bombus* species found in lower northern Pakistan with twenty four foraging floral host plants of thirteen plants families (Sheikh *et al*., 2015).

Spatio-temporal variation exists between bumblebees and their different floral host plants (Irwin and Maloof, 2002). In Southern Finland, foraging queens have been observed more in early May near stream and leys in late May (Alanen, 2008). Flight activity of workers was recorded from February to December and male from February to November (Thorp *et al*., 1983). They foraged the entire altitudinal range in summer, basal parts in spring and intermediate zone during autumn with more diversity during mid season (Lundberg and Ranta, 1980). Small workers mostly remain in the colony and perform nursing, cleaning and other inside nest jobs whereas larger workers move outside for foraging floral resources to collect more nectar and pollens (Kandori, 2002). Colony of *B. haemorrhoidalis* Smith consists about 250 to 300 workers and produce sexual individuals at the end of colony cycle (Chauhan *et al.,* 2014) However, variation in body size correlate with cold climate (Lundberg and Ranta, 1980).

Bumblebees play significant role as pollinators in agricultural and natural ecosystems (Kraus *et al*., 2009; Knight *et al*., 2009). Flowers which have jointed pollen only open with vibration and bumblebees open these pollens by their flight muscles with the frequency of about 400 Hz (King, 1993). These have to visit flowers for pollens and nectar for their survival that may take it to large area from some hundred meters foraging (Cressewll *et al*., 2000). These have ability to fly and forage in poor weather conditions even in cold and windy day. In North America, their queens were observed foraging at temperature below freezing point (Stanghellini *et al*., 2002).

Foraging activities of bees increase with the quantity and quality of food (Spaethe and Weidenmüller, 2002) with increased directionality of their flight highlighting their selection or identification of floral rewards and small scale habitat crumbling (Goverde *et al*., 2002). These have ability to differentiate the quality and quantity of pollens from different plant species during their collection but cannot differentiate plant species on the basis of floral characters of host plants (Rasheed and Harder, 1997). There, however, exist differences in their preferences for pollens and nectar (Connop *et al*., 2010) with more attraction to leguminous flowers (Carvell *et al*., 2007). Bumblebee species vary in their tongue length and mainly categorized into long- and short-tongue bumblebees. The former are predominantly important because of their ability to pollinate the deep flowers which make them more vulnerable than that of short-tongue ones (Goulson *et al*., 2008). Already visited inflorescence is generally avoided by other bumblebee species during their foraging of flowers (Goulson *et al.*, 1997). Large sized are better suited to foraging because they are able to transport more pollens and nectar to the nest, however, foraging trip times were observed inversely related to the bee size when collecting nectar but unrelated for pollen collection (Goulson *et al*., 2005). Almost one third foraging bumblebees has been recorded returning to nests with pollen loads after foraging (Martin *et al*., 2005).

Bumblebees have shown declining trend in many parts of the world mainly associated with the destruction of their natural habitats, reductions in the abundance of floral food plants and decrease in natural food resources (Williams *et al*., 2009). Foraging plants have declined in both large and local scale frequency in twentieth century reflecting serious reduction in quality of floral foraging (Carvell *et al*., 2006). These foraging ranges affected more by habitat fragmentation and agro-ecosystem on large scale (Rundlof *et al*., 2008). Strengthening of farming practices were also responsible for habitat destruction and forage resources leading to the their decline (Goulson *et al*., 2002b; Goulson and Hanley, 2004; Heard *et al*., 2007; Sabir *et al*., 2007; Goulson *et al*., 2008).

Probably underdeveloped flower rich grasslands and restricted distribution were the major causes of decline of many bumblebee species (Goulson *et al*., 2004). Fields with high pastures had more bumblebees as compared to those with little pasture lands (Morandin *et al*., 2006). Crop margins sown with strips containing pollen and nectar provide more effective foraging site (Carvell *et al*., 2007). Major decline in their fauna coincided with increased agricultural practices in Illinois (Jennifer *et al*., 2008). Bumblebee richness was positively associated with natural areas and negatively associated with areas disturbed by men (McFredrick *et al.,* 2006). Landscape and patch factors were linked with bumblebees positive richness in meadows (Hatfield and Gretchen, 2007).

Nest seeking queens were found more frequent along forest and field boundaries in an open cultivated area. These differences among species were attributed to both landscape type and habitat preferences (Svensson *et al*., 2000) because of differences in foraging range of different species which may be up to 9.8 km from their nest (Goulson and Jane, 2001). Bees chose to live in habitats with patchy distribution of nesting substrates and floral miscellany for maintaining their population (Kells *et al*., 2001) and best foraging habitat consisted of non-crop field margins (Pywell *et al*., 2005). These generally forage not near to their nests (within 50m) and commonly choose to forage for pollen and nectar at least 1.5 Km from the nest ((Dramstad, 1996; Osborne *et al*., 2008). Positive association between colony density and floral resources has been previously observed inside one kilometer range (Knight *et al*., 2009).

Crops in greenhouse and glasshouse growing systems require pollinators throughout the year and bumblebees serve this purpose effectively to maximize the crop yield. Maximum foraging flights of the buff tailed bumblebees (*Bombus terrestris* L.) on greenhouse tomato has been observed during temperature range of 19.6 to 24ºC (Roman and Szczesna, 2008). However, queens foraged at the lowest temperatures as compared to males and workers (Lundberg, 1980). Bumblebees are gifted with buzz pollination and excellent pollinators of Solanaceous crops like tomatoes (Van den Eijnde *et al*., 1990). They help pollinate highly commercial crops like tomato, brinjal and cucumber under greenhouse and tunnel farming (Goulson, 2003). Plants pollinated have shown greater number of young pods, mature pods, number of seeds and their weight that that of self pollinated ones (Al-Ghzawi *et al.*, 2003). It improved the fruit quality and enlarged total yield (Van der

Sande, 1990; Van Ravestijn and Van der Sande, 1991; Dogterom *et al*., 1998). A colony of bumblebee (*B. terrestris*) having approximately 80-120 workers can help to meet the pollination needs in glasshouses for two months (Eijnde *et al*., 1990).

Bumblebees have been reared in Europe as an Entomological industry to satisfy the need of crop pollination under greenhouses cultivation (Dogterom *et al*., 1998). Their commercial rearing started in 1987 and in two decades times, almost one million colonies started producing annually to pollinate highly commercial crops (Velthuis and Van Doorn, 2006). Colony development depends on the quality of pollens, nectar (Bucankova and Ptaeek, 2007) and temperature as a major factor (Btihler *et al*., 1983). Various stimulating treatments for colony initiation have been used like brood cells containing old pupae with some accompanying workers (Roseler, 1985) or 4 to 5 honeybee workers (Eijnde *et al*., 1991). Different *Bombus* species have been multiplied under laboratory conditions; however, *B. terrestris* is the most dominant species for commercial breeding.

Free introduction of non-native bumblebees can be a major factor of inter-specific competition between local and imported bumblebees. These imported bumblebees vary in their foraging behavior and activities in crop pollination (Inari *et al*., 2005). These also transfer pests and diseases to local bumblebee population and result in hazards to their survival. The need to study the local bumblebees of Pakistan to avoid these hazards from importation highlights the present work on ecological and biological aspects of indigenous bumblebee. *Bombus haemorrhoidalis* is only Bombus species of this study area and only one color pattern of this species was found in this study area (Sheikh *et al*., 2014; 2015).

# MATERIALS AND METHODS

The possible impacts of ecological and biological changes in indigenous bumblebee, *Bombus haemorrhoidalis* Smith were conducted for three different topographical areas of Northern Pakistan during two consecutive years of 2012 and 2013. These locations were further divided into sub-locations according to topography, vegetation, altitude and latitude.

## 3.1 STUDY AREAS

Three different areas from Northern Pakistan including, Rawalpindi-Islamabad, Rawalakot and Naran Kaghan valley were chosen for the present study based on different flora, topography, altitude, latitude and environmental conditions. Longitude, Altitude, latitude and temperature were measured with the Garmin e-trex 10 GPS device (Table 3.1: fig. 3.1, 3.2 and 3.3).

Rawalpindi and Islamabad are located in the range of Margalla hills, climate is sub-humid to subtropical and rainfall received from both monsoon and western climatic turbulence. Highest rainfall occurs in the monsoon season (more than 50%) extending almost from July to September with an average of about 1044 millimeters per annum. The temperatures in this area, however, vary from -1 to 46°C with an average low and high temperature of 23.8°C and 34.2°C during monsoon season and average rainfall of 289 mm (Fatimah and Tahira, 2012)

Rawalakot lies in the North-East of Pakistan under foothills of enormous Himalayas in the Poonch District of the charismatic Azad Jammu and Kashmir region. The landscape is mainly hilly and mountainous with valleys. It is characterized by moderate environment with average annual rainfall of 500–2000 mm, mostly uneven with intense storms especially during the monsoon and winter seasons. The mean annual temperature ranges from 0-30°C with average low and high temperature 23 and 33°C during monsoon season and average rainfall of 125 mm. In winter season, severe cold and snowfall hits the region which causes considerable variation in temperature and moisture because of high elevation (Nazar and Mahmood, 2011).

Naran and Kaghan valley is located in district Mansehra, Khyber Pakhtunkhwa province. The entire area is formed by high spurs of mountains along both side of the river Kunhar flowing in North-East to South-West direction. This area is marked by a distinct change of climate, soils and vegetation with temperature range of -5-28$^{o}$C with average low and high temperature of 17.5 and 28°C and average rainfall of 34 mm during monsoon season (Khan *et al.*, 2009).

Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley, each location was further classified into three sub-locations on the basis of different landscape types. Rawalpindi/Islamabad included Lake View Park, F9 Park and Bara Kahu areas. Poonch University area, Namnoota and Paniola were selected from Rawalakot and Naran Kaghan Valley included Mahandari, Kaghan and Naran area.

## 3.2. COLLECTION OF INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH

For collection of indigenous bumblebee *Bombus haemorrhoidalis*, monthly field surveys were conducted from March to November for two consecutive years.

Table 3.1 Global Positions of Study locations and sub-locations

| **Locations** | **Sub-Locations** | **Altitude** | **Global positioning** |
| --- | --- | --- | --- |
| **Rawalpindi/Islamabad** | Lake View Park | 542 m | 33° 43′ 05.16″ N<br>73° 08′ 00.22″ E |
| | F-9 Park | 564 m | 33° 42′ 35.01″ N<br>73° 01′ 21.72″ E |
| | Bara-Kahu | 679 m | 33° 45′ 14..57″ N<br>73° 11′ 19.72″ E |
| **Rawalakot** | Poonch University | 1614 m | 33° 50′ 48.25″ N<br>73° 46′ 28.10″ E |
| | Paniola | 1438 m | 33° 55′ 13.70″ N<br>73° 41′ 12.65″ E |
| | Namnoota | 1864 m | 33° 51′ 11.61″ N<br>73° 49′ 09.16″ E |
| **Naran Kaghan Valley** | Mahandri | 1673 m | 34° 41′ 40.56″ N<br>73° 34′ 27.09″ E |
| | Kaghan | 2095 m | 34° 46′ 40.81″ N<br>73° 31′ 31.80″ E |
| | Naran | 2772 m | 34° 55′ 25.86″ N<br>73° 46′ 00.99″ E |

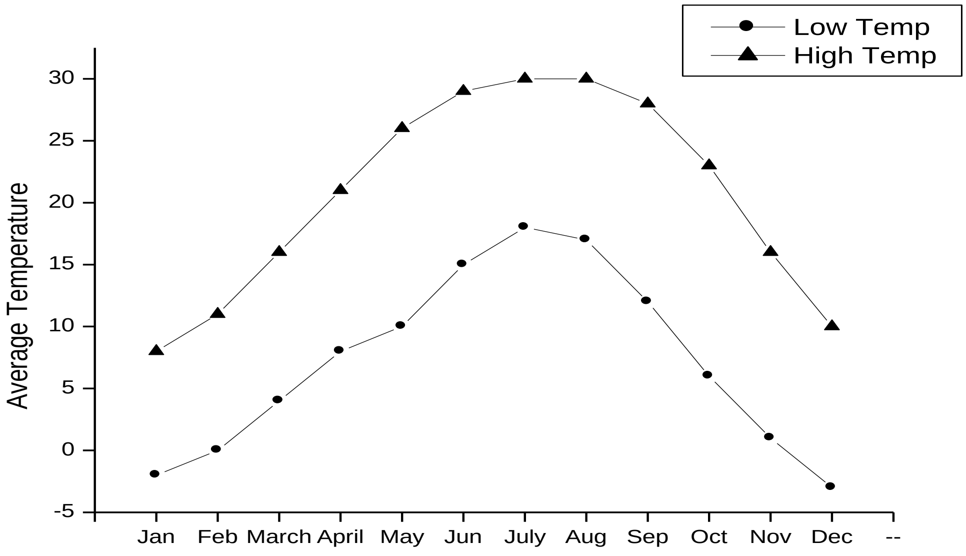


Figure 3.1 Monthly average low and high temperature of Rawalpindi/Islamabad

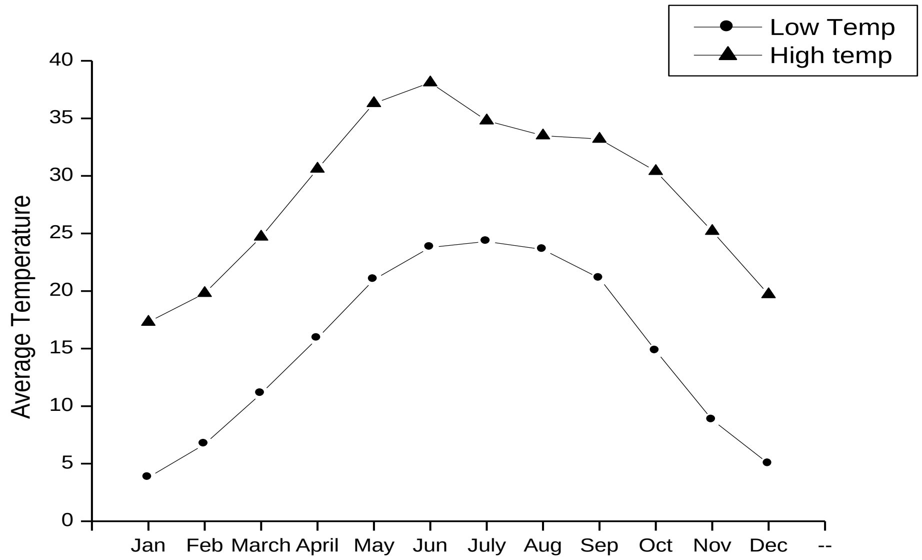


Figure 3.2 Monthly average low and high temperature of Rawalakot

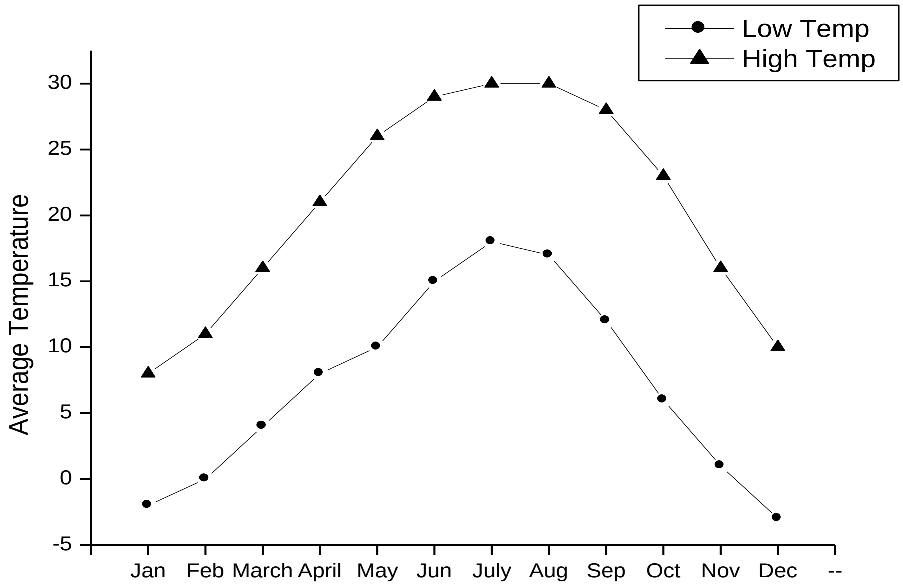


Figure 3.3 Monthly average low and high temperature of Naran Kaghan Valley

Arial hand net was used to collect the samples of bumblebee (Dafni, 1992) through walking in selected transact along roadside, crops, forest and surroundings of parks. Bumblebees were collected during the foraging activities in the morning time of sunny days. After collection, samples were killed in cyanide jars and preserved in transparent plastic jars or bags lined with tissue papers for protection during movement. Entomological pins were used to pin these specimens which properly set them on setting boards. Then they were labeled for locality, date, host plant and collector's name. Collected samples of bumblebees were identified by following the taxonomic literature available (Williams, 1991). All the specimens were deposited in the Insect Museum of Biosystematics Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan.

### 3.2.1 Relative Abundance of Indigenous Bumblebee, *Bombus haemorrhoidalis* Smith In Comparison With Other Pollinators and Species Diversity Indices

Relative abundance of indigenous bumblebee, *B.haemorrhoidalis* in comparison of other common pollinators was measured on monthly basis during the field surveys at all sub-locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley. Numbers of queens, workers and males of *B. haemorrhoidalis* with other common pollinator species were recorded at 9 to 11am in the morning and afternoon during 2-5pm. Monthly abundance of indigenous bumblebee was also measured to determine the population variation of *B. haemorrhoidalis* throughout the year. Diversity of each sub-location was calculated by using three species diversity indices. Diversity indices for each year were also determined and comparison was observed. These indices were calculated to compare the diversity of different locations and sub-locations.

1. The formula given by Margalef (1958) was used to calculate Shannon species diversity index (H) based on Shannon-Wiener function as:

$$H = \Sigma Pi (\ln Pi)$$

Where Pi = Ni/N

Ni = Total number of individuals in a species

N = Total number of individuals in all species

2. Evenness (j) was calculated to estimate the equitability component of diversity using the formula (Pielou, 1975):

$$J = H/\log 10S$$

Where H= Shannon species diversity index

S = total number of species

3. The following formula was used to calculate the Simpson's index of diversity:

$$D = \sum (n / N)^2$$

Where N= Total number of individuals of all species

n= Number of individuals of a species

### 3.2.2 Foraging Floral Range of *Bombus haemorrhoidalis* Smith

From Rawalpindi/Islamabad, Rawalakot and Naran Kaghan, floral host range of *B. haemorrhoidalis* was observed during the surveys throughout both years. Foraging floral host range was observed comprising weeds, ornamental plants, cultivated crops etc by modified bee walk transect method (Banaszak, 1980). The plant species visited by the bumblebee were recorded, photograph of each visiting plant was made and when in doubt, a herbarium was made for its proper identification by collecting two samples of each flowering plant species. Floral host plants were identified at species level by the existing literature (Sabir, 2011).

## 3.3 NEST SEEKING PREFERENCE OF INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH SPECIES

This study was conducted first time in this region and will be helpful to identify the bumblebee nesting sites for conservation of bumblebee fauna in this region. It will be helpful to collect the bumblebee queens to establish the year round rearing of this indigenous bumblebee species.

Study was conducted at three different locations including Rawalpindi/ Islamabad, Rawalakot and Naran Kaghan Valley from Northern Pakistan for both years. Surveys were conducted at all study locations during early spring season to observe maximum queens emerged after winter diapause. Surveys were focused on possible identification of indigenous bumblebee, *B. haemorrhoidalis* nests or hives habitat which they made mostly underground. The study was carried out within 12 $Km^2$ area of each location.

### 3.3.1 Landscape Types

**1. Open field (O)**: agricultural fields and forest boundaries with weeds and grasses

**2. Relatively open (RO)**: large agricultural fields mostly surrounded by other agricultural fields and roads but also by ditches, bushes, small woods and pastures

**3. Relatively wooded (RW)**: small agricultural fields surrounded by forests and roads with several small habitat types such as ditches, pastures and woods

**4. Wooded landscape (W)**: almost always surrounded by forest and weeds and grasses

### 3.3.2 Habitat Types

These four landscapes were further categorized into five different types of habitats. Three transacts (each of 800m×800m) were selected from each habitat type from each location. Each transact consisted number of intermingled habitats but it represented as the most covering habitat type area.

The habitats were classified as (Svensson *et al*, 2000);

**1. Field (F):** agricultural field, grass leys or uncultivated with a flora of weeds

**2. Field boundary (Fb):** a grass-dominated strip between an agricultural field and other open ground, pasture or clearing

**3. Pasture (P):** natural pasture somewhat covered with broad-leaved trees and bushes

**4. Forest boundary (Fob):** the vegetation mainly consisted of grass, herbs and bushes

**5. Forest (Fo):** diverse, well-grown forest where the bushes mainly consisted of dwarf-shrubs and herbs

### 3.3.3 Patch Transact Types

Bumblebee queens in search of a nesting site normally fly in a criss-cross pattern just above the ground within a patch rarely going down to explore the ground surface (Lundberg and Svensson, 1975). Queens were observed in small patches (transact) (1m × 1m) during flying with 5 minutes observation time for each patch (Sevnsson *et al.,* 2000) and counted following walk transact method (Banaszak, 1980). It is a rare phenomenon to find bumblebee nests in such large number. So number of nest-seeking queens in an area was used as indication of relative preference for nest in that area (Richards, 1973; Sevensson and Lundberg, 1977). Therefore, the number of queens performing nest-seeking activities in an area of a certain type was used as pointer of their relative preference for nesting site (Richards, 1973; Lundberg and Ranta, 1977). The patches characteristics were classified as:

1. Withered grass (WG).
2. New grass (NG).
3. Tussocks (Tus).
4. Stones and Moss (St & Mo)

   These four characteristics of patch type were randomly selected in all transact.

All transacts were inspected randomly between 07:00am and 04:00pm and no observation was made in rainy weather. Walking speed during the inspection was about 18-20m/ min. Time and length of transact was measured by Garmin e-trex 10 GPS device.

### 3.3.4. Observation of Queens

To compare the observations and behavior of queens in diverse landscape types and habitat types, all results were converted to total number of observation per 100 m during the inspection. For transformation of data into 100m, the formula was used as suggested previously (Svensson *et al.*, 2000). One factor ANOVA was used to analyses the data for landscape type and habitat type, respectively using Statistix 8.1 software. Means of number of queens observed in each landscape and habitat were compared with Least Significant Difference test.

$$\text{Total number of queens in 100m} = \frac{\text{No. of observed queens}}{\text{Total meters observed}} \times 100$$

## 3.4 BIOLOGICAL STUDIES OF *BOMBUS HAEMORRHOIDALIS* SMITH UNDER CONTROLLED LABORATORY CONDITIONS AND SEASONAL BIOLOGICAL VARIATION OF LOCAL BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH WORKERS, MALES AND QUEENS UNDER FIELD CONDITIONS

Present study provided information about the rearing techniques of indigenous *B. haemorrhoidalis* species under controlled laboratory conditions. It was the only species in reared in India there existed scarcity of information for biology of it under controlled laboratory conditions (Thakur *et al.,* 2005). It will be helpful in future possible mass rearing of this bumblebee as greenhouse crops pollinator alternative to imported bumblebees.

This study was performed in Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan. For biological studies under laboratory conditions, fecundated indigenous bumblebee *B. haemorrhoidalis* queens emerging in early spring after winter diapause were collected in March-April during years 2012 and 2013 from three locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan. Monthly surveys were conducted at all studied locations to find its seasonal biological variation for workers, males and queens under field conditions.

