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RESEARCH ARTICLE

SUCCESSFUL REARING OF BUMBLE BEE, *BOMBUS HAEMORRHOIDALIS* SMITH YEAR ROUND IN HIMACHAL PRADESH IN INDIA

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ABSTRACT

Bombus haemorrhoidalis Smith is a native bumble bee pollinator exhibits an annual life cycle but for commercial rearing, year round rearing is intended. Colonies of *B. haemorrhoidalis* first reared under laboratory conditions and then shifted in the field for further development. Sexual forms were isolated and mating was done artificially in the laboratory. Laboratory mated queens were allowed to start the progeny under controlled conditions. Out of total experimental queens, 83.33 per cent queens started rearing brood and raised the population upto 150-180 workers and thus for the first time in the country successfully reared the bumble bees for more than fifteen months without undergoing hibernation. Different pests were also recorded.

INTRODUCTION

Bumble bees are pollinators, belong to tribe Bombini and Genus *Bombus* found mainly under temperate to subtropical regions of the globe. While some perennial species of these pollinators are found in the tropical places also (Amir and Michener, 1977). These are considered as efficient pollinators because of long working hours, buzz pollination of crops, works well under adverse conditions of temperature and humidity (Corbet *et al*., 1988 and Erikson and Buchmann, 1983). In addition, due to low population and shorter flight range, these bees are very suitable for pollination under protected cultivation, especially solanaceous and cucurbitaceous crops (Velthuis and van Doorn, 2006; Shipp *et al*., 1994; Chauhan, 2011). Different *Bombus* species like *B. terrestris*, *B. impatiens*, *B. accidentalis* etc have been utilized for commercial pollination of different crops in Turkey, Holland, Japan, China, U.K., U.S.A. and many other countries (Kwon and Saeed, 2003; Velthuis and van Doorn, 2006; Klein *et al*., 2007). These species are quite costly to import for crops pollination (Asada and Ono, 2000). Their introduction can also cause different problems like competition in the local species and spread of diseases and pests which might be new to our environment (Couvillon *et al*., 2010; Stout and Goulson, 2000; Whittington and Winston, 2003). Worldwide only five species of bumble bees are commercially reared and in North America only two species, *B. impatiens* and *B. accidentalis* are used for pollination (Velthuis and van Doorn, 2006). Commercial enclosed farming has increased the use of the bumble bees as economically important pollinators for high economic return and offseason crops. Indigenous bumble bee species has been considered important to rear and use for pollination services than imported bumble bees to avoid possible environmental and pest problems. In India, *B. haemorrhoidalis* is natively available major bumble bee species spread from low lands to high altitude region of Himalayas (Saini and Ghattor, 2007) and attempts for its rearing have been made from last one decade (Dayal and Rana, 2004; Thakur, 2006; Chauhan, 2011). Partial success in domestication under laboratory conditions have been achieved but rearing throughout the year was not realized because of lack of diapause studies and unavailability of culture till onset of winters due to pests and diseases attacks (Chauhan *et al*., 2013). Successful attempt was made and present studies were formulated to study diapause for year round rearing of bumble bees.

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MATERIALS AND METHODS

The present investigations were carried out during 2012-2014 in the laboratory and experimental farm of the department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India at an altitude (1250m amsl), longitude of 77^0 $11^{'}$ $30^{''}$ E and a latitude of 30^0 $52^{'}$ $30^{''}$ N subtropical areas.

Rearing of bumble bee colonies

Post hibernated queens of *B. haemorrhoidalis* Smith were collected from different areas of Solan and Bilaspur districts of Himachal Pradesh and brought in the plastic vials to the laboratory. These queens were put solely in the wooden domiciles and maintained in the incubator at $26.9^0C \pm 1^0C$ temperature and 65%±5% relative humidity as per earlier practices (Dayal and Rana, 2004). Initially queens were fed with honey bee collected fresh corbicular pollen (2.68g±0.23g) and fifty per cent sucrose solution (2.22ml± 0.030ml). The colonies/queens were fed daily. Artificial domiciles were also cleaned daily under dark conditions using red light. Data on time taken by the bumble bee queens for wax secretion from the date of capturing was recorded along with the time required for the emergence of workers from the date of wax secretion in the bumble bee colony. Wax secretion pattern as well as the developmental period of different castes of bumble bee, *B haemorrhoidalis* were also studied.

Shifting and establishment of bumble bee colonies in the field

As per earlier practices, developing colonies were kept under laboratory conditions but these survived for four to five months only during 2012-2013 due to more disease and pests incidence under confinement. However, during 2013-2014, developing colonies were shifted to the field for further development when the population of the bumble bee workers in the colony reached to 10-25 workers/colony (Fig. 1). Such colonies were fed daily only till the start of collection of food (nectar and pollen). Foraging activity of the colony was recorded at every two hour interval from 600h-1800h subsequently for three days. Data on the development of colonies with regard to brood development were also gathered. Observations on the emergence of different castes worker, drone and queen were also collected. Studies on mating behavior were conducted.