### 3.4.1 Biological Studies of *Bombus haemorrhoidalis* Smith under Controlled Laboratory Conditions

Biological and life history parameters of *B. haemorrhoidalis* in colony under controlled laboratory conditions were studied.

#### 3.4.1.1. Collections of queens

Entomological aerial net were used to collect early season bumblebee queens on weekly basis throughout this period and brought to laboratory. Queens were assumed to be mated and collected mostly from the wild flowers of *Silybum marianum, Adhatoda zeylanica, Robinia pseudoacacia, Malus domestica, Lantana camara, Saussurea* sp., *Impatiens* sp. and *Mentha longifolia* during early and mid hours of the day. A total of 81 bumblebee queens were captured with 42 during year 2012 and 39 in 2013. These were brought to laboratory using water cooler with ice cubes to maintain the temperature and relative humidity during travelling.

**3.4.1.2. Rearing under controlled laboratory conditions**

Queens were shifted singly into the plastic starter boxes ($14\times19\times7cm^3$). Cardboard lined with false bumblebee pupa made of wax was provided and wax nectar cups were filled with sugar solution to initiate the egg-laying. Several small holes were made in the upper lid of box for proper ventilation and avoidance of moisture accumulation. Queens were fed with 50% sugar solutions mixed with 0.1% sodium benzoate in small jars with spongy wick placed in a plastic Petri dish on daily basis. Sugar solution concentration was measured by hand held refractometer to obtain the desired accuracy. Pollens collected from a local beekeeper were kept in refrigerator to store and provided in plastic Petri plates on every alternate day.

Queen starter boxes were kept in laboratory under controlled conditions maintaining temperature at 26-28$^{o}$C and relative humidity around 60-70%. Rearing room was kept dark with low intensity red light (10 lux) to avoid light disturbance (Bucankova and Ptacek, 2012). To activate the nesting behavior and colony start, two con-specific workers of *Bombus terrestris* were given to these queens (Salden, 1912: Ptacek *et al.*, 2000). When first worker of *B. haemorrhoidalis* emerged, *B. terrestris* workers were taken out.

With emergence of the first batch of workers, each colony was shifted in BioBest standard colony box separately for further biological studies. Biological parameters including pre-oviposition period (days), first egg laying period, first worker emergence, number of workers in first batch, egg beads, larvae, pupae, total number of workers, males and daughter queens, days of first male and daughter queen emergence and total mortality were recorded on daily basis throughout the colony development till the colony end.

#### 3.4.1.3 Life history parameters of indigenous bumblebee, *B. haemorrhoidalis*

Means of different life parameters pre-oviposition period, egg hatching period, larval and pupal period, first worker, male and daughter queen emerging period, colony foundation and maturation periods, mother queen life span, mortality rate and number of workers, males and daughter queens produced were subjected to statistical methods using means ± SE and compared in ANOVA at 5% probability for comparison of percentage values using Statistix 8.1.

### 3.4.2 Seasonal Biological Variation of Indigenous Bumblebee, *Bombus haemorrhoidalis* Smith Workers, Males and Queens under Field Conditions

To study the seasonal biological variations of all casts of *B. haemorrhoidalis*, field surveys were performed at three sub-locations of each Rawalpindi/Islamabad, Rawalakot and Naran Kaghan areas on monthly basis from March to November. From Rawalpindi/Islamabad, Lake View Park, F9 Park and Bara Kahu, from Rawalakot, Poonch University area, Namnoota and Paniola and from Naran Kaghan Valley, Mahandari, Kaghan and Naran sub-locations were selected to study the spatial distribution. Three transact of 500m long were inspected from each sub-location and transect were not always straight but consisted of curved paths along with different types of habitats. Number of queens, workers and males were recorded month-wise for each location.

Altitude, longitude and elevation from the sea level were recorded by using the Garmin e-trex 10 GPS device. Data was analyzed by using the Statistix 8.1 and Microl Origin software and means were compared with LSD test at 0.5% probability.

## 3.5 POLLINATION EFFECIENCY OF INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH IN COMPARISON WITH EUROPEAN BUMBLEBEE, *BOMBUS TERRESTRIS* L. AND THEIR FORAGING BEHAVIOR ON TOMATO CROP UNDER GREENHOUSE CONTROLLED CONDITIONS

The efficacy and foraging behavior of indigenous bumblebee species with commercially used European bumblebee species was evaluated for qualitative and quantitative fruit characters of greenhouse tomato crop. The efficient contribution of indigenous bumblebee species as active alternate can help to provide pollination services for the local needs of the greenhouse and tunnel growers in Pakistan.

### 3.5.1 Greenhouse Conditions

This experiment was conducted in a greenhouse ( 2000 sq m ) part of hydroponics farm (19,800m$^2$), Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi at Kalyam, Rawalpindi, Pakistan. The greenhouse was protected with Venlo insect screen having glass-aluminum structure of Dutch technology and computerized drip irrigation system. Average daily temperature was maintained between 18-26°C and relative humidity between 65-80%. Density of tomato plants was 2.5 plants per square meter. Grandella cultivar of tomato was grown in pots of rock wool of 12 $cm^2$ and placed in cocoa peat slab of 100 cm long and 23 cm wide placed 40 cm above the ground level. The experiment was performed in mid-September when plant age was five months maintained according to standard commercial practices. Test plants were selected in ten rows of plants for self pollination and manual pollination. Plants were selected randomly within the rows. For bumblebee pollination by each species, sections were made with poly film to enclose the plants and place a bee box inside. After 48 hours, the marked and visited flower trusses were tagged and covered with muslin cloth for further observations. After that, poly film was removed for normal bee visitation. Relative humidity and temperature were maintained throughout the experiment for better results and comparison of different pollination methods.

### 3.5.2 Pollinations Treatments

Two bumblebee species namely *B. terrestris* and *B. haemorrhoidalis* were used for bee pollination to compare their efficacy and activity in greenhouse crops. Colonies of European bumblebee, *B. terrestris* was imported from BioBest, Belgium and indigenous bumblebee, *B. haemorrhoidalis* colonies were developed under controlled condition in the Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. Colonies with 60-70 foraging work force were transferred from laboratory to the experimental glasshouse for their foraging and pollination role. Four colonies of each species were used in this experiment. Twenty trusses pollinated by both bumblebee species having at least one to two marks of bumblebee visit were selected and covered with muslin cloth to avoid further disturbance.

For self and manual pollination treatments, twenty newly developing trusses of twenty different plants were selected randomly from the greenhouse and covered with muslin cloth bags of 34cm long and 24cm wide for the avoidance of bumble bee interaction with them. Manual pollination was performed on daily basis for one week with a mechanical vibrator (manual pollinator) on ten covered trusses within muslin cloth bags and ten trusses covered were left undisturbed from manual and bumblebee pollination to be considered as self pollination. Fruits were picked when they matured at fruit color of orange-red or darker (Dogterom *et al.*, 1998).

### 3.5.3 Bumblebee Visitation Time and Trafficking

For the estimation of the pollination visitation of bumblebees, time spent on a single flower was measured (in seconds) by using stop watch starting with the bee's landing on flower through its working till leaving. An observation on bee trafficking was made for 5 minutes on each colony in first, third and seventh week four times in a week. During this period, incoming and outgoing bumblebees were recorded at three different timings: 8-9 am, 11-12 pm and 5-6 pm of the day and data were recorded from September 15 - November 05, 2013.

### 3.5.4 Qualitative and Quantitative Parameter of Tomato Fruits

Fruit size and quality parameters were measured after collection of the trusses from selected plants as per treatments. Vernier caliper was used to measure the diameter and height of tomatoes in millimeter and electric balance was used to calculate the weight of tomatoes in grams. A roundness index was measured by dividing maximum to minimum height of tomatoes. Every fruit was prepared for seed counting by thawing and removing the pulp. The remaining pulp was sieved by sieve of mesh size 20 and seeds were counted (Dogterom *et al.*, 1998).

Number of fruits per truss, number of seeds, weight and roundness of fruits were compared statistically and means were compared using LSD test ($P < 0.05$) with SPSS software (Norus ICE, SPSS Inc., 2006). Three observations of bee trafficking were also made and their means were also compared statistically at 5% probability using LSD test. Visitation rate of bumblebee foragers were presented graphically using Microl Origin software.

*Chapter 4*

# RESULTS AND DISCUSSION

## 4.1 RELATIVE ABUNDANCE OF *BOMBUS HAEMORRHOIDALIS* IN COMPARISON WITH THER INSECT POLLINATORS AND ITS FORAGING FLORAL HOST RANGE

### 4.1.1 Pollinators Species Composition Belonging to Different Insect Orders during Two Years (2012 and 2013)

In both years (2012 and 2013), Hymenoptera order was recorded with maximum (53%) population of pollinators followed by Diptera (30%) and Lepidoptera (17%) populations of pollinators, respectively (Fig. 4.1.1). At all three study locations, *B. haemorrhoidalis* was found with the highest numbers (17056) in comparison to other common pollinators followed by *Syrphid* sp. (13332). *Lucilia sericata*, *Apis cerana*, *Apis dorsata* and *Vespa orientalis* were observed with 10034, 9110, 5946 and 5598 number of individuals. From all locations, *Amegilla cingulata* was found minimum (735) during both years (Fig. 4.1.2).

### 4.1.2 Relative Abundance of *Bombus haemorrhoidalis* in Comparison with Other Insect Pollinators

Relative abundance of *B. haemorrhoidalis* in comparison with other pollinators was recorded in three study locations, Rawalpindi/Islamabad, Rawalakot and Naran Kaghan and each location was further classified in to three sub-locations for comparison. Selection of these locations was based on evidence from literature and our experience about bumblebee especially *B. haemorrhoidalis*.

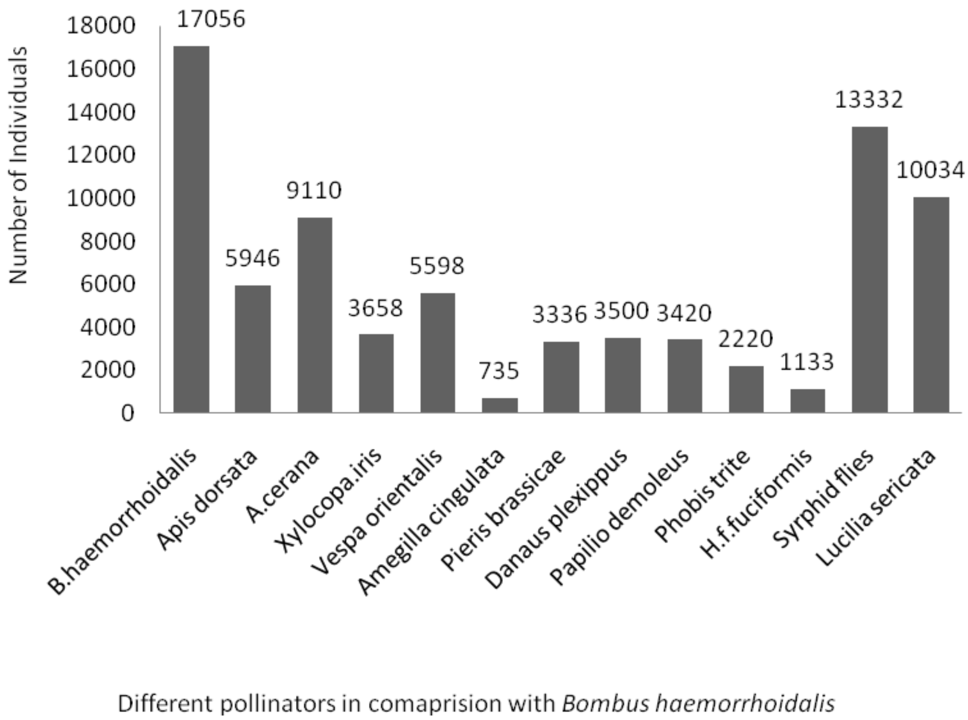


Figure 4.1.1 Overall comparative abundance of *Bombus haemorrhoidalis* Smith and other pollinators belonging to different insect orders

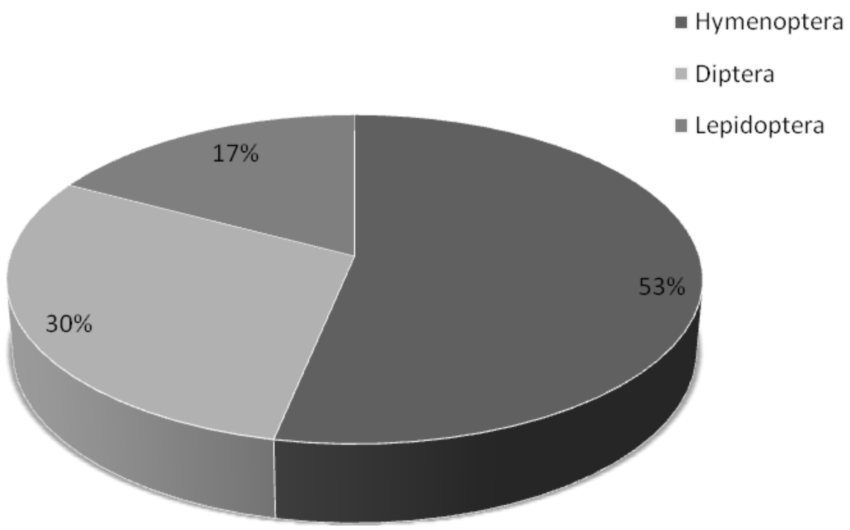


Fig 4.1.2 Species composition of pollinators belonging to different insect orders from all study locations of three main regions

There was significant difference in relative abundance among all species of pollinators at all study locations and sub-locations.

#### 4.1.2.1 Relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other insect pollinators at sub-locations of Rawalpindi/Islamabad

*B. haemorrhoidalis* was the most abundant pollinator (20.29, 18.65 and 19.92%) followed by *Syrphus* sp. (16.71, 17.47 and 13.58%) at Lake View Park, F9 Park and Bara-Kahu, respectively. *A. cingulata* was found (0.98, 0.93 and 0.72%) minimum at Lake View Park, F9 Park and Bara-Kahu, respectively. At Lake View Park, relative abundance of four pollinators, *Papilio demoleus*, *A. cerana*, *L. sericata* and *A. dorsata* ranged between 6.33 to 13.71% while other seven pollinators not exceeded 6.11%. At F9 Park, relative abundance of seven pollinators, *P. demoleus*, *Pieris brassicae*, *Danaus plexippus*, *V. orientalis*, *Xylocopa* sp., *A. cerana* and *L. sericata* ranged between 5.28 to12.29% but other three did not exceed 4.31%. At Bara-Kahu, five pollinators including *A. cerana*, *A. dorsata*, *V. orientalis*, *Syrphus* sp and *L. sericata* ranged between 7.85-13.89% and other five were less than 6.63% (Table 4.1.1).

#### 4.1.2.2 Relative abundance of *Bombus haemorrhoidalis* smith in comparison with other insect pollinators at sub-locations of Rawalakot

At Poonch University area, relative abundance of four pollinators including *L. sericata*, *Syrphus* sp., *A. cerana*, and *B. haemorrhoidalis* ranged from 13.35-18.57%. *Phobis trite*, *D. plexippusi*, *P. brassicae* and *V. orientalis* were from 5.08-9.02%. Remaining five pollinators, *A. dorsata*, *Xylocopa* sp, *A. cingulata* and *Hemaris fuciformis fuciformis* were less than 2.89% reflecting their paucity in this sub-locality. *B. haemorrhoidalis* was most abundant with 18.57% relative abundance at this sub-locality. At Paniola, *Syrphus* sp. was higher in abundant with 21.94% followed by *B. haemorrhoidalis* (17.76%). Relative abundance of three pollinators i.e., *V. orientalis*, *L. sericata* and *A. cerana* ranged from 10.82-17.59%. Other eight pollinators did not exceed than 3.68% and *A. cingulata* was least 0.45% abundant in this sub-locality. *B. haemorrhoidalis* was the most abundant pollinator (19.12%) followed by *A. cerana* (19.05%) at Namnoota. Relative abundance of three pollinators i.e., *V. orientalis*, *L. sericata* and *Syrphus* sp. ranged from 9.99-16.25%. Other eight pollinators at this sub-location not exceeded more than 4.64% (Table 4.1.2).

#### 4.1.2.3 Relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other insect pollinators at sub-locations of Naran Kaghan Valley

At Mahandari, *B. haemorrhoidalis* was showed the highest (26.87%) relative abundance followed by *Syrphus* sp. (16.52%). Relative abundance of other pollinators i.e., *X.* sp., *A. cerana*, *A. dorsata* and *L. sericata* ranged from 5.14-11.24%. Other seven pollinators at sub-location not exceeded more than 4.80% and minimum abundant pollinator was *A. cingulata* (1.31%). *B. haemorrhoidalis* was again the most abundant (28.22%) pollinator at Kaghan followed by *Syrphus* sp. (15.47%). Relative abundance of other pollinators at Kaghan were less than 11.37% and *H. fuciformis* was the least abundant. At Naran, relative abundance of four pollinators, *Xylocopa* sp., *A. cerana*, *A. cingulata* and *A. dorsata* ranged between 5.15-11.20%. Abundance of other seven pollinators was less than 4.51%.

*B. haemorrhoidalis* (28.85%) remained the highest abundant pollinator followed by *Syrphus* sp. (15.07%) (Table 4.1.3).

#### 4.1.2.4 Comparison of relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other insect pollinators in Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley

*B. haemorrhoidalis* was relatively abundant (19.59, 18.48 and 27.96%) than *Syrphus* sp. (15.94, 18.95 and 15.70% at Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley, respectively. *A. cingulata*, on other hand, was the least abundant pollinator (0.88, 0.56 and 1.42% at Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley, respectively Hymenoptera insect order was found with maximum abundance of pollinators at all locations (Table 4.1.4).

### 4.1.3 Comparison of Diversity Indices at Rawalpindi/Islamabad, Rawalakot and Naran Kaghan of different insect pollinators

Shannon diversity index, Simpson diversity index and Evenness index for common insect pollinators were recorded at three sub-locations of each Rawalpindi/ Islamabad, Rawalakot and Naran Kaghan Valley during years 2012 and 2013. Overall comparison of diversity indices at all locations and sub-locations during both years was also recorded.

#### 4.1.3.1 Comparison of diversity indices of different sub-locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2012

During year 2012, maximum Shannon and Evenness indices (1.18 and 1.06) were observed for Naran valley,and the highest Simpson index

(0.882) at Bara-Kahu area of Rawalpindi/Islamabad. At Paniola, minimum Shannon and Evenness indices (0.904 and 1.06) were found with least Simpson index at Mahandari area (0.713). From all other localities, insignificant variations were recorded (Table 4.1.5).

#### 4.1.3.2 Comparison of diversity indices of different locations of Rawalpindi/ Islamabad, Rawalakot and Naran Kaghan Valley during the year 2013

For the year 2013, both Naran and F9 Park areas were found with higher Shannon index (1.00) and maximum Evenness (0.902) at Naran. From Poonch University area, Rawalakot minimum Shannon index (0.911) and Evenness (0.809) were found. In case of Simpson index, F9 Park was with (0.884) highest value and all other localities were found with insignificant variations (Table 4.1.6).

#### 4.1.3.3 Overall comparison of diversity indices of different locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the years 2012 and 2013

Comparison of indices for both years (2012 and 2013) for both Naran and F9 Park were the highest for Shannon index (1.03 and 1.00). Maximum Simpson and Evenness indices were again found at Naran. Paniola area of Rawalakot was recorded with the least (0.910, .0851 and .0817) Shannon, Simpson and Evenness indices for both years (2012 and 2013). All other localities were found with insignificant variations for all three indices (Table 4.1.7).

Table: 4.1.1 Relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other pollinators in different locations of Rawalpindi/Islamabad

| **Pollinator Species (insect order)** | **Lake View** | | **F9 Park** | | **Bara-Kahu** | |
|---|---|---|---|---|---|---|
| | **Number** | **Relative abundance** | **Number** | **Relative abundance** | **Number** | **Relative abundance** |
| *Bombus haemorrhoidalis* (Hymenoptera) | 2013 | 20.29 | 2036 | 18.65 | 2067 | 19.92 |
| *Apis dorsata* (Hymenoptera) | 1360 | 13.71 | 471 | 4.31 | 1309 | 12.62 |
| *Apis cerana* (Hymenoptera) | 974 | 9.81 | 1197 | 10.96 | 815 | 7.85 |
| *Xylocopa* spp (Hymenoptera) | 607 | 6.11 | 885 | 8.10 | 688 | 6.63 |
| *Amegilla cingulata* (Hymenoptera) | 98 | 0.98 | 102 | 0.93 | 75 | 0.72 |
| *Vespa orientalis* (Hymenoptera) | 412 | 4.15 | 729 | 6.67 | 830 | 8.00 |
| *Syrphus* spp (Diptera) | 1658 | 16.71 | 1908 | 17.47 | 1409 | 13.58 |
| *Lucilia sericata* (Diptera) | 1023 | 10.32 | 1342 | 12.29 | 1441 | 13.89 |
| *Danaus plexippus* (Lepidoptera) | 355 | 3.57 | 647 | 5.92 | 514 | 4.95 |
| *Pieris brassicae* (Lepidoptera) | 360 | 3.62 | 557 | 5.28 | 436 | 4.20 |
| *Papilio demoleus* (Lepidoptera) | 728 | 6.33 | 596 | 5.45 | 375 | 3.61 |
| *Phobis trite* (Lepidoptera) | 203 | 2.043 | 244 | 2.23 | 284 | 2.73 |
| *Hemaris fuciformis fuciformis* (Lepidoptera) | 128 | 1.29 | 182 | 1.66 | 129 | 1.24 |
| **Total** | **9919** | | **10916** | | **10372** | |

Table: 4.1.2 Relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other insect pollinators at sub-locations of Rawalakot

| Pollinator Species (insect order) | Poonch University Area | | Paniola | | Namnoota | |
|---|---|---|---|---|---|---|
| | **Number** | **Relative abundance** | **Number** | **Relative abundance** | **Number** | **Relative abundance** |
| *Bombus haemorrhoidalis* (Hymenoptera) | 1688 | 18.57 | 1514 | 17.76 | 1570 | 19.12 |
| *Apis dorsata* (Hymenoptera) | 112 | 1.23 | 118 | 1.38 | 96 | 1.16 |
| *Apis cerana* (Hymenoptera) | 1342 | 14.76 | 1499 | 17.59 | 1565 | 19.05 |
| *Xylocopa* spp (Hymenoptera) | 161 | 1.77 | 82 | 0.962 | 65 | 0.791 |
| *Amegilla cingulata* (Hymenoptera) | 42 | 0.462 | 39 | 0.457 | 65 | 0.791 |
| *Vespa orientalis* (Hymenoptera) | 832 | 9.15 | 922 | 10.82 | 821 | 9.99 |
| *Syrphus* spp (Diptera) | 1688 | 18.57 | 1817 | 21.94 | 1335 | 16.25 |
| *Lucilia sericata* (Diptera) | 1213 | 13.35 | 1257 | 14.75 | 1328 | 16.17 |
| *Danaus plexippus* (Lepidoptera) | 479 | 5.27 | 305 | 3.57 | 381 | 4.64 |
| *Pieris brassicae* (Lepidoptera) | 664 | 7.30 | 314 | 3.68 | 308 | 3.75 |
| *Papilio demoleus* (Lepidoptera) | 263 | 2.89 | 205 | 2.40 | 222 | 2.70 |
| *Phobis trite* (Lepidoptera) | 462 | 5.08 | 292 | 3.42 | 328 | 3.99 |
| *Hemaris fuciformis fuciformis* (Lepidoptera) | 140 | 1.54 | 103 | 1.20 | 127 | 1.54 |
| **Total** | **9086** | | **8520** | | **8211** | |