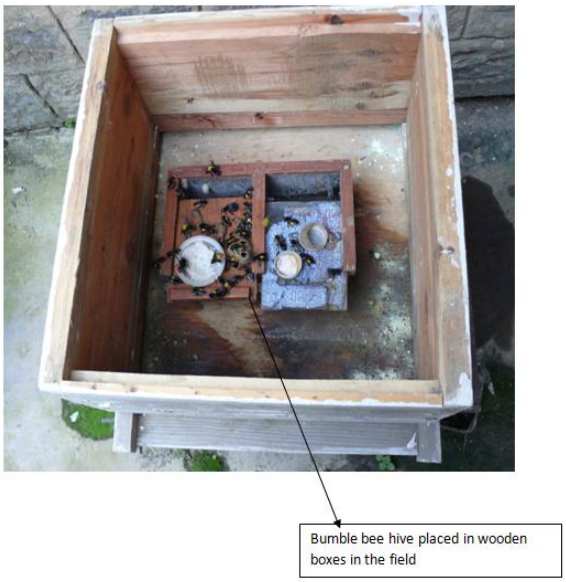

Fig.1 Bumble bee field shifted nest for establishment

Isolation of daughter queen and drones for mating

Five colonies of bumble bees were kept under observation from June 2013 till December 2013 after their shifting to field. Period of drones and queens cell formation and their emergence were observed critically. Mating behavior observations were also recorded under laboratory and field conditions. Twelve queens were isolated from the three mother colonies, four from each colony during the last week of November. These were then allowed to mate under laboratory conditions in the transparent plastic vials (8cmx4cm) (Fig.2 and Fig. 2a). Four queens were later isolated in the first week of December and were not mated under laboratory conditions assuming that they were already mated naturally. Other queens were allowed to remained there in the remaining two colonies to study the fate of colonies in the winter months.

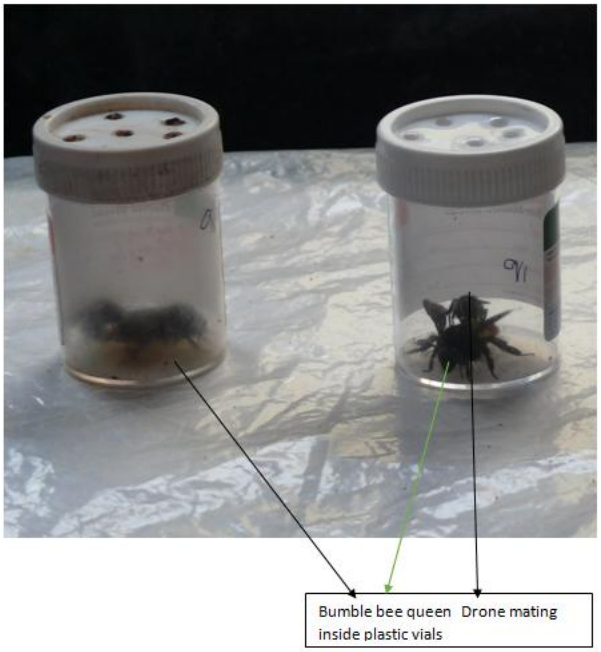

Fig.2. Mating of bumble bees in plastic vials under laboratory conditions

Fig. 2a Mating under open conditions

Diapause in *B. haemorrhoidalis*

Sixteen newly mated queens were maintained under incubator conditions. Out of these, four were $C0_2$ treated and remaining twelve were untreated. $C0_2$ treatment was given as per the method used by Rosler (1985). Similarly, queens in the field established nests were observed till first week of January, 2014 for studying diapause under natural conditions.

RESULTS AND DISCUSSION

Rearing of bumble bee

Fecundated bumble bee foraging queens were captured from various flora listed in Table 1. *Caryopteris bicolor*, *Helichrysum bracteatum, Lupinu shartwegi, Lavendula* sp., *Scutellaria linearis, Salvia moorcroftiana*, *Jakranda mimmosaefolia, Adhatoda vasica and Punica granatum* was found to be the most important flora for sustaining queen population under subtropical areas in Himachal Pradesh. Queens started wax secretion after 7.9±0.74 days of their confinement and workers emerged after 36.0 ±0.84 days after caging queens. First batch of worker bees was emerged on an average 28.1±1.00 days after the laying. The number of worker bees emerged in the first batch was ranged between 1 and 4 bees (average 2.4±0.88 bees).