Table: 4.1.3 Relative abundance of *Bombus haemorrhoidalis* Smith in comparison with other insect pollinators at sub-locations of Naran Kaghan Valley

| Pollinating Species (Insect Order) | Mahandari | | Kaghan | | Naran | |
|---|---|---|---|---|---|---|
| | Number | Relative abundance | Number | Relative abundance | Number | Relative abundance |
| *Bombus haemorrhoidalis* (Hymenoptera) | 2026 | 26.87 | 2066 | 28.22 | 2076 | 28.85 |
| *Apis dorsata* (Hymenoptera) | 841 | 11.15 | 833 | 11.37 | 806 | 11.20 |
| *Apis cerana* (Hymenoptera) | 632 | 8.38 | 548 | 7.48 | 538 | 7.47 |
| *Xylocopa* spp (Hymenoptera) | 408 | 5.41 | 391 | 5.34 | 371 | 5.15 |
| *Amegilla cingulata* (Hymenoptera) | 99 | 1.31 | 105 | 1.43 | 110 | 1.52 |
| *Vespa orientalis* (Hymenoptera) | 337 | 4.47 | 356 | 4.86 | 359 | 4.91 |
| *Syrphus* spp (Diptera) | 1246 | 16.52 | 1133 | 15.47 | 1085 | 15.07 |
| *Lucilia sericata* (Diptera) | 848 | 11.24 | 807 | 11.02 | 775 | 10.77 |
| *Danaus plexippus* (Lepidoptera) | 285 | 3.78 | 273 | 3.72 | 261 | 3.62 |
| *Pieris brassicae* (Lepidoptera) | 240 | 3.18 | 215 | 2.39 | 222 | 3.08 |
| *Papilio demoleus* (Lepidoptera) | 362 | 4.80 | 344 | 4.69 | 325 | 4.51 |
| *Phobis trite* (Lepidoptera) | 109 | 1.44 | 148 | 2.02 | 150 | 2.08 |
| *Hemaris fuciformis fuciformis* (Lepidoptera) | 105 | 1.39 | 102 | 1.39 | 117 | 1.62 |
| **Total** | **7538** | | **7321** | | **7195** | |

Table: 4.1.4 Comparative relative abundance of *B. haemorrhoidalis* Smith in comparison with other Insect pollinators in Rawalpindi/Islamabad, Rawalakot and Naran Kaghan

| **Pollinator Species (Insect Order)** | **Rawalpindi/ Islamabad** | | **Rawalakot** | | **Naran Kaghan Valley** | |
|---|---|---|---|---|---|---|
| | **Number** | **Relative abundance** | **Number** | **Relative abundance** | **Number** | **Relative abundance** |
| *Bombus haemorrhoidalis* (Hymenoptera) | 6116 | 19.59 | 4772 | 18.48 | 6168 | 27.96 |
| *Apis dorsata* (Hymenoptera) | 3140 | 10.06 | 326 | 1.26 | 2480 | 11.24 |
| *Apis cerana* (Hymenoptera) | 2986 | 9.56 | 4406 | 17.06 | 1718 | 7.78 |
| *Xylocopa* spp (Hymenoptera) | 2180 | 6.98 | 308 | 1.19 | 1170 | 5.30 |
| *Amegilla cingulata* (Hymenoptera) | 275 | 0.88 | 146 | 0.564 | 314 | 1.42 |
| *Vespa orientalis* (Hymenoptera) | 971 | 6.31 | 2575 | 9.97 | 1052 | 4.77 |
| *Syrphus* spp (Diptera) | 4975 | 15.94 | 4893 | 18.95 | 3464 | 15.70 |
| *Lucilia sericata* (Diptera) | 3806 | 12.19 | 3798 | 14.71 | 2430 | 11.01 |
| *Danaus plexippus* (Lepidoptera) | 1516 | 4.85 | 1165 | 4.51 | 819 | 3.71 |
| *Pieris brassicae* (Lepidoptera) | 1373 | 4.39 | 1286 | 4.98 | 677 | 3.06 |
| *Papilio demoleus* (Lepidoptera) | 1699 | 5.44 | 690 | 2.67 | 1031 | 4.67 |
| *Phobis trite* (Lepidoptera) | 731 | 2.34 | 1082 | 4.19 | 407 | 1.84 |
| *Hemaris fuciformis fuciformis* (Lepidoptera) | 439 | 1.40 | 370 | 1.43 | 324 | 1.46 |
| **Total** | **31207** | | **25817** | | **22054** | |

#### 4.1.3.4 Comparison of diversity indices of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2012

From Naran Kaghan Valley in 2012, maximum Shannon and Evenness indices (1.311 and 1.117) were observed followed by Rawalpindi/Islamabad with Shannon index (0.911). In case of Simpson index, Rawalpindi/Islamabad area was found with maximum (0.885) followed by Rawalakot and Naran, respectively with (0.877 and 0.876). From Rawalakot, the least Shannon and Evenness (0.911 and 0.818) indices were observed, respectively (Table 4.1.8).

#### 4.1.3.5 Comparison of diversity indices of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2013

In 2013, Naran Kaghan Valley was again found with highest Shannon and Evenness indices (0.988 and 0.890) followed by Rawalpindi/Islamabad with (0.966 and 0.840). At Rawalakot, minimum Shannon and Evenness indices (0.0.938 and 0.840) were recorded. In case of Simpson index, maximum index was found at Rawalpindi/Islamabad (0.910) followed by both Rawalakot and Naran Kaghan Valley (0.862 and 0.855) (Table 4.1.9).

#### 4.1.3.6 Overall comparison of diversity indices of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the years 2012 and 2013

In overall comparison for both years, Naran Kaghan Valley and Rawalpindi/ Islamabad were found with higher Shannon indices (1.02 and 1.00),

Table 4.1.5 Comparison of diversity indices of different locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2012

| **Location** | **Sub-location** | **Shannon Index** | **Simpson Index** | **Evenness** |
|---|---|---|---|---|
| Rawalpindi/Islamabad | Lake View Park | 1.07 | 0.861 | 0.967 |
| | F9 Park | 1.11 | 0.831 | 1.00 |
| | Bara-Kahu | 0.998 | 0.882 | 0.896 |
| Rawalakot | Poonch University | 1.00 | 0.863 | 0.905 |
| | Paniola | 0.904 | 0.850 | 0.811 |
| | Namnoota | 0.923 | 0.860 | 0.827 |
| Naran Kaghan Valley | Mahandari | 1.06 | 0.713 | 0.958 |
| | Kaghan | 0.954 | 0.855 | 0.856 |
| | Naran | 1.182 | 0.864 | 1.06 |

Table 4.1.6 Comparison of diversity indices of different locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2013

| **Location** | **Sub-location** | **Shannon Index** | **Simpson Index** | **Evenness** |
|---|---|---|---|---|
| Rawalpindi/Islamabad | Lake View Park | 0.985 | 0.876 | 0.884 |
| | F9 Park | 1.00 | 0.884 | 0.896 |
| | Bara-Kahu | 0.957 | 0.883 | 0.890 |
| Rawalakot | PoonchUniversity | 0.901 | 0.879 | 0.809 |
| | Paniola | 0.911 | 0.851 | 0.818 |
| | Namnoota | 0.928 | 0.858 | 0.833 |
| Naran Kaghan Valley | Mahandari | 0.957 | 0.857 | 0.859 |
| | Kaghan | 0.953 | 0.854 | 0.856 |
| | Naran | 1.00 | 0.853 | 0.902 |

Table 4.1.7 Overall Comparison of diversity indices of different locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2012-2013

| Location | Sub-location | Shannon Index | Simpson Index | Evenness |
|---|---|---|---|---|
| Rawalpindi/Islamabad | Lake View Park | 0.987 | 0.877 | 0.886 |
| | F9 Park | 1.00 | 0.884 | 0.903 |
| | Bara-Kahu | 0.999 | 0.883 | 0.897 |
| Rawalakot | Poonch University | 0.960 | 0.870 | 0.861 |
| | Paniola | 0.910 | 0.851 | 0.817 |
| | Namnoota | 0.928 | 0.858 | 0.833 |
| Naran Kaghan Valley | Mahandari | 0.956 | 0.858 | 0.858 |
| | Kaghan | 0.956 | 0.855 | 0.858 |
| | Naran | 1.03 | 0.893 | 0.959 |

Table 4.1.8 Comparison of diversity indices of Rawalpindi/Islamabad,

| Locations | Shannon Index | Simpson Index | Evenness |
|---|---|---|---|
| Rawalpindi Islamabad | 1.0059 | 0.8856 | 0.9030 |
| Rawalakot | 0.9118 | 0.8772 | 0.8186 |
| Naran Kaghan Valley | 1.3117 | 0.8765 | 1.1770 |

Rawalakot and Naran Kaghan Valley during the year 2012

Table 4.1.9 Comparison of diversity indices of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2013

| Locations | Shannon Index | Simpson Index | Evenness |
|---|---|---|---|
| Rawalpindi Islamabad | 0.9661 | 0.9100 | 0.840 |
| Rawalakot | 0.9389 | 0.8622 | 0.842 |
| Naran Kaghan Valley | 0.9880 | 0.8553 | 0.890 |

Table 4.1.10 Overall Comparison of diversity indices of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley during the year 2012-2013

| Locations | Shannon Index | Simpson Index | Evenness |
|---|---|---|---|
| Rawalpindi Islamabad | 1.00 | 0.885 | 0.903 |
| Rawalakot | 0.937 | 0.861 | 0.841 |
| Naran Kaghan Valley | 1.02 | 0.855 | 0.959 |

respectively. At Naran Kaghan Valley, maximum Evenness index (0.959) was observed. From Rawalpindi/ Islamabad, maximum Simpson index ( 0.885 ) was observed followed by Rawalakot and Naran Kaghan Valley (0.861 and 0.855), respectively (Table 4.1.10).

### 4.1.4 Floral Host Range Of *Bombus Haemorrhoidalis* Smith During Years 2012 and 2013

During both years (2012 and 2013), floral host range of *Bombus haemo-rrhoidalis* was recorded at species level in all three study locations including Rawalpindi/Islamabad, Rawalakot and Naran Kaghan valley. Different floral host plants species and plant families were observed at different study locations. From Rawalpindi/Islamabad, maximum of 48 floral host plants species *B. haemorrhoidalis* belonging 24 plant families were found. Asteraceae family was recorded with maximum host plants from all three study areas followed by Fabaceae and Lamiaceae as major host plant families of *B. haemorrhoidalis.*

#### 4.1.4.1 Floral host range of *Bombus haemorrhoidalis* smith from Rawalpindi/Islamabad during the years 2012 and 2013

Asteraceae family was found with maximum twelve plants as major host plant family followed by Fabaceae with six plant species. Lamiaceae, Malvaceae and Solanaceae were observed with three plant species each. Aceanthaceae, Cucurbitaceae, and Convolvulaceae were recorded with two host plants. Apocynaceae, Asclepiadaceae, Cisteraceae, Iridaceae, Liliaceae, Lytheraceae, Leguminosae, Myrtaceae, Plantaginaceae, Polygonaceae, Rosaceae, Rublaceae, Verbenaceae and Violaceae were recorded with one host plant for each (Table 4.1.11).

#### 4.1.4.2 Floral host range of *Bombus haemorrhoidalis* smith from Rawalakot during the years 2012 and 2013

From Rawalakot, total 43 plant species within twenty plant families were observed as floral host plants of *B. haemorrhoidalis*. Maximum 10 plant species of Asteraceae plant family were found followed by 5 plants of Fabaceae family as major floral host plants of *B. haemorrhoidalis*. Cucurbitaceae, Lamiaceae and Malvaceae were recorded with three plant species each. Acanthaceae, Convovulaceae, and Solanaceae families were recorded with two plant species each. From plant families including Balsaminaceae, Bignoniaceae, Cisteraceae, Ebenaceae, Iridaceae, Myrtaceae, Plantaginaceae, Rosaceae, Ranunculaceae, Verbenaceae and Violaceae, one floral host plant was observed for each family (Table 4.1.12).

#### 4.1.4.3 Floral host range of *Bombus haemorrhoidalis* Smith from Naran Kaghan valley during both years (2012 and 2013)

At Naran Kaghan, total 42 plant species belonging to 18 plant families were found as floral host plants of *B. haemorrhoidalis*. Asteraceae was found as major host plant family with maximum 10 plants followed by Lamiaceae and Fabaceae with 7 and 5 plants species, respectively. Rosaceae, Solanaceae and Amaranthaceae were observed with 2 plant species each. Apocynaceae, Apiaceae, Bignoniaceae, Cisteraceae, Cucurbitaceae, Cannaceae, Caprifoliaceae, Iridaceae, Oxalidaceae, Primulaceae and Ranunculaceae were found with one plant species each (Table 4.1.13).

### 4.1.5 Monthly Abundance of *Bombus Haemorrhoidalis* in Rawalpindi/ Islamabad, Rawalakot and Naran Kaghan Valley

From Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley, monthly population trend of *B. haemorrhoidalis* was recorded throughout the life cycle. Abundance of different castes (worker, male and queens) of *B. haemorrhoidalis* were also recorded. From all localities, workers were found (11961) maximum in abundance followed by males (4731) and young queens (364), respectively (Fig. 4.1.3).

#### 4.1.5.1 Monthly abundance of *Bombus haemorrhoidalis* Smith at Rawalpindi/ Islamabad

At Lake View Park and its surroundings area in March and April, minimum population of *B. haemorrhoidalis* was found but its population was highest in September followed by August. There was no population in December, January and February. At F9 Park and Bara-Kahu areas, same pattern of population trend of *B. haemorrhoidalis* was observed (Fig. 4.1.4, 4.1.5 and 4.1.6).

#### 4.1.5.2 Monthly abundance of *Bombus haemorrhoidalis* smith at Rawalakot

At Poonch University and its surrounding areas, maximum number of *B. haemorrhoidalis* was recorded in September followed by August. March, April and November months were observed with very low population and no population in

Table 4.1.11 Floral host range of *Bombus haemorrhoidalis* Smith form Rawalpindi/Islamabad

| **Host Plants** | **Scientific name** | **Family** |
|---|---|---|
| Zinnia | *Zinnia sp* | Asteraceae |
| Wild daisy | *Bellis perennis* | Asteraceae |
| Lesser knapweed | *Centaurea nigra* | Asteraceae |
| Centaurea blue | *Centaurea cyanus* | Asteraceae |
| Sunflower | *Helianthus annuus* | Asteraceae |
| Milk thistle | *Silybum marianum* | Asteraceae |
| Gusato | *Xanthium strumarium* | Asteraceae |
| Gainda | *Tagetes erecta* | Asteraceae |
| Saw-wort | *Saussurea spp* | Asteraceae |
| Daisy | *Chrysanthemum leucanthemum* | Asteraceae |
| Globe theistle | *Echinops echinatus* | Asteraceae |
| Gule- asharfi | *Calenda officinalis* | Asteraceae |
| Yellow Oleande | *Thevetia neriifolia* | Apocynaceae |
| Sodom apple | *Calotropis procera* | Asclepiadaceae |
| Baikhar | *Adhatoda zeylanica* | Acanthaceae |
| Dicliptera | *Dicliptera roxburghiana* | Acanthaceae |
| Tori | *Luffa cylindrical* | Cucurbitaceae |
| Cucumber | *Cucumis sativus* | Cucurbitaceae |
| Field bindweed | *Convolvulus arvensis* | Convolvulaceae |
| Pink morning glory | *Ipomoea carnea* | Convolvulaceae |
| Rock rose | *Cistaceae sp* | Cisteraceae |
| Brachychiton | *Brachychiton diversifolius* | Malvaceae |
| Kachnar | *Bauhinia variegate* | Fabaceae |
| Amaltas | *Cassia fistula* | Fabaceae |
| Lupin flower | *Lupinus sp* | Fabaceae |
| Black locust | *Robinia pseudoacacia* | Fabaceae |
| Clover | *Trifolium repens* | Fabaceae |
| Gladiolus | *Gladiolus sp.* | Iridaceae |
| Sage | *Salvia officinalis* | Lamiaceae |
| Dead-nettle white | *Lamium sp* | Lamiaceae |
| Pudina | *Mentha piperita* | Lamiaceae |
| Lily flower | *Lilium sp* | Liliaceae |
| Lagerstroemia | *Lagerstroemia indica* | Lythraceae |
| Amaltas | *Casia fistula* | Leguminosae |
| Hollyhock | *Alcea rosea* | Malvaceae |
| Rose of Sharon | *Hibiscus syriacus* | Malvaceae |
| Guava | *Psidium guajava* | Myrtaceae |
| Fox glove pink | *Digitalis sp* | Plantaginaceae |
| Polygonum sp | *Polygonum affine* | Polygonaceae |
| Apple | *Malus domestica* | Rosaceae |
| Butter cup | *Ranunculus muricatus* | Ranunculaceae |
| Galium | *Galium apa* | Rublaceae |
| Tomato | *Solanum lycopersicum* | Solanaceae |
| Brinjal | *Solanum melongena* | Solanaceae |
| Brinjal | *Solanum nigrum* | Solanaceae |
| White clover | *Trifolium repens* | Trifolium repens |
| Lantana | *Lantana camara* | Verbenaceae |
| Banafsha | *Vioila pilosa* | Violaceae |

Table 4.1.12 Floral host Plants of *Bombus haemorrhoidalis* Smith form Rawalakot

| **Host Plants** | **Scientific name** | **Family** |
|---|---|---|
| Globe thistle | *Echinops echinatus* | Asteraceae |
| Mary thistle | *Silybum marianum* | Asteraceae |
| Wild daisy | *Bellis perennis* | Asteraceae |
| Lesser knapweed | *Centaurea nigra* | Asteraceae |
| Centaurea blue | *Centaurea cyanus* | Asteraceae |
| Sunflower | *Helianthus annuus* | Asteraceae |
| Blue Thistle | *Carduus sp* | Asteraceae |
| Saw-wort | *Saussurea spp* | Asteraceae |
| Zinnia | *Zinnia sp* | Asteraceae |
| Daisy | *Chrysanthemum leucanthemum* | Asteraceae |
| Dicliptera sp | Dicliptera roxburghiana | Acanthaceae |
| Baikhar | *Adhatoda zeylanica* | Acanthaceae |
| Sichuan Gold | *Impatiens sp* | Balsaminaceae |
| Yellow bells | *Tecoma stans* | Bignoniaceae |
| Cucumber | *Cucumis sativus* | Cucurbitaceae |
| Musk melon | *Cucurbita pepo* | Cucurbitaceae |
| Tori | *Luffa cylindrical* | Cucurbitaceae |
| Field bindweed | *Convolvulus arvensis* | Convolvulaceae |
| Pink morning glory | *Ipomoea carnea* | Convolvulaceae |
| Rock rose | *Cistaceae sp* | Cisteraceae |
| Brachychiton | *Brachychiton diversifolius* | Asteraceae |
| Persimmon | *Diospyros kaki* | Ebenaceae |
| Kachnar | *Bauhinia variegate* | Fabaceae |
| Amaltas | *Cassia fistula* | Fabaceae |
| Lupin flower | *Lupinus sp* | Fabaceae |
| Black locust | *Robinia pseudoacacia* | Fabaceae |
| Clover | *Trifolium repens* | Fabaceae |
| Gladiolus | *Gladiolus sp.* | Iridaceae |
| Sage | *Salvia officinalis* | Lamiaceae |
| Dead-nettle white | *Lamium sp* | Lamiaceae |
| Pudina | *Mentha piperita* | Lamiaceae |
| Okra | *Abelmoschus esculentus* | Malvaceae |
| Hollyhock | *Alcea rosea* | Malvaceae |
| Rose of Sharon | *Hibiscus syriacus* | Malvaceae |
| Guava | *Psidium guajava* | Myrtaceae |
| Sleeping beauty | *Oxalis corniculata* | Oxalidaceae |
| Fox glove pink | *Digitalis sp* | Plantaginaceae |
| Apple | *Malus domestica* | Rosaceae |
| Butter cup | *Ranunculus muricatus* | Ranunculaceae |
| Tomato | *Solanum lycopersicum* | Solanaceae |
| Brinjal | *Solanum melongena* | Solanaceae |
| Lantana | *Lantana camara* | Verbenaceae |
| Banafsha | *Vioila pilosa* | Violaceae |

Table 4.1.13 Floral host plants of *Bombus haemorrhoidalis* Smith form Naran Kaghan Valley

| **Host Plants** | **Scientific Name** | **Family** |
|---|---|---|
| Milk thistle | *Silybum marianum* | Asteraceae |
| Gold thistle | *Echinops echinatus* | Asteraceae |
| Wild daisy | *Bellis perennis* | Asteraceae |
| Dehlia | *Dahlia variabilis* | Asteraceae |
| Saw-wort | *Saussurea spp* | Asteraceae |
| Cosmos | *Cosmos bipinnatus* | Asteraceae |
| Tarragon | *Artemisia dracunculus* | Asteraceae |
| Zinnia | *Zinnia sp* | Asteraceae |
| Cornflower | *Centaurea cyanus* | Asteraceae |
| Gule- asharfi | *Calenda officinalis* | Asteraceae |
| Yellow Oleander, | *Thevetia Peruvians* | Apocynaceae |
| Chervil | *Anthriscus cerefolium* | Apiaceae |
| Lesua | *Digera muricata* | Amaranthaceae |
| Choleri | *Amaranthus ovalifolius* | Amaranthaceae |
| Chinese Trumpet Vine | *Campsis grandiflora* | Bignoniaceae |
| Rock rose | *Cistaceae sp* | Cisteraceae |
| Tori | *Luffa cylindrical* | Cucurbitaceae |
| Siri | *Cana indica* | Cannaceae |
| Honeysuckle | *Lonicera periclymenum* | Caprifoliaceae |
| Persimmon | *Diospyros kaki* | Ebenaceae |
| Lupin flower | *Lupinus sp* | Fabaceae |
| Black locust | *Robinia pseudoacacia* | Fabaceae |
| Lotus | *Lotus corniculatus* | Fabaceae |
| Clover | *Trifolium medium* | Fabaceae |
| Clover | *Trifolium montanum* | Fabaceae |
| Iris | *Iris aitchisonii* | Iridaceae |
| Sage | *Salvia officinalis* | Lamiaceae |
| Dead-nettle white | *Lamium sp* | Lamiaceae |
| Stachys | *Stachys spp* | Lamiaceae |
| Wild mint | *Mentha longifolia* | Lamiaceae |
| Basil | *Ocimum basilicum* | Lamiaceae |
| Blue bugle | *Ajuga reptans* | Lamiaceae |
| Self-heal | *Prunella vulgaris* | Lamiaceae |
| Sleeping beauty | *Oxalis corniculata* | Oxalidaceae |
| Larg leaf primose | *Primula macrophylla* | Primulaceae |
| Red rose | *Rosa indica* | Rosaceae |
| Himalayan Blackberry | *Rubus armeniacus* | Rosaceae |
| Apple | *Malus domestica* | Rosaceae |
| Rose | *Rosa webbiana* | Rosaceae |
| Musk Larkspur | *Delphinium brunonianum* | Ranunculaceae |
| Brinjal | *Solanum nigrum* | Solanaceae |
| Snakbarry | *Solanum dulcamara* | Solanaceae |

December, January and February. At Namnoota, Paniola and their surrounding areas, population trend of *B. haemorrhoidalis* was similar to that of Poonch University area (Fig. 4.1.7, 4.1.8 and 4.1.9).

#### 4.1.5.3 Monthly abundance of *Bombus haemorrhoidalis* smith at Naran Kaghan Valley

At Mahanhdari and its surrounding areas, maximum population of *B. haemorrhoidalis* was recorded in August followed by July. In March and October, there existed minimum population whereas not found during November, December, January and February. At Kaghan and Naran Valley, the same population trend was observed (Fig. 4.1.10, 4.1.11 and 4.1.12).

Different insect pollinator species play vital role in pollination, survival of plant species in nature and enhance biodiversity to protect life. Some species have been used for crops pollination and increase in crop yield to meet food requirements of humans and economical system. Bumblebees are used under enclosed farming of crops especially vegetables and fruits. Their natural population depends on plant species composition, flowering patterns and abundance. During present study, relative abundance of thirteen insect pollinators of three different insect orders comprised six from Hymenoptera, two from Diptera and five from Lepidoptera when compared with *B. haemorrhoidalis* Smith from Rawalpindi/ Islamabad, Rawalakot and Naran Kaghan valley. There existed significant differences among relative abundance of all insect pollinators at different locations under observation. Insect pollinators of order Hymenoptera were more in


Figure 4.1.3 Overall abundance of all castes of *Bombus haemorrhoidalis* Smith

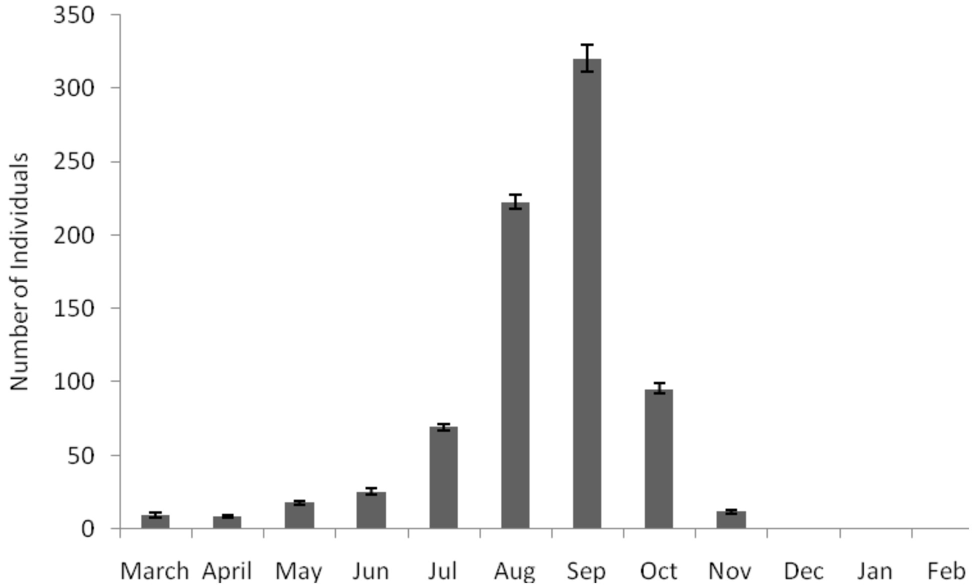


Figure 4.1.4 Monthly abundance of *Bombus haemorrhoidalis* Smith in Lake View Park and its surroundings for the years 2012-2013

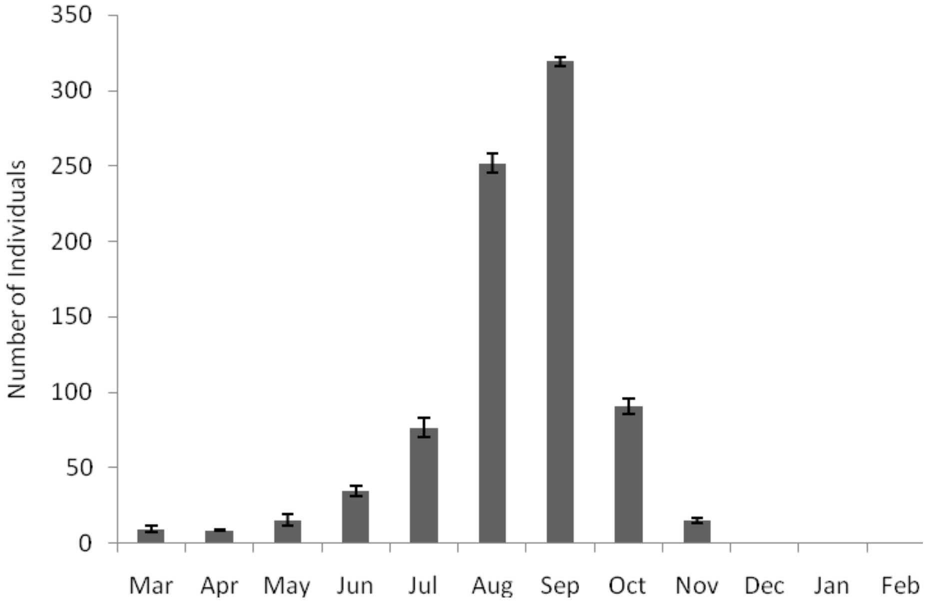


Figure 4.1.5 Monthly abundance of *Bombus haemorrhoidalis* Smith in F9 Park and its surroundings for the years 2012-2013

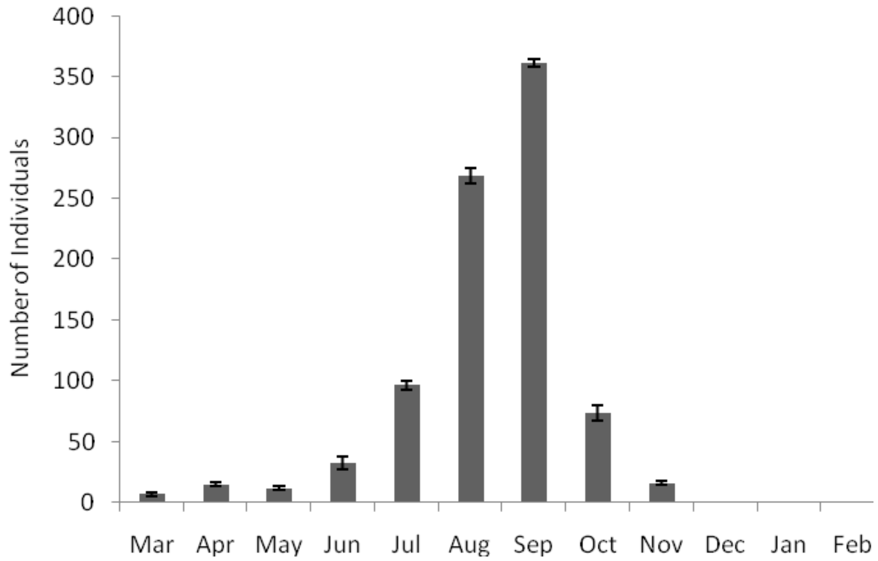


Figure 4.1.6 Monthly abundance of *Bombus haemorrhoidalis* Smith in Bara-Kahu and its surroundings for the years 2012-2013

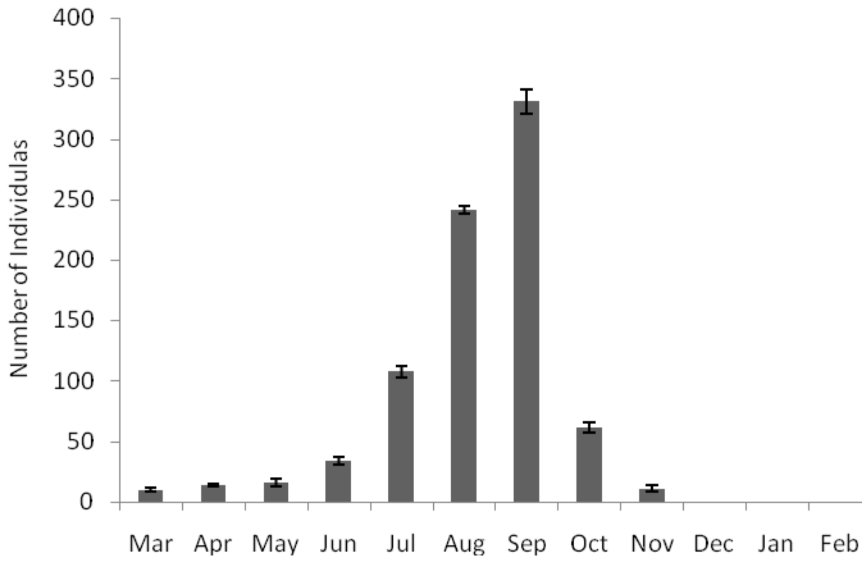


Figure 4.1.7 Monthly abundance of *Bombus haemorrhoidalis* Smith in Poonch University and its surroundings for the years 2012-2013

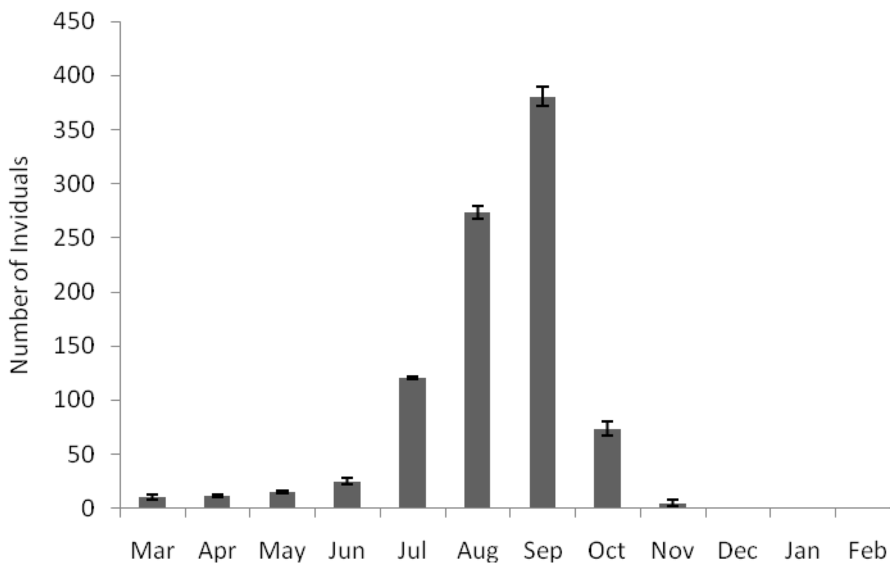


Figure 4.1.8 Monthly abundance of *Bombus haemorrhoidalis* Smith in Namnoota and its surroundings for the years 2012-2013

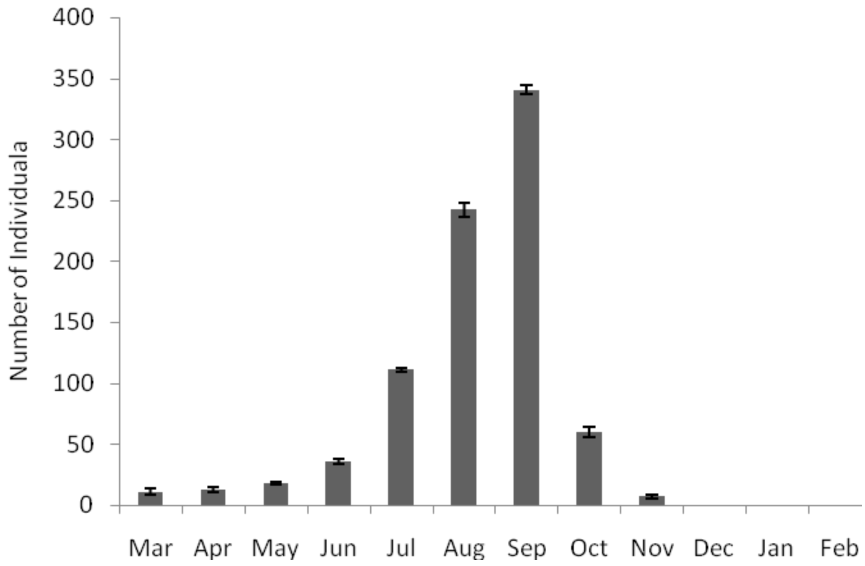


Figure 4.1.9 Monthly abundance of *Bombus haemorrhoidalis* Smith in Paniola and its surroundings for the years 2012-2013

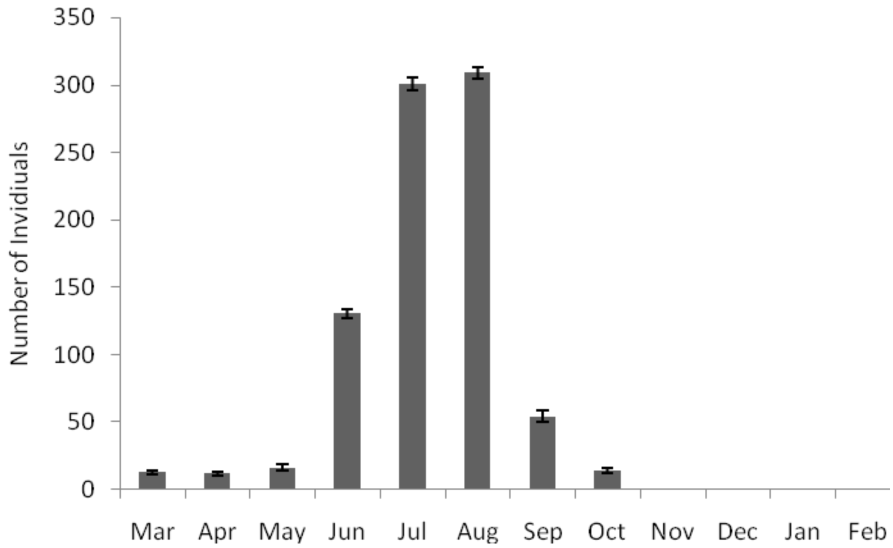


Figure 4.1.10 Monthly abundance of *Bombus haemorrhoidalis* Smith in Mahandari and its surroundings for the years 2012-2013

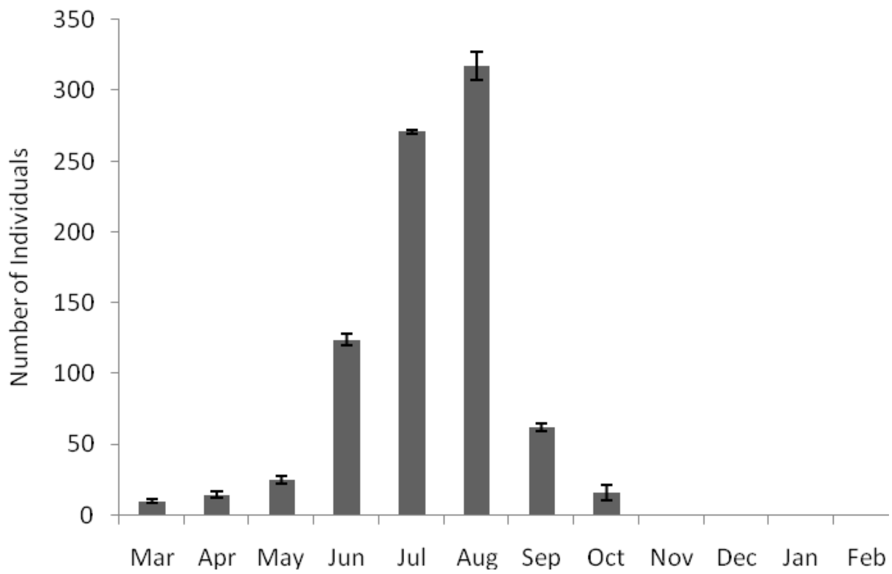


Figure 4.1.11 Monthly abundance of *Bombus haemorrhoidalis* Smith in Kaghan and its surroundings for the years 2012-2013

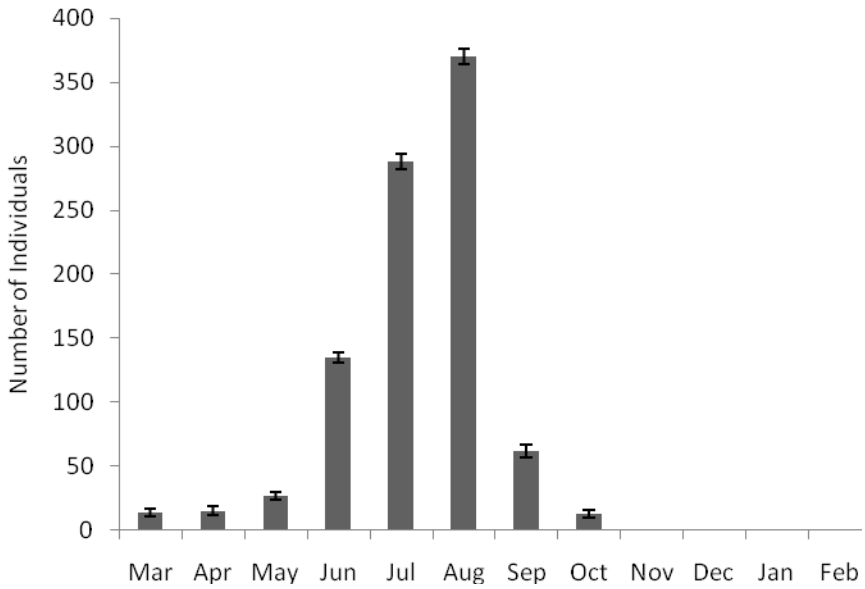


Figure 4.1.12 Monthly abundance *Bombus haemorrhoidalis* Smith in Naran and its surroundings for the years 2012-2013

abundance followed to that of Diptera and Lepidoptera. Variation in vegetation pattern or altitude may account for this difference. From Himalayan foot hills of Azad Jammu and Kashmir of Pakistan, nine insect pollinator species of Hymenoptera were recorded with dominant in abundance and significantly diverse among pollinators (Hussain *et al.*, 2012). In Haryana, state of India, Hymenoptera was found with maximum abundance of pollinators followed by Lepidoptera and Diptera (Devi *et al*., 2014).

In Kashmir of India, Hymenoptera was dominant group of insect pollinators with the highest abundance (Kumar, 1995). On plum flowers in Kashmir, Hymenoptera was also with highest abundance of pollinators with nine species (Abrol et al., 2005). In Uttarakhand of India, 12 pollinators, 9 from Hymenoptera and one from each Diptera, Lepidoptera and Coleoptera order were recorded on sunflower with Hymenoptera as most abundant followed by Diptera (Goswami et al., 2013). In Himachal Himalaya, similarly, Hymenoptera was the most abundant along with other three insect orders of Diptera, Lepidoptera and Coleoptera in temperate fruit crops (Raj and Mattu, 2014). However, long tongued *B. haemorrhoidalis* species was the most abundant at all study locations and the only *Bombus* pollinator of lower northern Pakistan (Sheikh *et al.*, 2014). It is most important and common pollinator across the plantation in central Himalayas of India and different altitude throughout the region (Sinu *et al.*, 2011).

The results of diversity indices showed significant differences among locations. That might be due to variation in altitude, weather conditions, natural flora and vegetation pattern. Some variation for both years was also observed linked with weather fluctuation and environmental factors. Maximum diversity indices were for Naran Kaghan Valley might be due to rich natural flora in this region and fact of less disturbed by human activities in comparison to that in Rawalpindi/Islamabad and Rawalakot. From Rawalpindi/Islamabad, F9 Park was more diverse than other sub-locations due to variety of year round vegetation and ornamental flowers gardens. In Rawalakot, minimum diversity indices were noted might be due to thick alpine forest area with less floral vegetation. The inadequate divergence in diversity indices of different locations in Kashmir Valley, Pakistan indicated uniform distribution of pollinators throughout the Valley (Hussain *et al*., 2012). Abundance and diversity of pollinators depends on temporal and spatial unevenness (Ollerton and Louise, 2002). The abundance of pollinators at any location corresponds with the presence of flora and seasonal weather changes (Sajjad *et al.*, 2010),

Present studies, however, were performed on monthly basis and such possibility can be excluded for collections and observations in the specified regions under study. These bumblebees have developed an interaction with their natural floral host plants for centuries to meet their pollen and nectar needs. From present study, 48 floral hosts of 24 plant families from Rawalpindi/Islamabad, 43 of 20 plant families from Rawalakot and 42 of 18 plant families from Naran Kaghan as foraging floral plants for *B. haemorrhoidalis* were recorded, respectively. Floral host plants of Asteraceae family were found as major family followed by Fabaceae and Lamiaceae plant families. Their foraging preference areas included sloppy regions with floral plants rather than densely covered wild trees with flowers beneath and bilaterally symmetrical flowers preferred over asymmetrical flowers

(Moller and Sorci, 1998). *Cana indica*, *Dahlia variabilis*, *Rosa sp.*, *Thevetia neirifolia*, *Saussoria lappa* and *Tamarix gaelic* were the main visited floral plants by these bumblebees in other Northern Pakistan (Sabir, 2011).

A total of 23 floral plant species from 13 families were recorded with four major plant families (Asteraceae, Lamiaceae, Papilionaceae, Ranunculaceae) mostly visited by bumblebees (Sabir, 2011). Asteraceae was the major plant family for *B. haemorrhoidalis* in lower northern Pakistan (Sheikh *et al*., 2014). The first two families were responsible to attract around seventy percent bumblebees in crop area within agricultural habitat (Suhail *et al*., 2009). Twenty four host plants of ten plant families were found as foraging host plants of *Bombus* spp. from Naran Kaghan Valley and Asteraceae as the major floral plant family visited (Sheikh *et al*., 20015).

Presence of perennials and annual plants throughout the observation range showed their preference of different plant species belonging to different families. However, perennials showed higher preference due to their ability to produce more nectar and pollens based on their longer growth periods (Fussel and Corbet, 1992). Choice of flowers as minor, medium or major source of visitation by these bumblebees might be attributed to variation of sucrose concentration which decreases their foraging time with maximum reward (Cnaani *et al*., 2006). The collection of the food resources has been considered as important factor not only for their survival but also for their fecundity (Raine *et al*., 2006). Honeybees and bumblebees keep the record of suitable host plants for food reserves and develop interactive responses with their quick learning abilities (Ali *et al*., 2014). Body size of the foraging bumblebees has been observed an important factor in foraging competitiveness yet *B. haemorrhoidalis* workers showed insignificant size variation with longer proboscis than *B. terrestris* workers (Ings *et al*., 2005). Pollens collected by bumblebees can also help to identify the food supply for these important pollinators (Teper, 2006). Identified foraging plants of *B. terrestris* by pollen analysis from the bumblebee feces showed 56 pollen grains types from 28 plant families with most of the pollens collected from the entomophilous plants (Teper, 2004).

Twenty nine pollen sources comprised diverse sources of protein which is necessary to build the cells and use as food (Teper, 2004). Such a long floral host range provides suitable development of interactions for long term survival for the bumblebee pollinators. Food, nest sites and other resources may be shared or competed with exotic *Bombus* species imported for pollination as observed previously in different regions of the world (Couvillon *et al*., 2010; Whittington and Winston, 2003; Stout and Goulson, 2000). Diversity of indigenous bumblebees with information of their floral host plants and foraging range are considered important for their services identification to manage the diverse plant families. They play vital role in conservation of natural floral resources.

The lowest altitude where this indigenous bumblebee was observed was around 540 m from the parks of Rawalpindi area and the highest was 2772 m from Naran Valley. However, another species, *B. terrestris* widely used in commercial crop pollination has been observed from zero altitude in the East Mediterranean region showing their diverse climatic adaptations (Gurel *et al*., 2008). Thirteen bumblebee species have been observed at altitude more than 3000 m from Northern areas of Pakistan (Sabir *et al*., 2011). Their absence in our areas below this level might be due to the harsh summer in the subtropical environment and their limitation in the mountainous region with harsh winter months to meet their hibernation needs. Our results showed maximum population of *B. haemorrhoidalis* at Rawalpindi/Islamabad and Rawalakot in August and September whereas it was the lowest in October. There was no population in December, January and February in these locations due to severe winter used as hibernation period of *B. haemorrhoidalis*. Maximum population in August and September might be due to favorable average temperature range in these months or maximum colony development stage and lowest population in October due to end of life cycle of this species in this region. At Naran Kaghan, it was maximum in July and August and started decreasing after that might be due to the advent of winter in these locations after August. There was no population of *B. haemorrhoidalis* during four months i.e., November to February due to harsh and severe winter. Population of different castes was found more in August and September then declined (Sheikh *et al*., 2014). Present studies highlighted the importance of this only bumblebee species of this region observed at different altitudes and habitats. Its seasonal distribution in different locations with different flora showed its diverse floral host range for collection of nectar and pollens. It has established interaction with certain plant species as a major source of pollination and there existed quite variable population trends at all locations.

## 4.2. NEST SEEKING PREFERENCE OF LOCAL INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH SPECIES

In early spring, newly emerged queens from winter diapause were recorded during nest seeking activities for nest habitat selection in nature. There was significant difference among habitat and landscape selection for nesting. Total 411 queens were recorded in all three study locations. From Naran Kaghan Valley, maximum of 153 queens were found followed by Rawalakot and Rawalpindi/Islamabad with 139 and 119 queens, respectively (Fig.4.2.1).

### 4.2.1. Habitat Selection of Queens for Nesting at Rawalpindi/Islamabad

At Rawalpindi /Islamabad, relative open field type landscape was observed with maximum (0.51 queens/100 m) nest seeking queens followed by those in open field type landscape (0.34 queens/100 m) and wooded type landscape (0.26 queens/100 m; Fig. 4.2.2). In habitat types, forest boundary was the most suitable place to look for the queen bumblebees found with 0.56 and 0.52 queens/100 m. Minimum queens (0.12 queens/100 m) were observed in forest type habitat (Fig. 4.2.3). Among patch characteristics, withered grass type was recorded with 37.8% queens followed by tussocks type patches with 30.9%. From new grass and stone and moss type patches, 20.1% and 10.1% nest seeking queens were recorded, respectively (Fig. 4.2.4).

### 4.2.2. Habitat Selection of Queens for Nesting at Rawalakot

At Rawalakot, the highest nest seeking queens i.e., 0.57 queens/100m were found in relative open type landscape followed by open field type landscape whereas minimum queens (0.21 queens/100 m) were recorded from wooded (Fig. 4.2.5). Among the habitat type, forest boundary was observed with maximum (0.65 queens/100m) number of queens followed by field boundary (0.56 queens/100 m). Forest type habitat showed the least nest seeking queens i.e., 0.15 queens/100 m) (Fig. 4.2.6). Maximum of 38.84% nest seeking queen were recorded from withered grasses followed by tussocks type with 30.9% whereas the least were recorded with 9.35% in stone and moss type patches (Fig. 4.2.7).

### 4.2.3 Habitat Selection of Queens for Nesting at Naran Kaghan

From Naran Kaghan valley, maximum nest seeking queens (0.64 queens/100 m) were found at relative open field type landscape followed by open field type. Wooded type landscape show minimum (0.21 queens/100 m) nest seeking queens (Fig. 4.2.8). In habitat type, the highest queens (0.66 queens/100 m) were recorded in forest boundary followed by field boundary (0.58 queens/100 m), respectively and minimum (0.18 queens/100 m) from forest type habitat (Fig. 4.2.9). Among patch characteristics, withered grass patch type was found with maximum (38.56%) and stone and moss type patches with minimum nest seeking queens (9.1%), respectively (Fig. 4.2.10).

### 4.2.4 Comparison of Different Landscape and Habitat Type

At Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley, all landscape and habitat types were significantly different (P= 0.00) with each other. Maximum nest seeking queens were recorded from relative open field type landscape (141 ± 1.77) followed by open field type (106±1.10) while minimum

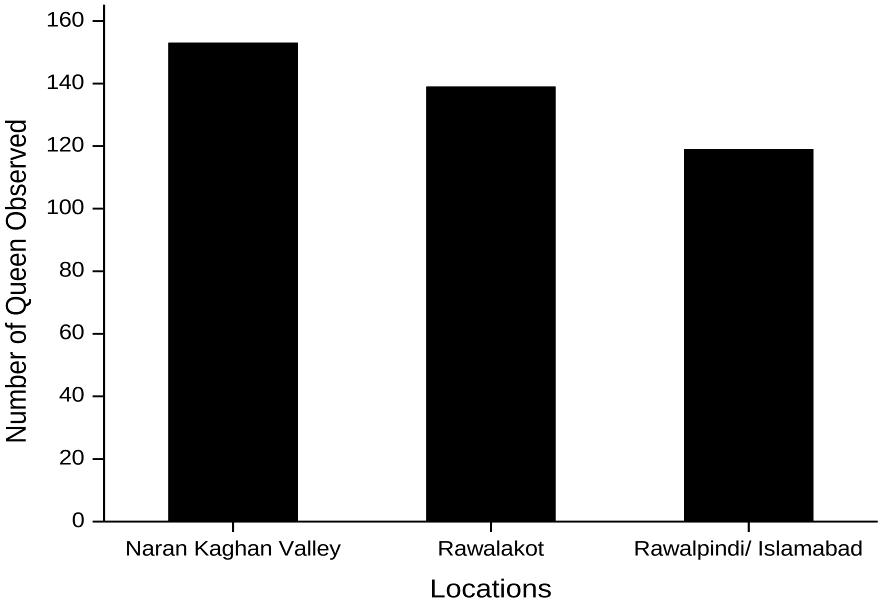


Figure 4.2.1 Total number of nests seeking queens observed in different localities of study area during the year 2012 and 2013

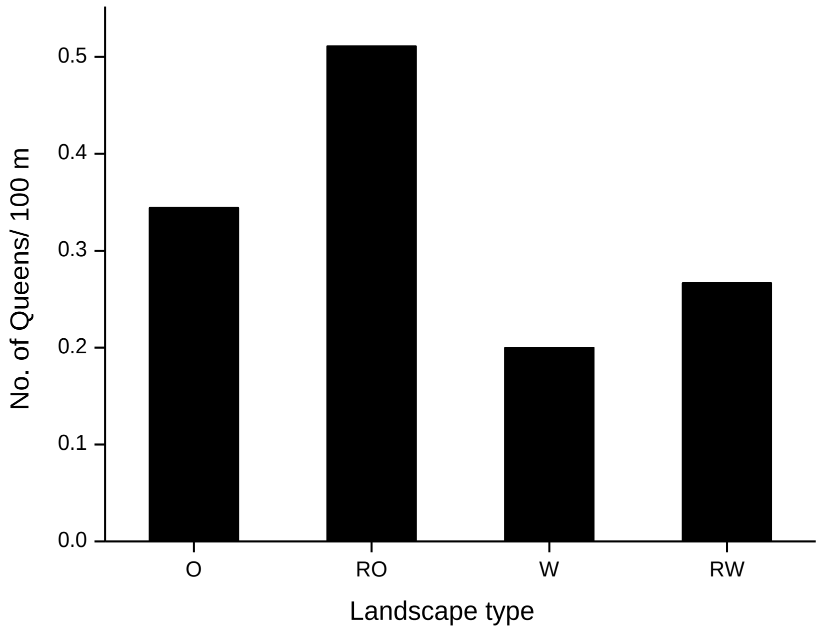


Figure 4.2.2 Number of nest-seeking queens observed per 100 m transect in different landscape of Rawalpindi/ Islamabad

O, open field; RO, relative open field; W, wooded land; RW, relative wooded land

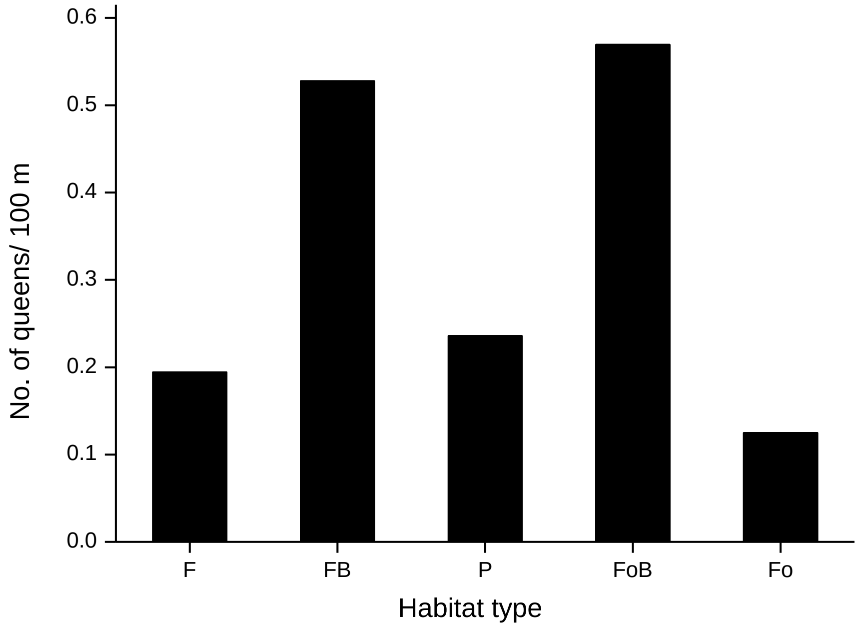


Figure 4.2.3 Number of nest-seeking queens observed per 100 m transect in different habitat type of Rawalpindi/ Islamabad

F, field; FB, field boundary; P, posture; FoB, forest boundary; Fo, forest

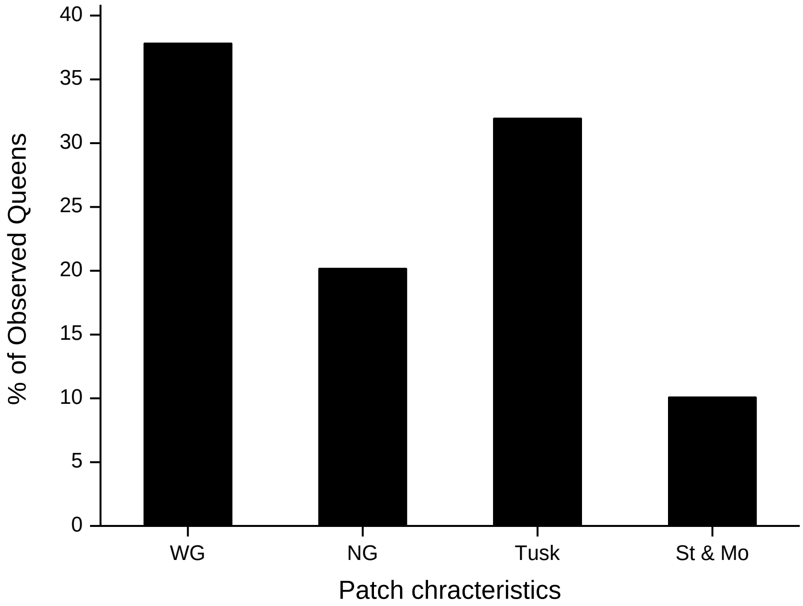


Figure 4.2.4 Percentage of nest-seeking queens observed in different type of patch characteristics in Rawalpindi/ Islamabad

WG, withered grass; NG, new grass; Tusk, tussocks; St & Mo, stone and moss

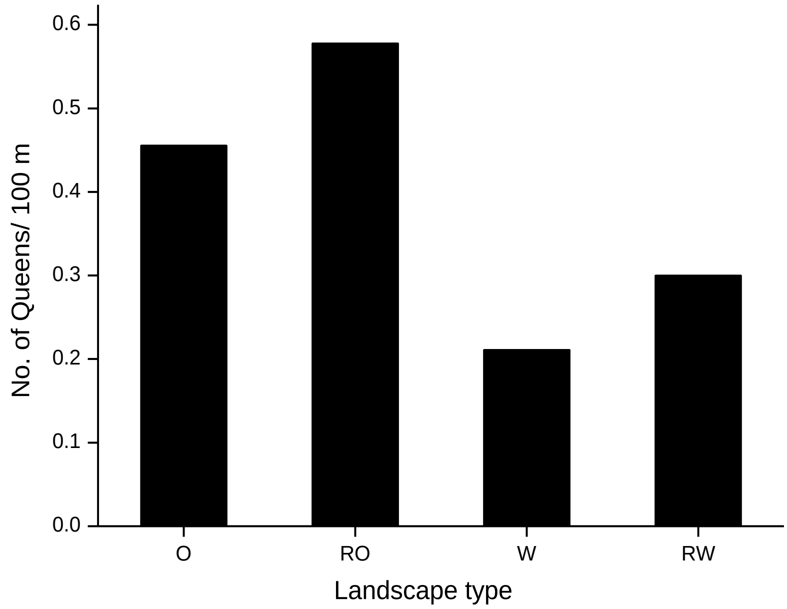


Figure 4.2.5 Number of nest-seeking queens observed per 100 m transect in different landscape type of Rawalakot

O, open field; RO, relative open field; W, wooded land; RW, relative wooded land

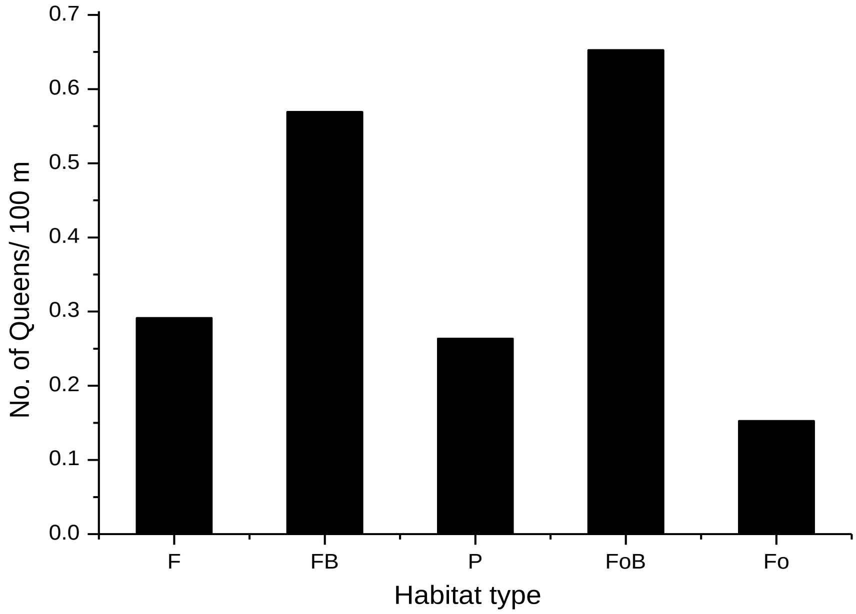


Figure 4.2.6 Number of nest-seeking queens observed per 100 m transect in different habitat type of Rawalakot

F, field; FB, field boundary; P, posture; FoB, forest boundary; Fo, forest

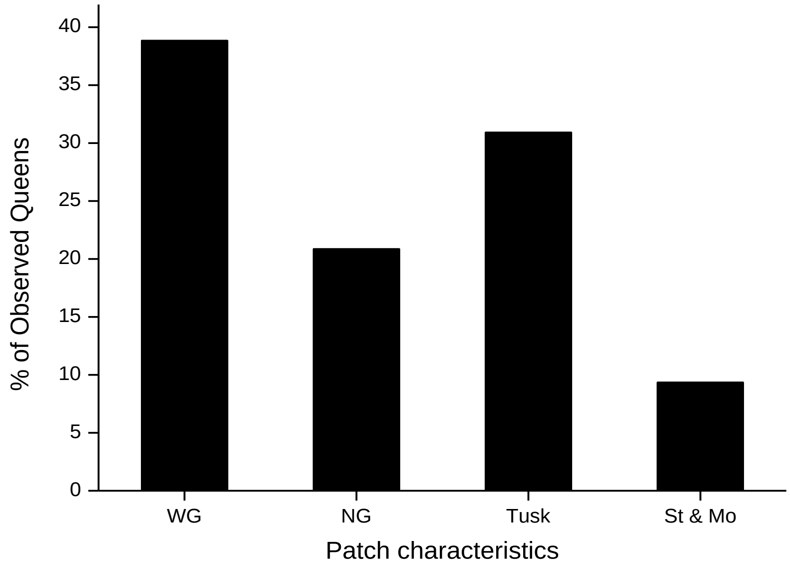


Figure 4.2.7 Percentage of nest-seeking queens observed in different type of patch characteristics in Rawalakot

WG, withered grass; NG, new grass; Tusk, tussocks; St & Mo, stone and moss.

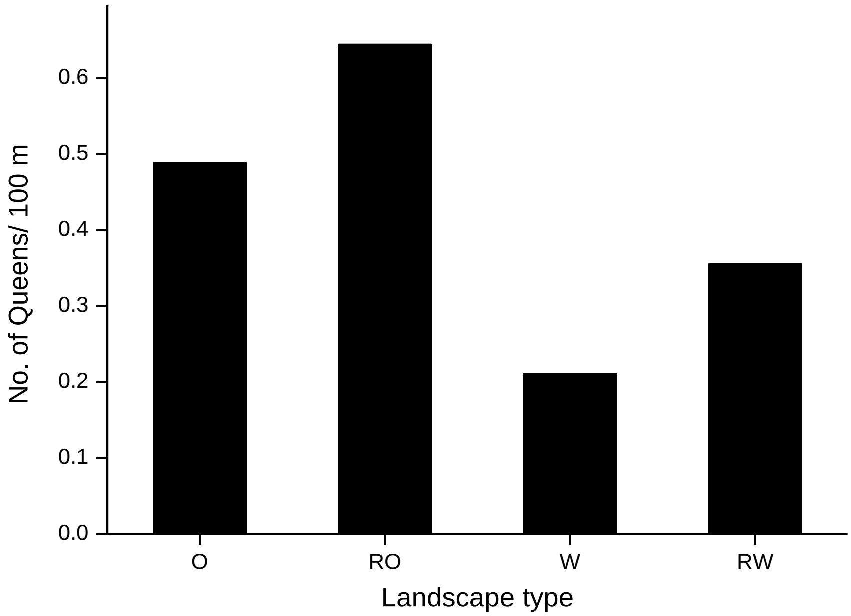


Figure 4.2.8 Number of nest-seeking queens observed per 100 m transect in different landscape type Naran Kaghan Valley

O, open field; RO, relative open field; W, wooded land; RW, relative wooded land

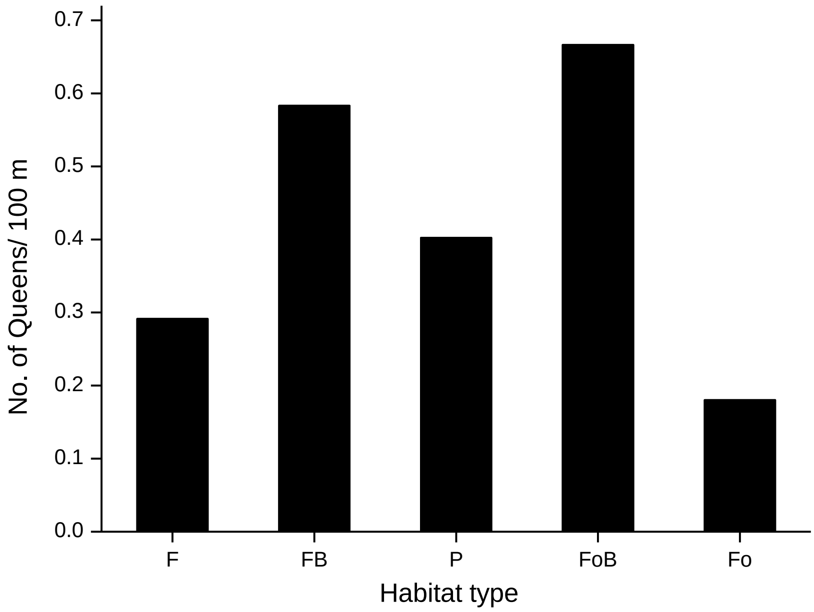


Figure 4.2.9 Number of nest-seeking queens observed per 100 m transect in different habitat type Naran Kaghan Valley

F, field; FB, field boundary; P, posture; FoB, forest boundary; Fo, forest

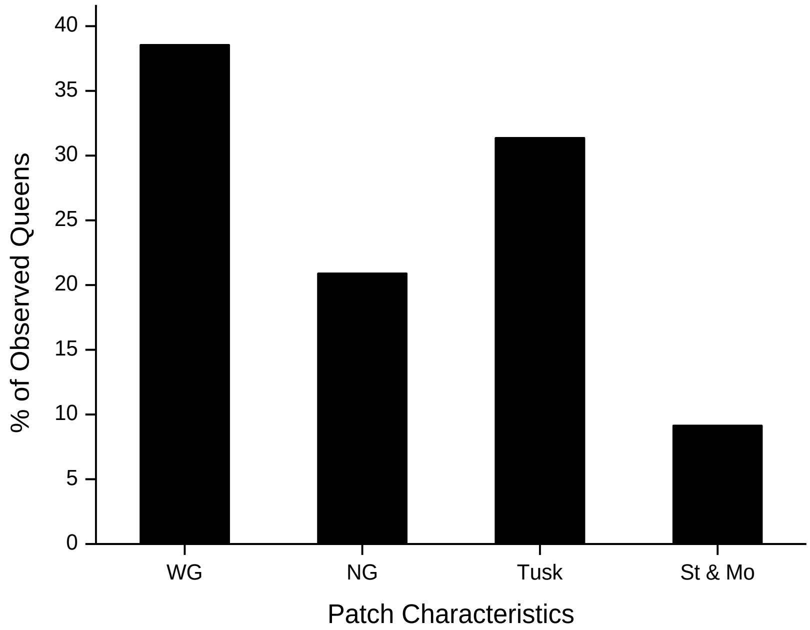


Figure 4.2.10 Percentage of nest-seeking queens observed in different type of patch characteristics in Naran Kaghan Valley

WG, withered grass; NG, new grass; Tusk, tussocks; St & Mo, stone and moss

Table: 4.2.1 Comparison of different habitat types

| Habitat type | No. of Observed Queens |
|---|---|
| Field | 76 ± 1.08 C |
| Field boundaries | 103 ± 1.08 B |
| Posture | 69 ± 1.22 D |
| Forest boundaries | 109 ± 1.80 A |
| Forest | 54 ± 1.29 E |
| P | 0.00 |

The table stand for mean ± SE followed by the different letter in the column are significantly different at $p<0.05$ by Least Significant Difference Test.

Table: 4.2.2 Comparison of different landscape types

| Landscape type | No. of Observed Queens |
|---|---|
| Open field | 106 ± 1.10 B |
| Relative open | 141 ± 1.77 A |
| Wooded | 72 ± 1.29 D |
| Relative wooded | 91 ± 1.82 C |
| P | 0.00 |

The table stand for mean ± SE followed by the different letter in the column are significantly different at $p<0.05$ by Least Significant Difference Test

(72±1.29) from wooded landscape, respectively (Table 4.2.1). In habitat type, maximum (109 ± 1.80) queens were found from the forest boundaries followed by (103 ± 1.08) field boundaries while minimum (54 ± 1.29) from forest type habitat (Table 4.2.2).

Landscape and habitat type of locations under study was almost similar in semi-hilly to hilly areas of Northern Pakistan with somewhat difference in altitude (range from 542 m to 2772 m). Being similar environmental conditions which increase in intensity from Rawalpindi/Islamabad to Rawalakot to Naran Kaghan valley and wild vegetation is also in similar fashion. B. haemorrhoidalis was found to be the most common bumblebee species in these areas in present study suggested to look for the possible nest sites and nesting behavior of the diapause queens after emergence in spring season (Sheikh et al., 2014, 2015).

Variations in response of different bumblebee species has previously been observed for different landscape and vegetation type which showed differences in such preferences with species-specific characteristics (Kells and Goulson, 2003; Svensson *et al*., 2000).

Thirteen different bumblebee species were observed in the Northern regions of Pakistan (Sabir *et al*., 2011), however, the study areas differ in present study and only five species were observed with only one species limited to lower Northern region (Sheikh *et al*., 2014). The number of species may also depend upon the type of floral resources, their diversity and abundance in the region (Saini *et al*., 2012). Landscape, habitat and patch preferences help to identify the possible foraging and nest seeking behavior of queens emerging after winter diapause. Relative open field was the most preferred site for nest seeking bumblebee queens of *B. haemorrhoidalis* at the three study areas.

Degree of shelter, underground or subterraneous nest with effective soil drainage and proper sun heat absorption are some limiting factors which has evolved with time and entail in species for nest selection site (Bowers, 1985). Selection of withered grass of tussock type was the choice of different species in Europe where different bumblebee species showed different preference for these patch types (Kells and Goulson, 2003). Their preference was similar for the under observation species which showed maximum preference for withered grass followed by tussock type. This preference suggested their selection of the type of patch where nest hidden can be least observed with maximum protection. *B. lucorum* and *B. pascuorum* showed preference for banks of field and forest boundaries near tussock type of vegetation proving the behavioral preference for shelter site and amount of sunlight required (Svensson *et al*., 2000).

Small burrows of mammals around field or tussocks are the preferred sites for nest searching bumblebee queens (Svensson *et al*., 2000). These sites are most common near borders of field or forest lines suggesting their possible nesting sites. Such areas are also under stress of intensive cultivation responsible for decrease in their numbers due to farming of open fields for agriculture crops. Most queens were found near shady place searching for possible nest sites for observed species. *Bombus lapidarius* previously showed preference for low temperature and shady places for foraging and left early before sunset (Hasserlot, 1960; Prys-Jones and

Corbet, 1991). However, their foraging workers showed early to late evening foraging behavior. Such shady and hiding nest searching might be due to selection for least disturbed nest place out of reach for others and avoidance from environmental hazards like excessive rains in these areas. Relatively open or open areas for nest sites might be required to get maximum heat benefit from sunlight for the nest and their progeny development (Banaszek, 1983; Fussel and Corbet, 1992).

Nesting site selection does not means the exact number of bumblebee queens to develop and multiply their progenies as different environmental hazards like parasitism, human interruption and harsh environmental conditions like variation in floral disturbance, environment change etc. These factors, however, are considered important which maintain the natural population levels of these bumblebees stressing their genetic and phenotypic characters to maintain them for changing environment. Such environmental hazards in different years showed variation in their nests and foragers abundance which might be due to nest sites, floral resources and change in environment (Pyke, 1982; Ranta, 1982). Their success of nest sites and their multiplication will depend upon the floral resources and their availability in range of the foraging bumblebees (Cresswell *et al*., 2000). Conservation of banks or field margins of open or forest areas will help these bees to refuge these sites to maintain the ecological stability and provision of such valuable pollinators for crops and wild plants (Lagerlof and Wallin, 1993; Kells *et al*, 2001).

## 4.3 BIOLOGY OF *BOMBUS HAEMORRHOIDALIS* UNDER CONTROLLED LABORATORY AND SEASONAL BIOLOGICAL VARIATION OF DIFFERENT CASTES IN FIELD CONDITIONS

This experiment was designed to study the biology, different life stages and development in *Bombus haemorrhoidalis* nests under controlled laboratory and field conditions. In early spring, *B. haemorrhoidalis* queens were collected from Rawalpindi/Islamabad, Rawalakot and Naran Kaghan during foraging activates after emerging from winter diapause. These queens were brought to the Laboratory, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, for biological studies under controlled conditions. Monthly surveys were conducted to study the biological variations of *B. haemorrhoidalis* workers, males and queens at different sub-locations of Rawalpindi/Islamabad, Rawalakot and Naran Kaghan Valley. There existed significant variations among all castes of *B. haemorrhoidalis* during all the months in field conditions.

### 4.3.1 Biological Events in Collected Queens from Rawalpindi/Islamabad, Rawalakot and Naran Kaghan

In early spring of 2012 and 2013, eighty one queens emerging from winter diapause were collected during foraging in sunny day morning’s from natural fields. Forty two queens were collected in 2012 and 39 in 2013, respectively which were brought to the laboratory for further biological study. Twenty four queens died in first week after arrival in the laboratory and 37 died without laying any egg bead. Only 24% collected queens laid egg beads successfully and larvae produced from their first egg beads of 21% queens. From all collected queens, 17% reached

Table 4.3.1 Biological events observed in collected queens under controlled laboratory conditions

| **Year** | **T.Q** | **D.F.W %** | **W.E.B %** | **E.B %** | **P.L %** | **P.P %** | **P.W %** | **C.F %** | **C.M %** | **P.S %** |
|---|---|---|---|---|---|---|---|---|---|---|
| **2012** | 42 | 31 | 50 | 19 | 19 | 12 | 7 | 7 | 3 | 3 |
| **2013** | 39 | 28 | 41 | 31 | 23 | 23 | 15 | 15 | 10 | 5 |
| **Total** | 81 | 30 | 46 | 25 | 21 | 17 | 11 | 11 | 6 | 4 |

T.Q, total queens; D.F.W, died in first week; W.E.B, without egg beads; E.B, egg beads; P.L, produced larvae; P.P, produced pupae; P.W, produced worker; C.F, Colony foundation stage; C.M, colony maturation stage; P.S, produced sexuals at pupal stage, 11% produced workers and reached at colony foundation stage. Only 6% colonies of *B. haemorrhoidalis* reached at colony maturation stage and 4% colonies produced male and daughter queens (Table 4.3.1).

### 4.3.2 Biology of *Bombus haemorrhoidalis* under Controlled Laboratory Conditions

Under controlled laboratory conditions, pre-oviposition period of *B. haemorrhoidalis* took 12 ± 0.57 days. Eggs hatched in 3.14 ± 0.31 days and became larvae which changed into pupae after 16.42 ± 1.2 days. Pupil stage took 11.23 ± 0.74 days for their full development (Fig. 4.3.1). From starting of colony till foundation stage, there produced 92.33±7.44 of larvae followed by 62.3±7.75 pupae. At foundation stage, total 57±3.45 workers were present in the colony. There were 21± 3.44 egg beads from first bead formation to foundation stage (Fig. 4.3.2).

First worker, male and daughter queen in the colony emerged after 30.33±1.85, 79±7.21 and 81± 6.04 days of first egg bead, respectively. Life span of mother queen was of 125.33±5.92 days (Fig. 4.3.3). During complete life of *B. haemorrhoidalis* colony, 125.3 ± 4.08 males, 28.6 ± 1.84 daughter queens and 270 ± 7.8 workers were produced (Fig. 4.3.4). During colony development, colonies reached foundation stage (about 50 workers) after 62±5.68 days and maturation stage after 84±5.14 days of first egg beads (Fig. 4.3.5). Mortality of *B. haemorrhoidalis* in colony at initial stage was zero percent and reached maximum

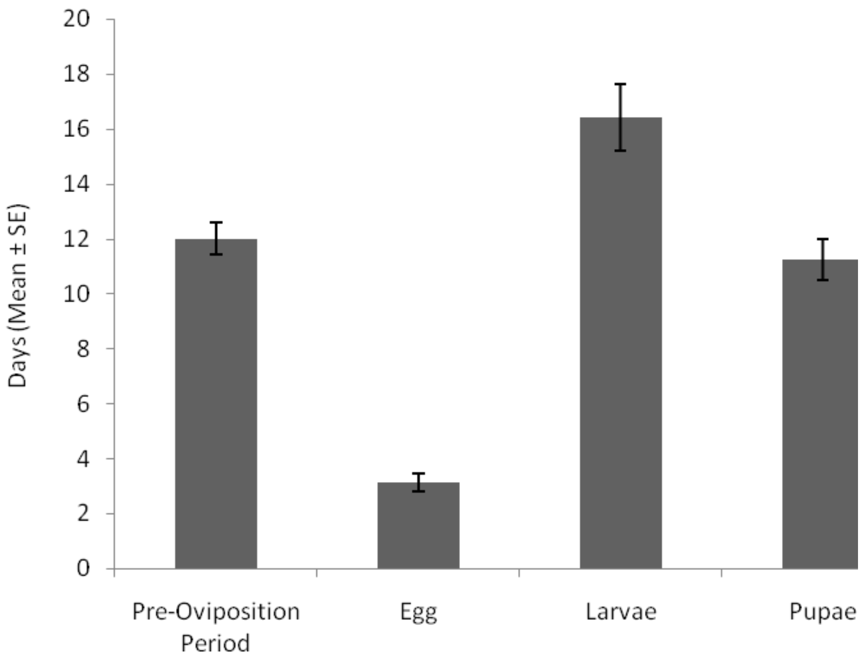


Figure 4.3.1 Life development stages of bumblebee (*Bombus haemorrhoidalis*) at colony initiation under controlled laboratory conditions

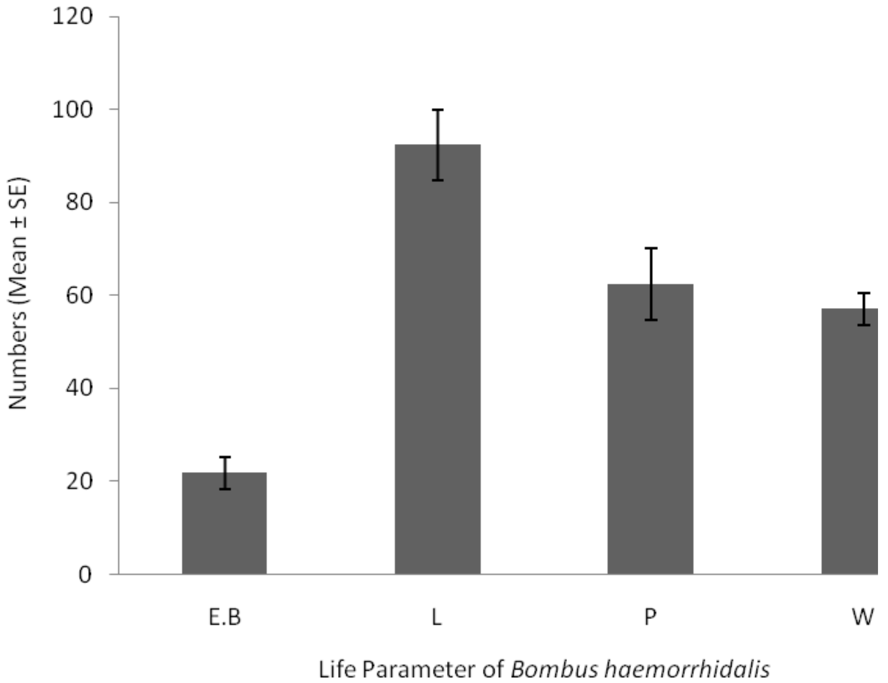


Figure 4.3.2 Life history parameters of bumblebee (*Bombus haemorrhoidalis*) at colony foundation stage under controlled laboratory conditions

E.B, egg beads; L, larvae; P, pupae; W, workers

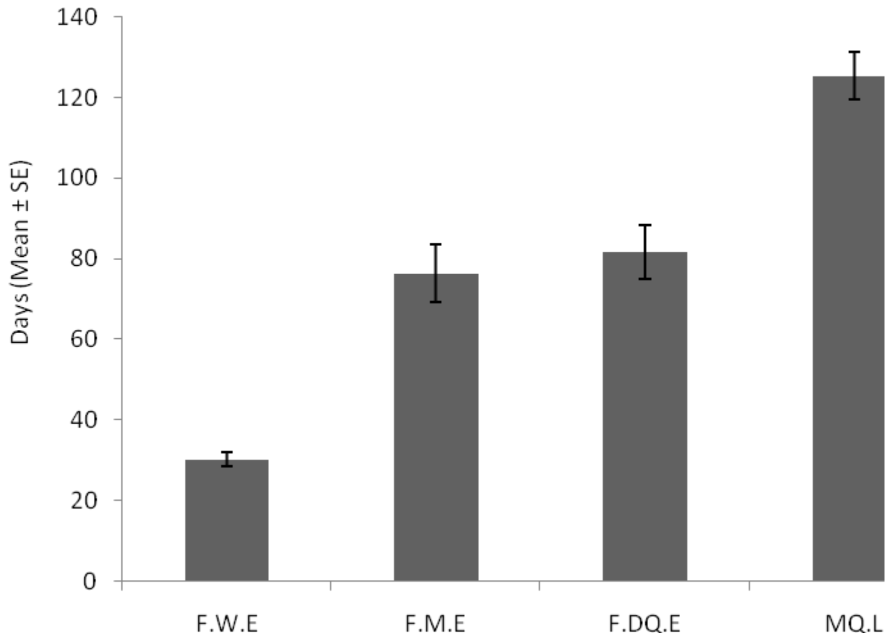


Figure 4.3.3 Life history parameters of bumblebee (*Bombus haemorrhoidalis*) during colony development under controlled laboratory conditions

F.W.E, first worker emergence; F.M.E, first male emergence; F.DQ.E, first daughter queen emergence; MQ.L mother queen live

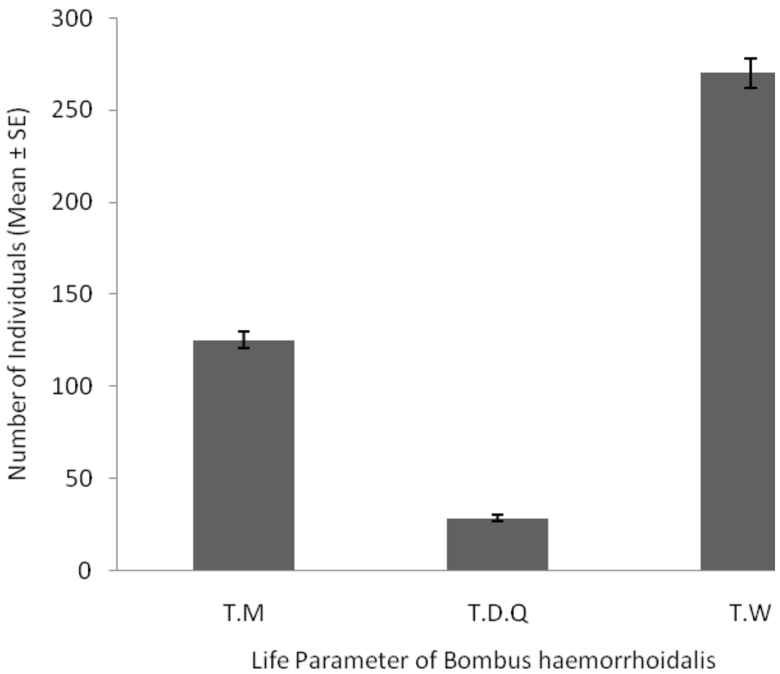


Figure 4.3.4 Life history parameters of bumblebee (*Bombus haemorrhoidalis*) at colony maturation stage under controlled laboratory conditions

T.M, total males; T.D.Q, total daughter queens; T.W, total workers

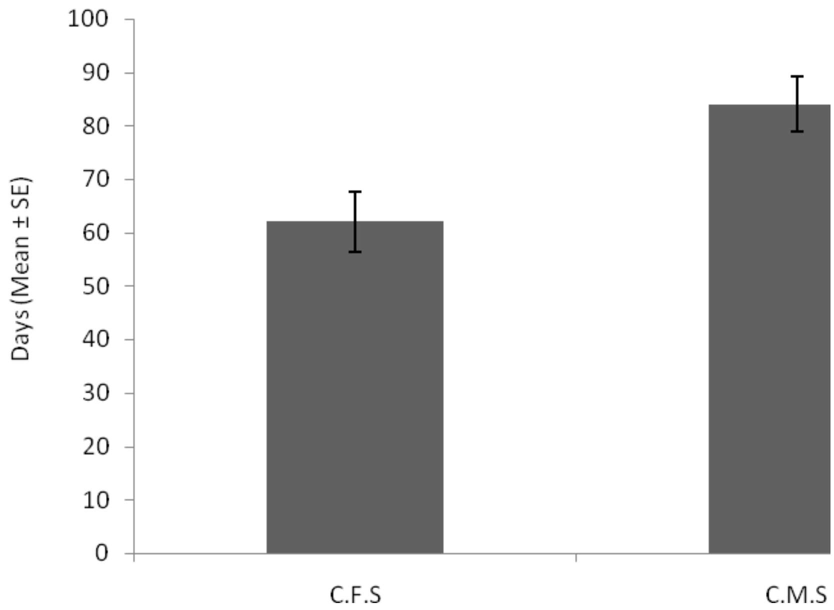


Figure 4.3.5 Life history parameters of bumblebee (*Bombus haemorrhoidalis*) during colony development under controlled laboratory conditions

C.F.S; colony foundation stage, C.M.S; colony maturation stage

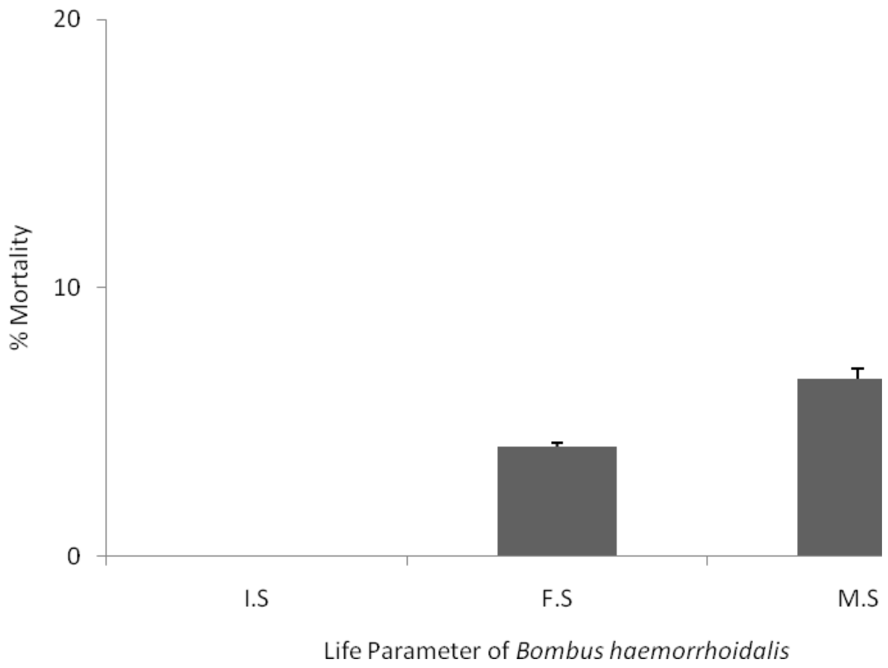


Figure 4.3.6 Mortality percentages of bumblebee (*Bombus haemorrhoidalis*) at colony initiation, foundation and maturation stages under controlled laboratory conditions

I.S, initiation stage; F.S, foundation stage (50 workers); M.S, maturation stage (150 workers) of 8% mortality atcolony maturation stage (150 workers). At colony foundation stage the mortality was 4 % (Fig. 4.3.6).

### 4.3.3 Seasonal Biological Variations of *Bombus haemorrhoidalis* Workers, Male and Daughter Queens

In natural field conditions, life cycle of *B. haemorrhoidalis* started in March when queens emerged from end of winter diapause and completed in October-November with sexual productions. Queens emerged from winter diapause were found in March, April and even early May. From June to October, workers were recorded and daughter queens were observed in October and November. Queens spent December, January and February. During winter diapause, there were no

population of workers, males and daughter queens at all study locations.

#### 4.3.3.1 Seasonal biological variations of *Bombus haemorrhoidalis* workers, male and daughter queens at sub-locations of Rawalpindi/Islamabad

At Rawalpindi/Islamabad, queens emerging from winter diapause were found throughout March, April and May at sub-locations of Lake View and Bara-Kahu area; At F9 Park area, queens were found in March and April. From June to September, only workers were found and maximum population of workers was observed in September at all sub-locations of Rawalpindi/Islamabad. Among the sexuals, males were found in September and October and reached the highest in October. Daughter queens were found only in November at all sub-locations of Rawalpindi/Islamabad. During the months of December, January and February, there were no populations of B. haemorrhoidalis (Fig. 4.3.7, 4.3.8 and 4.3.9).

#### 4.3.3.2 Seasonal biological variations of *Bombus haemorrhoidalis* workers, male and daughter queens at sub-locations of Rawalakot

At all sub-locations (Poonch University, Paniola and Namnoota) of Rawalakot, emerging queens from winter diapause were found in March, April and May. April was found with maximum numbers of emerging queens. Workers of *B. haemorrhoidalis* were recorded throughout June, July, August, September and October and the highest population of workers was found in September. Sexuals including males and daughter queens were observed in September to October and October to November, respectively. October was with the highest population of males and least that of workers. There were no populations of workers, males and queens during January, February and December at all sub-locations of Rawalakot (Fig. 4.3.10, 4.3.11 and 4.3.12).

#### 4.3.3.3 Seasonal biological variations of *Bombus haemorrhoidalis* workers, male and daughter queens at sub-locations of Naran Kaghan Valley

From all sub- locations of Naran Kaghan Valley, queens emerging from winter diapause were found from March to May while maximum queens emerged in April. Workers of B. haemorrhoidalis, males and daughter queens were found from June to September, August to September and September to October, respectively. July recorded the highest population of workers and September with that of male population. There was no population of workers, males and daughter queens in January-February and November-December at all sub- locations of Naran Kaghan Valley (fig. 4.3.13, 4.3.14 and 4.3.15).

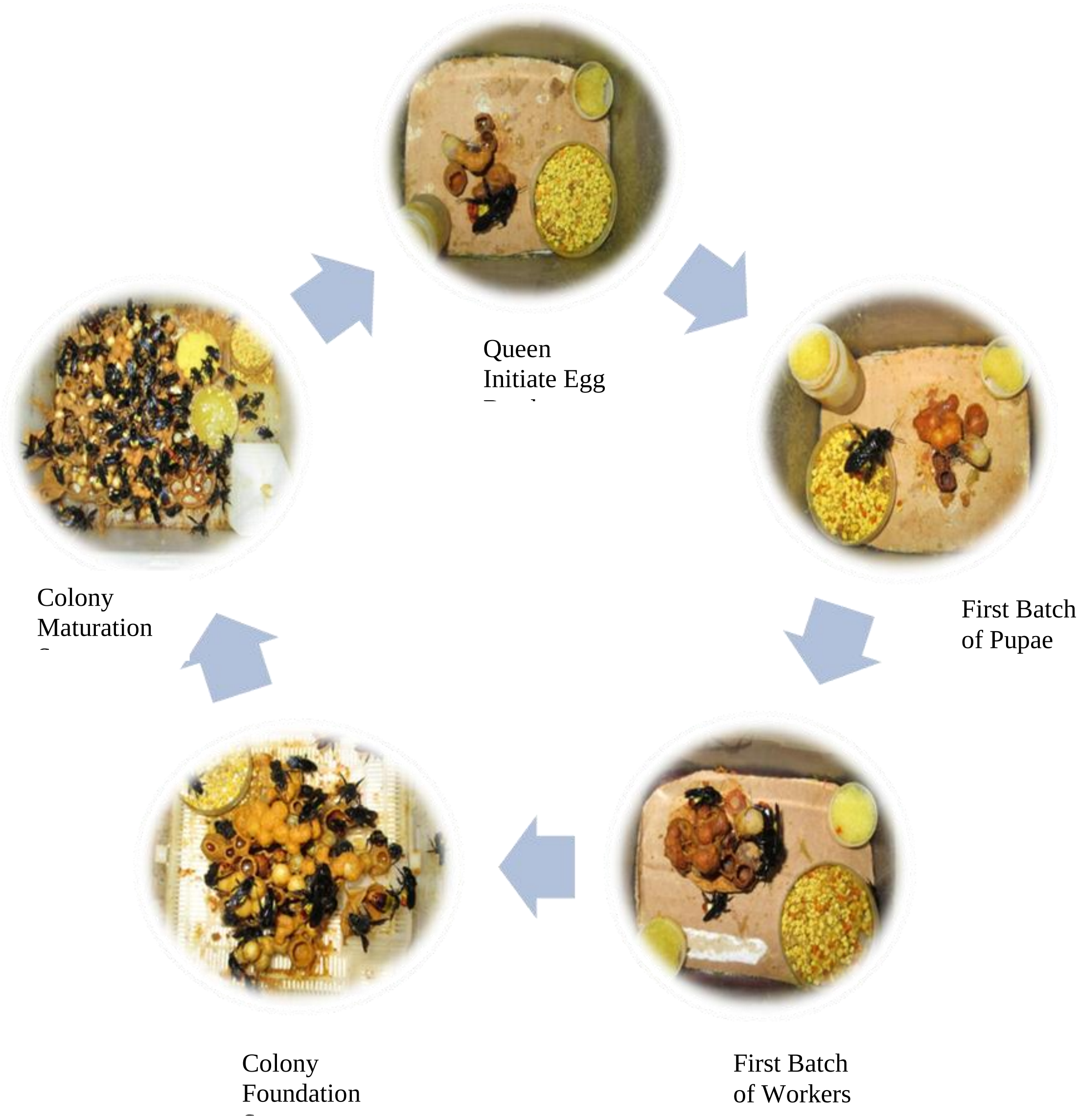


Figure 4.3.7 Different life stages of bumblebee (*Bombus haemorrhoidalis*) under controlled laboratory conditions

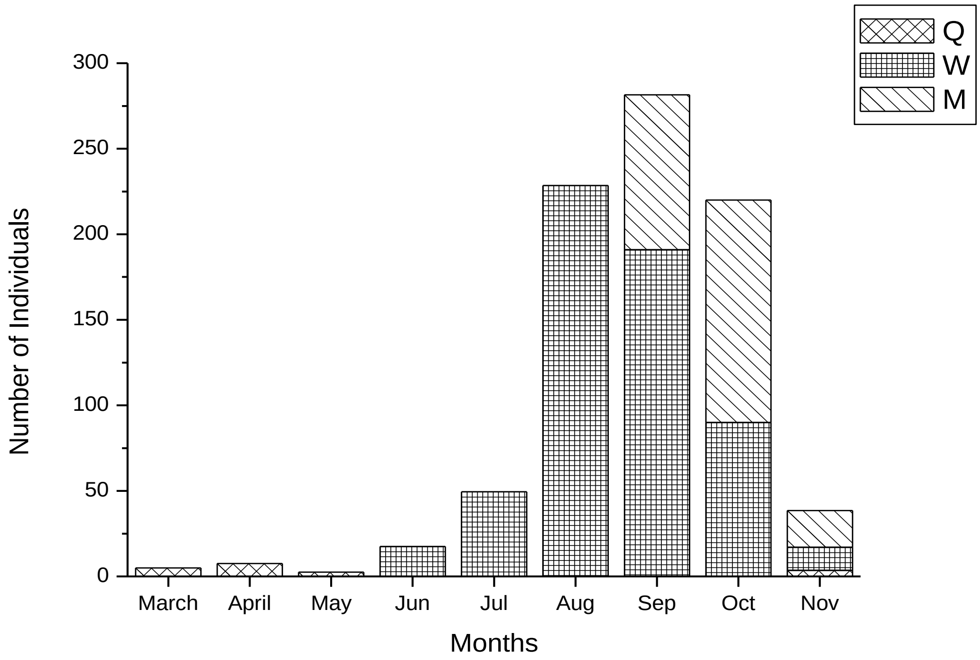


Figure 4.3.8 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Lake View Park (Rawalpindi/Islamabad) and its surroundings for the year 2012-13 (each bar with three divisions show males, workers and queens from top to bottom)

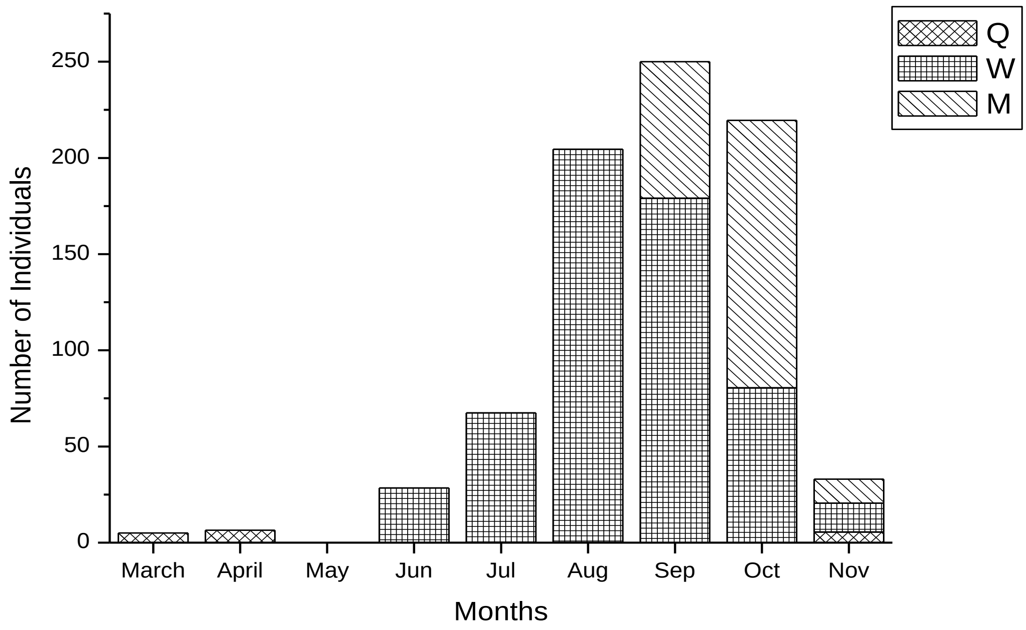


Figure 4.3.9 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at F9 Park (Rawalpindi/Islamabad) and its surroundings for the year 2012-13 (each bar with three divisions show males, workers and queens from top to bottom)

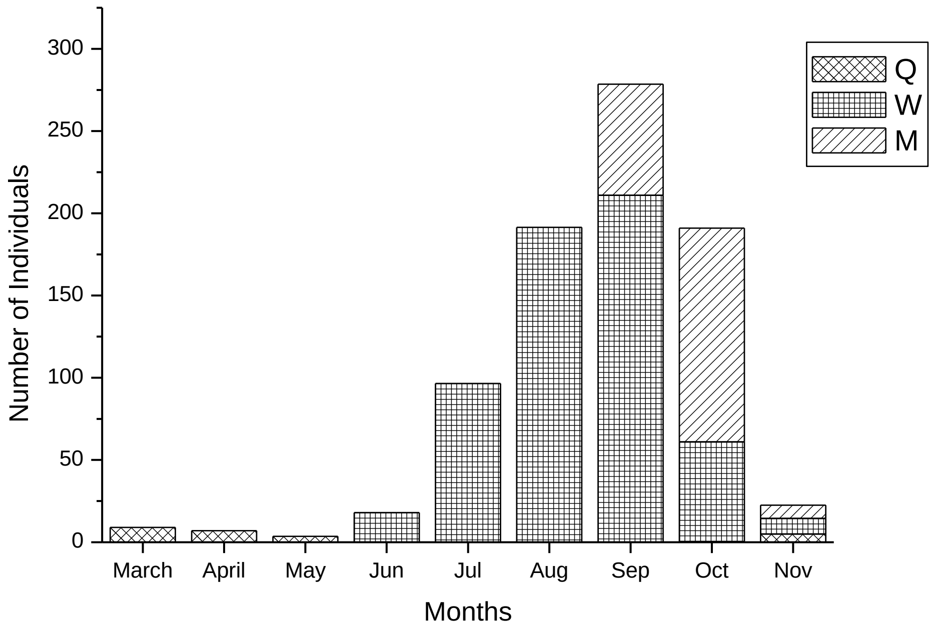


Figure 4.3.10 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Bara Kahu and (Rawalpindi/Islamabad) and its surroundings for the year 2012-13 (each bar with three divisions show males, worker and queens from top to bottom)

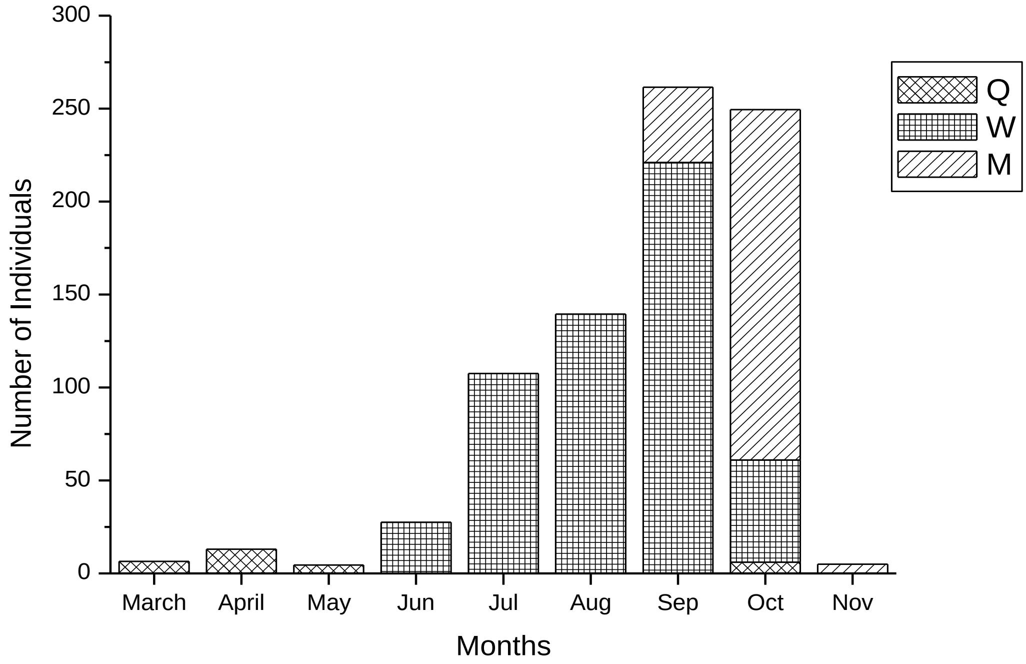


Figure 4.3.11 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Poonch University area (Rawalakot) and its surroundings for the year 2012-13 (each bar with three divisions show males, worker and queens from top to bottom)

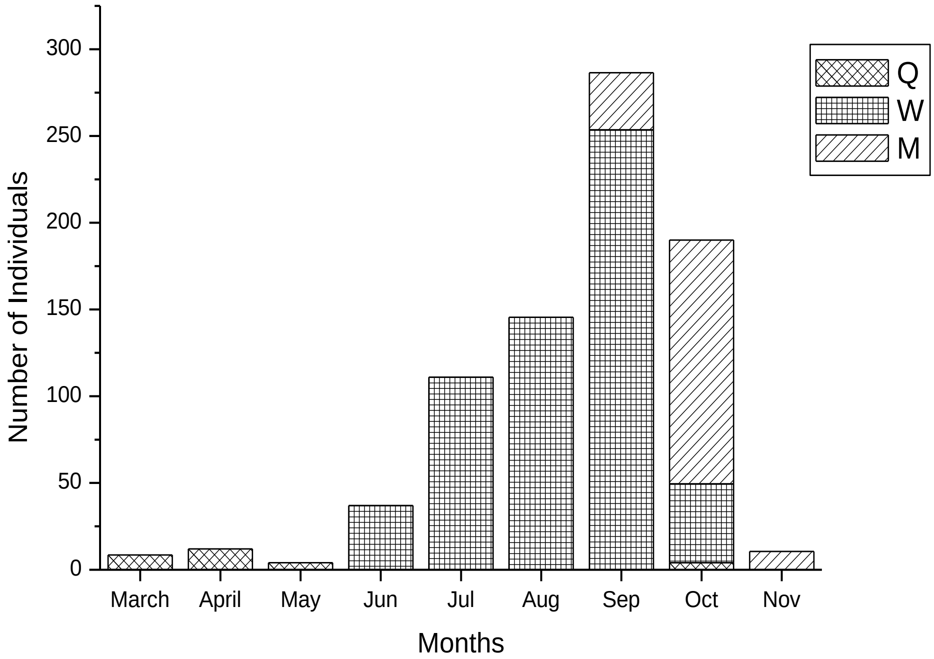


Figure 4.3.12 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Paniola (Rawalakot) and its surroundings for the year 2012-13 (each bar with three divisions show males, worker and queens from top to bottom)

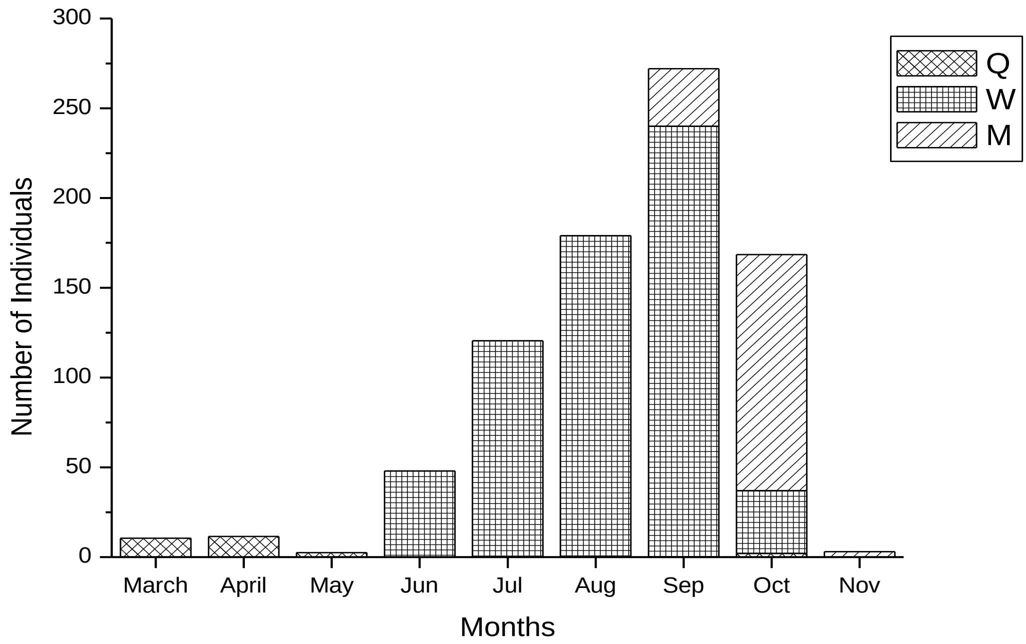


Figure 4.3.13 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Namnoota (Rawalakot) and its surroundings for the year 2012-13 (each bar with three divisions show males, workers and queens from top to bottom)

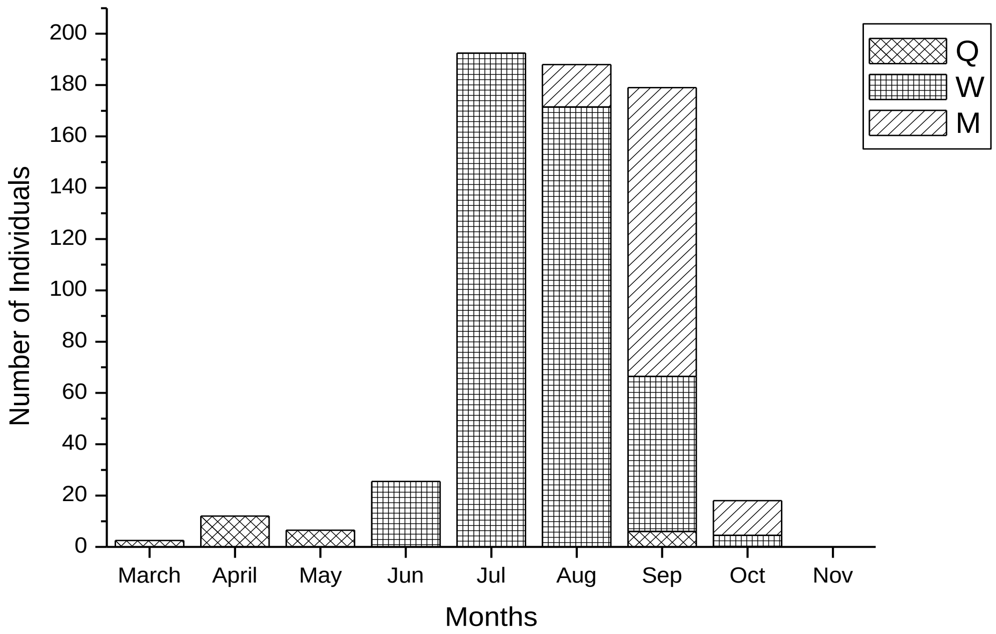


Figure 4.3.14 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Mahandari (Naran Kaghan Valley) and its surroundings for the year 2012-13 (each bar with three divisions show males, worker and queens from top to bottom)

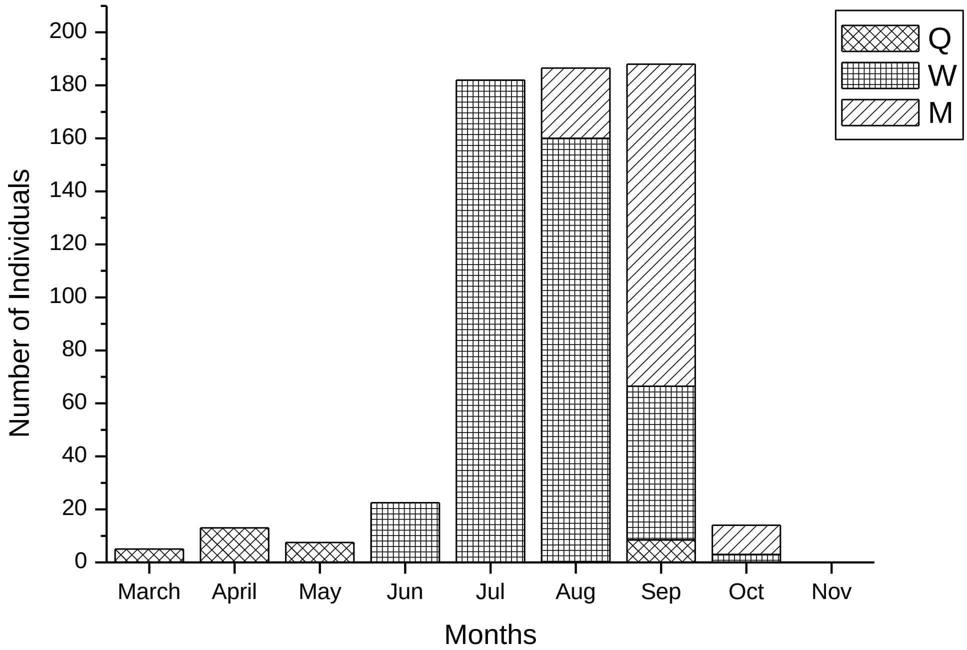


Figure 4.3.15 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Kaghan (Naran Kaghan Valley) and its surroundings for the year 2012-13 (each bar with three divisions show males, worker and queens from top to bottom)

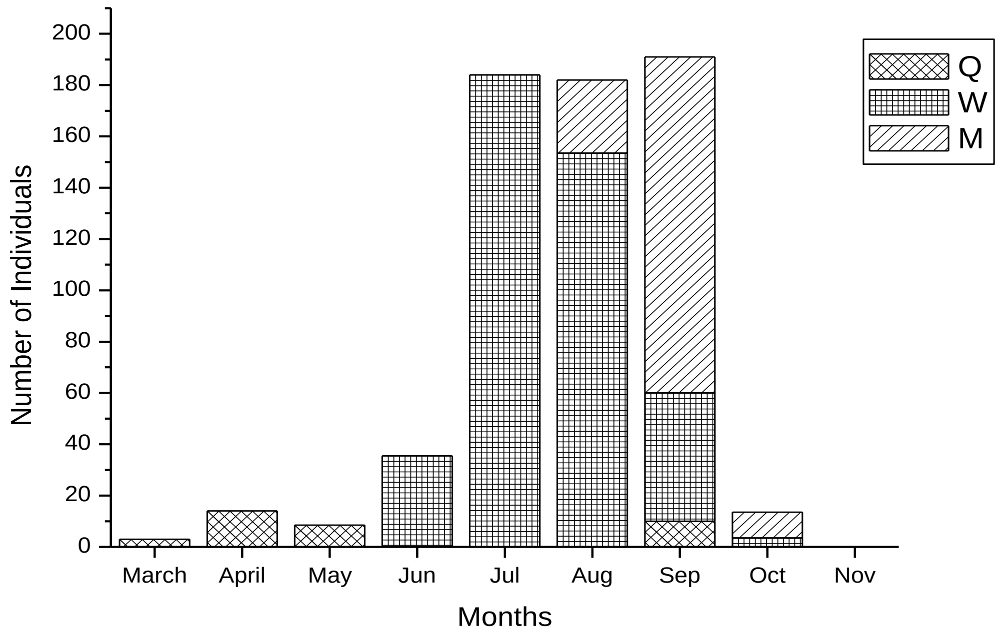


Figure 4.3.16 Seasonal biological variation of local bumblebee, *Bombus haemorrhoidalis* Smith workers, males and queens at Naran (Naran Kaghan Valley) and its surroundings for the year 2012-13 (each bar with three divisions show males, workers and queens from top to bottom)

Present study is based on the first attempt of rearing this indigenous bumblebee species, *B. haemorrhoidalis* in Pakistan. In India, some researchers tried to rear this species but could not fully succeed. Rearing of indigenous bumblebee will decrease the risk of transportation of diseases and parasites carried by imported bees to indigenous fauna. Research on bumblebee rearing started with the work of Sladon in 1912 and after this many researchers started working in mid 20th century. Now, many bumblebee species are reared throughout the world for commercial purpose. Among those species, *B. terrestris* in Belgium, Netherland, Israel and Korea, Spain and *Bombus impatiens* in the United States of America, Canada and Mexico were reared on large scale and imported throughout the world for greenhouse and tunnel cropping system (Velthuis and Doorn, 2006).

Results from present study showed that 24% collected queens started egg laying for colony initiation and only 11% of them reached the foundation stage. This indicated low success rate which might be due to the attempt being made first time to domesticate this native bumblebee species, B. haemorrhoidalis. The most important character in colony initiation stage of B. terrestris was considered to be the laying of first egg batch or bead with first eggs showing the reproductive success of the foundation queen (Yoon *et al.*, 2004a). Pre-oviposition period was 12±0.57 days while in case of *B. terrestris*, it was 9.1±1.64 (Imran *et al*., 2015) while eggs hatched in 3.14 ±0.31 days insignificantly varied with 2.6 days in case of *B. haemorrhoidalis* in India. Larval period took 16.42±1.2 days, pupal period 11.23±0.74 days and first worker emerged in 30.33±1.85 days which remained insignificant with the previous observations for this species (Chuhan *et al*., 2013). For *B. terrestris*, first worker emerging time was 26.3±0.83 days which remained somewhat sorter than that of the indigenous species (Imran *et al*., 2015). In colony of *B. haemorrhoidalis*, sexuals were produced at the end of life cycle same as for *B. terrestris* colony (Gosterit, 2011). Total workers (270 ± 7.8) were produced in bumblebee colony during the complete life cycle while in India it was about 200 workers. Queen longetivity, duration of complete life cycle were the same as in case of *B. haemorrhoidalis* rearing practice in India (Chuhan *et al*., 2013).

Different rearing conditions like temperature, humidity, sugar concentration and pollen sources have effect on life parameters of bumblebee and such requirements vary with species to species. Temperature, duration of necrosis time, artificial hibernation duration has immense effect on pre-oviposition period. Concentration of sugar solution has also effect on colony initiation time and colony development stages (Velthuis and Doorn, 2006). Pre-oviposition period, time of workers emerged and sexual productions of *B. terrestris* changed with different concentration of wax coated Pollen patties (Imran *et al*., 2015). Material type of starter boxes for colony initiation effect the life parameter in colony of *B. terrestris* (Imran *et al*., 2014).

Biological cycle of *B. haemorrhoidalis* in natural field conditions showed one generation per year. However, it lasted longer than *B. terrestris* observed previously where diapause period was longer i.e., about 5–8 months (Gurel *et al*., 2008) than for the observed species lasting no longer than three months (personal observations). According to present study, fecundated queens emerged in early spring in March and April after spending winter diapause. Workers started emerging in early May and continued till October while males and daughter queens in late October and early November at Rawalpindi/Islamabad and Rawalakot. December, January and February were hibernation months for *B. haemorrhoidalis* queens. At Naran Kaghan, queens emerged in late April and early May, workers started emerging in June and continued till September while males and daughter queens emerged in late September and early October. November, December, January and February were hibernation months for *B. haemorrhoidalis* queens at this location. This variation in seasonal distributions of different castes at different locations may be due to different environmental factors like temperature and humidity. Previous study of seasonal distribution of different castes of *B. haemorrhoidalis* from lower northern Pakistan had the same results as those of the present study (Sheikh *et al*., 2014).

In Turkey, queens of *B. terrestris* emerged in February-March at the Termessos site after hibernation while at Phassalis site which was coastal area, queens emerged in November-December after aestivation (Gurel *et al*., 2008). Different environmental factors including temperature, moisture, photoperiod and availability of food limit normal colony development. Variation in temperature might also hinder their foraging potential when used for crop pollination (Kwon and Saeed, 2003). Under natural field conditions, these factors not only activated diapause but also helped wake up and initiated the colony which must be synchronized with flowering plant species (Wuellner, 1999; Danks, 2007). Decreased or delayed rainfall could be an important factor for plant development and flower phenology ultimately affecting the visiting insect pollinators (Ogaya and Penuelas, 2007) and mainly responsible to break the aestivation in bumblebee queens (Rasmont *et al*., 2005).

## 4.4 POLLINATION EFFICIENCY OF INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHOIDALIS* SMITH IN COMPARISON WITH EUROPEAN BUMBLEBEE, *BOMBUS TERRESTRIS* L. AND THEIR FORAGING BEHAVIOR ON TOMATO CROP UNDER GREENHOUSE CONTROLLED CONDITIONS

This experiment was designed to evaluate the efficiency of indigenous bumblebee, *Bombus haemorrhoidalis* with the European bumblebee, *B. terrestris* and foraging behavior of both species on tomato crop under green house conditions. A comparison for tomato crop production using self pollination, manual and that with both bumble bee species was also performed. Differences for qualitative and quantitative characters of tomato fruits among these treatments were analyzed for comparative performance of indigenous species to already used one. Foraging behavior of both bumblebee species was observed at different time and weeks for effective time and pollination services performed by these bee species.

### 4.4.1 Qualitative and Quantitative Comparison of Two Bumblebee Species Pollination, Manual Pollination and Self Pollination of Tomato Cultivar Grandella under Hydroponic Conditions

Maximum fruit weight was recorded (116.9 ± 2.01 g/fruit) from crop pollinated by *B. terrestris* followed by (111.0 ± 2.05g/fruit) indigenous *B. haemorrhoidalis*. Crop pollinated by *B. haemorrhoidalis* was found with highest (132.5 ± 2.29, 55.36 ± 0.43 and 58.73 ± 0.37) seed number per fruit; fruit height and fruit diameter followed by *B. terrestris* (129.3 ± 2.02, 54.3 ± 0.46 and 57.33 ± 0.36). Self pollinated crop was found with minimum (16.76 ± 2.33, 12.8 ± 2.15,

Table 4.4.1 Qualitative and quantitative comparison of two bumblebee species pollination, manual pollination and self pollination of tomato cultivar (Grandella) under hydroponic conditions

| **Characters** | **Self Pollination** | **Manual Pollination** | ***Bombus haemorrhoidalis* Pollination** | ***Bombus terrestris* Pollination** | **P** |
|---|---|---|---|---|---|
| **Weight (gm)** | 16.76 ± 2.33 C | 79.677 ± 9.12 B | 111.0 ± 2.05 A | 116.9 ± 2.01 A | 0.000 |
| **Seed (count)** | 12.8 ± 2.15 C | 88.59 ± 5.41 B | 132.5 ± 2.29 A | 129.3 ± 2.02 A | 0.000 |
| **Height (mm)** | 16.93 ± 1.75 C | 43.66 ± 1.86 B | 55.36 ± 0.43 A | 54.3 ± 0.46 A | 0.000 |
| **Diameter (mm)** | 19.7 ± 1.95 C | 47.68 ± 2.09 B | 58.73 ± 0.37 A | 57.33 ± 0.36 A | 0.000 |
| **Roundness** | 3.92 ± 0.094 A | 2.21 ± 0.44 B | 1.18 ± 0.11 C | 1.14 ± 0.11 C | 0.000 |

The table stand for mean ± SE followed by the different letter in the column are significantly different at $p<0.05$ by Least Significant Difference Test

16.93 ± 1.75 and 19.7 ± 1.95) fruit weight, seed numbers per fruit, height of fruit and fruit diameter. In case of self pollinated crop, fruit weight was seven times, seed numbers ten times and fruit height three times less than bumblebee pollinated crop. Maximum fruit roundness (3.92 ± 0.094) was observed in case of self pollinated crop and minimum (1.14 ± 0.11) in *B. terrestris* (Table 4.4.1).

Comparison of both bumblebee pollinated tomato crop proved *B. haemorrhoidalis* as an alternate pollinator of tomato plants under greenhouse conditions. There existed significant differences in qualitative and quantitative characters of tomato fruits in comparison with bumblebee pollinated crop with manual and self pollinated plants.

### 4.4.2 Foraging Behavior of Both Bumblebee Species On Tomato Flowers Under Greenhouse Controlled Conditions

Comparison of foraging preference of both species of bumblebees on Grandella cultivar was equally good.

#### 4.4.2.1 Interaction between different observation weeks and times for *Bombus haemorrhoidalis* and *Bombus terrestris* workers foraging for greenhouse tomato pollination

In case of both bumblebees, maximum number of bumblebees were observed coming back to their hives after flowers visitation in first weak during morning time (0800-0900 am) and minimum in seventh week (0500-0600pm). In fourth week of arrival of colonies, incoming bee traffic decreased than that in first week and negligible during seventh week (Table 4.4.2)

Maximum outgoing movement of both bumblebees was during first week (0800-0900am) while minimum in seventh week (0500-0600pm). Decrease in outward movements of bees from their hives was observed in fourth week. It was less than first week but sufficient for pollination of flowers and negligible in seventh week for both bumblebee species (Table 4.4.3).

#### 4.4.2.2 Time spent by *Bombus terrestris* and *Bombus haemorrhoidalis* on tomato flower during the foraging of pollens

Time spent on single flower during visitation by both bumblebee species was measured in seconds. Linear regression analysis showed great variation in visitation time by *B. haemorrhoidalis* and *B. terrestris* ($Y = 0.028x + 2.623$; $R^2 = 0.190$) and ($Y = 0.029x + 2.452$; $R^2 = 0.199$). Time spent by *B. haemorrhoidalis* lasted from 1 to 8.7s and 1.02 to 8.63s by *B. terrestris*. This variation might be due to different body size or sticky wet pollens on flower height on trusses. Average time spent by *B. haemorrhoidalis* was (4.03 ± 0.19s) more than that by *B. terrestris* (3.93 ± 0.19s) on single flower in single visit (Fig 4.4.1, 4.4.2 and 4.4.3).

Use of *B. haemorrhoidalis* as crop pollinator under hydroponic crop system was first time practiced and no case for its use pollinator in closed crop system was reported previously.

Our results suggested *B. haemorrhoidalis* as an alternative pollinator of *B. terrestris* under greenhouse and hydroponic crop conditions. Pollination of tomato

Table 4.4.2 Interaction between different observation weeks and times for *Bombus haemorrhoidalis* and *Bombus terrestris* incoming trafficking for greenhouse tomato pollination

| **Weeks** | ***Bombus haemorrhoidalis*** | | | ***Bombus terrestris*** | | |
|---|---|---|---|---|---|---|
| | **0800-0900 am** | **1100-1200 am** | **0500-0600 pm** | **0800-0900 am** | **1100-1200 am** | **0500-0600 pm** |
| **1st** | 4.75 a | 3.25bc | 1.500 de | 5.50 a | 3.50 b | 1.50cd |
| **4th** | 4.25 ab | 3.25bc | 1.00 e | 3.25 b | 2.25bc | 0.05 ef |
| **7th** | 1.50 cd | 1.00 e | 0.25 e | 1.25de | 0.75 ef | 0.00 F |

Table 4.4.3 Interaction between different observation weeks and times for *Bombus haemorrhoidalis* and *Bombus terrestris* outgoing trafficking for greenhouse tomato pollination

| Weeks | *Bombus haemorrhoidalis* | | | *Bombus terrestris* | | |
|---|---|---|---|---|---|---|
| | **0800-0900 am** | **1100-1200 am** | **0500-0600 pm** | **0800-0900 am** | **1100-1200 am** | **0500-0600 pm** |
| **1st** | 6.00 a | 3.00 b | 1.00 cd | 6.00 a | 2.75 bc | 0.50 de |
| **4th** | 5.50 a | 2.25 bc | 0.25 d | 4.25 b | 2.00 cd | 1.00 de |
| **7th** | 1.00 bc | 0.75 cd | 0.25 d | 1.25 cd | 0.75 de | 0.00 e |

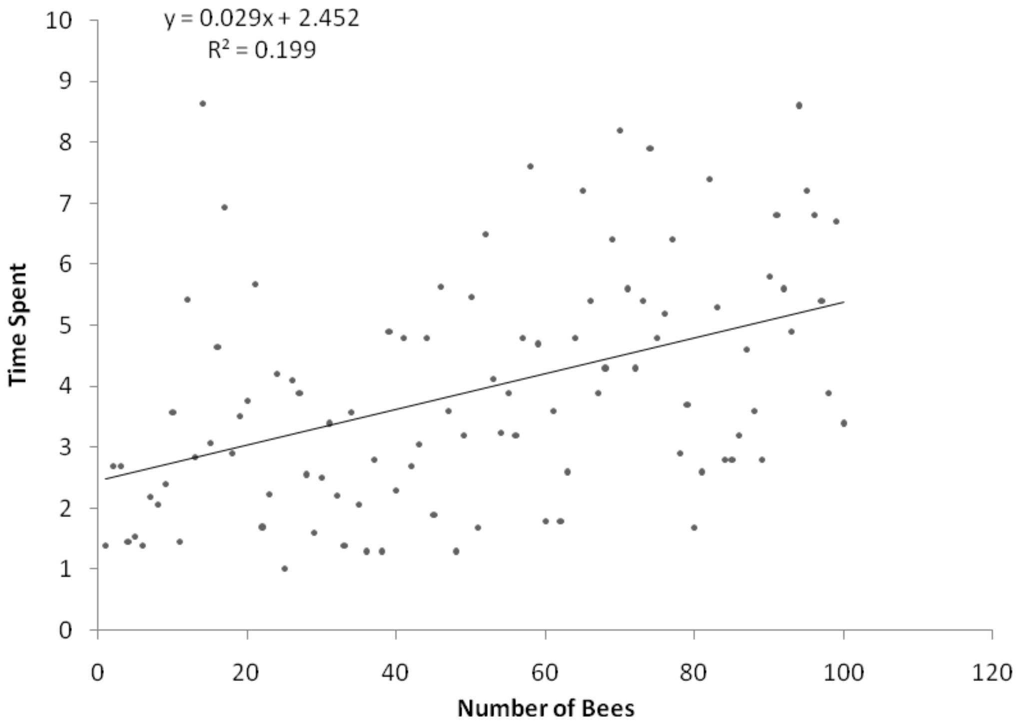


Figure 4.4.1 Time spent by *Bombus terrestris* on tomato flower during foraging of flowers

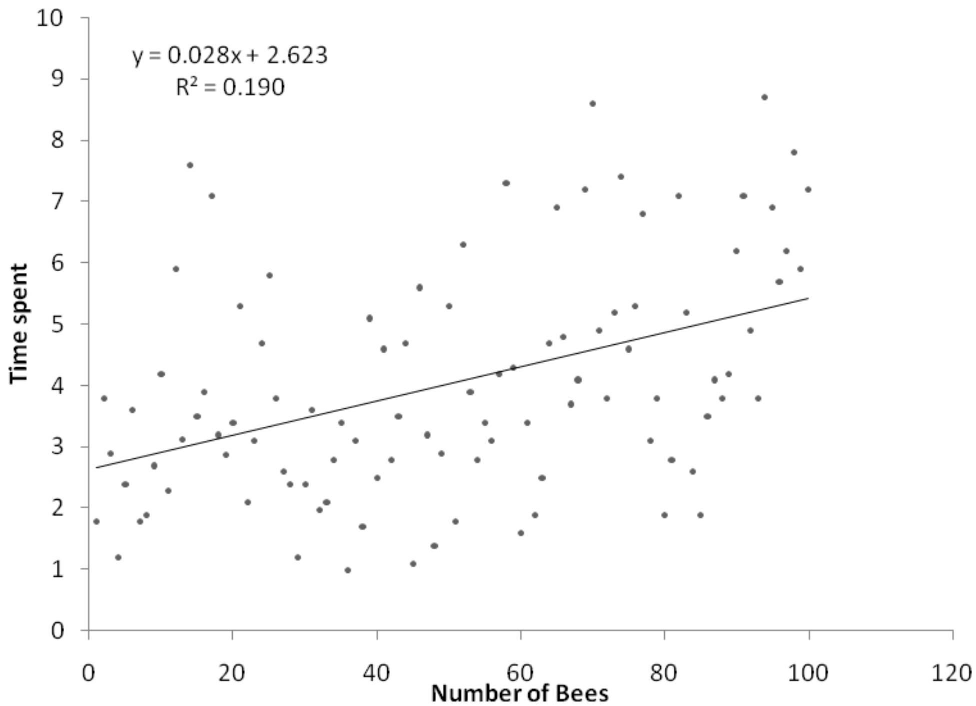


Figure 4.4.2 Time spent by *Bombus haemorrhoidalis* on tomato flower during foraging of flowers

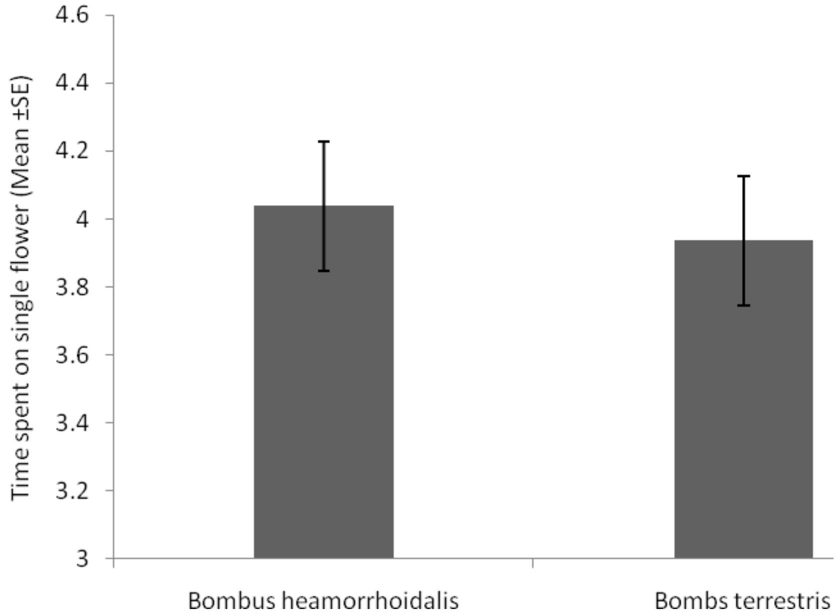


Figure 4.4.3 Comparative time spend (Mean± SE) by *Bombus haemorrhoidalis* and *Bombus terrestris* on tomato flowers during their foraging behavior

crop by *B. haemorrhoidalis* gave similar results as those with the *B. terrestris*. Both species helped produce more yield with high quality of fruit like fruit size, fruit weight and seed count than manual (with electric vibrator) and self pollination. Tomato fruit size, number of seeds and fruit quality enhanced more by bee pollination than manual or self pollination (Morandin *et al*., 2001). Manual pollination with electric vibrator increased fruit quality and chemical characters of fruits but bee pollination was considered an easier and better pollination method (Paydas *et al*., 2000).

Bumblebees foraging was more important than that of honeybees to serve as successful pollinators under greenhouse conditions (Corbet, 1996). These bees were able to fly and pollinate flowers under cool conditions due to their better thermoregulatory abilities than honeybees (Corbet, 1996).

Different bumblebee species including *B. terrestris, B. impatiens, B. ignitus* and *B. ephippiatus* are reared year round and widely used to meet the pollination needs in glasshouse cropping systems. Import of *B. terrestris* is more than all other commercially used bumblebee species throughout the world for greenhouse pollination followed by *B. impatiens* (Vergara and Buendia, 2012).

About one million bumblebee hives have been used meeting pollination needs of the crops. Various techniques like manual vibrators, honey bees and bumble bees have been used to increase fruit set and yield in tomatoes and other solanaceous crops, however, the latter were the best in performance (Paydas *et al*., 2000; Sun *et al.,* 2006). *B. haemorrhoidalis* is the only bumblebee species in lower northern Pakistan and native pollinator in Pakistan (Sheikh *et al.,* 2014).

Quantitative and qualitative parameters like high yield, more number of seeds, fruit roundness and high specific gravity resulted from bumblebee pollinated tomatoes compared to manual vibration and plant growth regulators support present study (Morandin *et al.*, 2001; Al-Attal *et al.*, 2003) and bumble bees produced heavier fruit than manual pollination (Kevan *et al.*, 1991). Quantity of seeds increased by bumblebee pollination than that with manual or no pollination (Carlos and Paula., 2012) and number of seeds might be the true method for determining the levels of pollination because other measures may be affected by environment (Picken 1984; Parmentier *et al*., 2014). Qualitative and quantitative characters of tomato fruit were enhanced with visitation of *B. terrestris* foragers in greenhouse condition, Pakistan. This species also gave better results for different cultivars of tomato crops in this region (Ahmad *et al.*, 2015).

According to present study, forager of both *B. terrestris* and *B. haemorrhoidalis* preferred to visit flowers during morning to midday and evening time. Decrease in midday might be due to increased temperature and light intensity inside greenhouses. Such variation might be for different *Bombus* species and environmental conditions like temperature or relative humidity inside greenhouse conditions (Heinrich, 1975). Activity of both species decreased with the passage of time and to negligible level after four weeks. It may be due to decrease in the number of workers in the colony or due to completion of life cycle inside. Foraging activities depended on number of workers, brood, stage of colony and amount of food inside the colony (Kwon and Saeed, 2003). *B. haemorrhoidalis* spent more time on flowers than *B. terrestris* foragers. Different bumblebee species have different foraging behavior and different subspecies belonging to different origin and genetics adaptability (Roman and Szczêsna, 2008).

Import of bumblebee colonies for pollination purposes might cause transportation of mites in different regions (Goka *et al.*, 2001) which can infect the native bees. There is need to increase the use of indigenous bumblebees as crop pollinators in glasshouse and greenhouse conditions helpful to decrease the risk of pathogens transportation. This study encouraged use of indigenous *B. haemorrhoidalis* to avoid such risks of exotic pathogens and parasites from imported species. Number of bumblebee hives, their active duration and preference for a specific cultivar may serve as important factors to maintain their optimum activity. Selection of high yielding cultivars can help to increase the economic returns of 100% crop pollination. We suggest the use of indigenous bumblebees as crop pollinators for better and improved tomato crop under tunnel farming and greenhouse enclosed farming in Pakistan.

# SUMMARY

Bumblebees provide important services for crop pollination especially for tomato, pepper, cucumber, strawberries and other crop grown under tunnel farming or glasshouse conditions for better crop yields. Bumblebees fauna comprises 267 species worldwide among which 13 are reported from Pakistan. *Bombus haemorrhoidalis* Smith is the most common species found in Pakistan and only available in the Lower Northern Pakistan. Ecological and biological aspects need experimentation both in field and laboratory conditions. Therefore, present study was designed to determine its abundance in comparison with other pollinators, foraging floral plant species and habitat selection for nest making from three different Northern areas including Rawalpindi/Islamabad, Rawalakot and Naran Kaghan; each location was divided into three sub-localities. Biological studies were conducted in laboratory conditions and its efficiency as crop pollinator was compared with *Bombus terrestris* in tomato crop grown under glasshouse controlled conditions.

Abundance of native bumblebee in relation to other pollinators confirmed *B. haemorrhoidalis* the most abundant *Bombus* pollinator in comparison with other pollinators in all sub-localities. Diversity of *B. haemorrhoidalis* and other pollinators was determined with the help three commonly used indices; Shannon index, Simpson index and Evenness to compare the diversity of different study locations. In 2012, maximum indices and evenness values were recorded in Naran Kaghan valley and minimum in Rawalpindi/Islamabad. In 2013, maximum

Shannon index and evenness were recorded in Naran Kaghan valley while Simpson index was more in Rawalpindi/Islamabad and minimum Shannon index in Rawalakot. Foraging floral resources of native bumblebees comprised 42, 43 and 48 floral plant species in Naran Kaghan, Rawalakot and Rawalpindi/Islamabad, respectively. Maximum species (11) belonged to plant family Asteraceae from all locations. Fabaceae and Lamiaceae were also recorded as major foraging floral species for *B. haemorrhoidalis*. Population fluctuation studies indicated increasing trend in population from June to onwards reaching at peak in September and declining afterwards at all localities. There was no population of bumblebees in December, January and February at Rawalpindi/ Islamabad and Rawalakot and none in November to February at Naran Kaghan Valley.

Nest seeking queens emerged after spending winter diapause in nature and preferred relatively open field landscape followed by open fields. Forest boundaries proved more populated habitats followed by field boundaries for nesting sites. Withered grasses remained most favorite patches and stone and moss the least ones for nesting sites of *B. haemorrhoidalis* at all locations. Maximum nest seeking queens were found in Naran Kaghan while minimum in Rawalpindi/Islamabad.

Biological parameters of *B. haemorrhoidalis* were explored by rearing in laboratory at $25 \pm 2^{o}C$ temperature and 65% relative humidity. Queens started egg laying in 12±0.57 days after shifting from field to laboratory (March-April), egg hatching, larval and pupal periods took 3±1.4, 16±1.2 and 11 days, respectively. First workers emerged after 30 days of egg-laying and colony reached at foundation stage after 57 ± 3.45 days. First males and daughter queens emerged after 79 ± 7.21 and 81 ± 6.04 days, respectively. Mother queens remained alive about 125 days. Among field collected queens, 24 % started bead formation, 7% attained colony maturation stage and 4% were successful to produce sexual morphs. Seasonal fluctuation of sexual morphs indicated first emergence of winter diapause queens in March–April. Maximum population of workers was observed in September while that of males and daughter queens in October in field.

Comparative pollination performance of indigenous *B. haemorrhoidalis* with European bumblebee, *B. terrestris* was observed for tomato plants in quantitative and qualitative way under Hydroponic Research Farm. Insignificant differences existed for number of seeds per fruit, fruit weight and height and fruit diameter for both pollinators. These findings advocated that if successfully reared in laboratory, the indigenous bumblebee can be used as alternate pollinator of exotic species in glasshouse or greenhouse conditions.

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