Thus the total period of emergence of worker bees from the date of capturing was recorded to be 36.0±0.84 days. Similarly, the second batch of worker bees/ brood was found to be emerged after 57.9 ± 1.10 days of the confinement of the queens. The number of worker bees emerged in second batch was 8.8 ± 1.02 bees (6-12 bees) (Table 2). Similar results were found by Chauhan and Thakur (2011) who observed emergence of workers after wax secretion to be 27.22 days. Wax secretion pattern revealed that 53.04% queens secreted the wax in the center of the bottom of wooden domicile while 31.14% queens laid wax in the corners of the bottom of rearing cages. Only 15.82% queens secreted wax in the pollen lids and moulded the brood cells. After the emergence of first batch of workers or before emergence, queens shifted the brood cells from the pollen lid to the bottom of the domicile.

Comparative duration of development stages in different castes

The average egg incubation period of different castes varied between 2.10 and 2.90 days. The development periods of larval stage of drone, queen and worker bees were 16.32, 21.13 and 18.96 days, respectively. Similarly, respective pupal stages were completed in 7.74, 9.74 and 8.74 days. In this way, respective castes took 26.41, 32.96 and 30.60 days to complete the development cycle (Table 3).

Table 1. Important flora from which bumble bee queens were collected

Month	Botanical Name	Common name	Source	Place of collection
February	*Caryopteris bicolor*	Caryopteris	Pollen and nectar	Bilaspur
	Helichrysum bracteatum, Lupinus hartwegi	Paper flower, Lupin	Nectar Nectar and pollen	Bilaspur
March	*Lavendula* sp.	Lavendula	Pollen	Bilaspur
	Delphinium sp.	Larkspur	Pollen	Solan
	Scutellaria linearis	Scutellaria	Pollen	Ramshehr
	Jasminum humile	Chameli,	Pollen and Nectar	Ramshehr
	Viola tricolor	Viola	Nectar	Solan
April	*Salvia moorcroftiana*	Salvia	Nectar	Ramshehr
	Nicotiana tobaccum	Wild tobacco	Pollen and nectar	Ramshehr
	Punica granatum	Wild pomegranate	Pollen	Ramshehr
May	*Jakranda mimmosaefolia*	Gulmohar,	Pollen and Nectar	Ramshehr
	Adhatoda vasica	Basuti	Pollen and Nectar	
	Sonchus sp.	Thisle	Nectar	

Table 2. Oviposition and emergence of workers brood of bumble bee, *B. haemorrhoidalis* under laboratory conditions

	Developmental period (in days)			
Colony number	Nest initiation after caging queen (days)	Duration of emergence of first batch workers after caging queens (days)	Developmental period of first batch (days)	Emergence of second workers batch after caging queens (days)
Colony 1	9	39 (2)	30	62 (11)
Colony 2	6	38 (1)	32	57 (9)
Colony 3	11	35 (2)	24	52 (8)
Colony 4	5	34 (3)	29	60 (12)
Colony 5	13	39 (2)	26	54 (6)
Colony 6	6	37 (4)	31	57 (8)
Colony 7	6	35 (2)	29	54 (7)
Colony 8	8	33 (1)	25	60 (11)
Colony 9	7	39(3)	32	60 (9)
Colony 10	8	31 (4)	23	63 (7)
Mean ± S.E.	7.9±0.74	36.0 ±0.84	28.1±1.00	57.9 ± 1.10

* Values in parenthesis are number of workers

Table 3. Duration of developmental stages of different castes of *B. haemorrhoidalis* under laboratory conditions

Caste	Duration of development of different stages (days)			
	Egg	Larval	Pupal	Total period
Drone	2.35	16.32	7.74	26.41
Queen	2.10	21.13	9.74	32.96
Worker	2.90	18.96	8.74	30.60

Field establishment

Twenty one colonies of bumble bees were shifted in the field in the months of May- July. All colonies established themselves in the field after 3.49±0.22 days of their shifting and started foraging for pollen and nectar as found in natural colonies. Data in Table 4 revealed that activity of bumble bees started at 0600h (6.44) which then increased to maximum at 1000h (14.78) in the morning and after that it started decreasing till evening (10.17).

Table 4. Foraging activity of bumble bee at the nest entrance during July, 2013

Day hours (h)	Incoming workers/15min	Outgoing workers/15min	Mean
0600	2.44	10.44	6.44
0800	4.77	14.56	9.66
1000	8.88	20.67	14.78
1200	8.33	14.22	11.28
1400	11.89	10.11	11.00
1600	14.00	6.88	10.44
1800	17.11	3.22	10.17
Mean	9.63	11. 44	

$CDp=_{0.05}$	
TIME	0.81
INCOMING/OUTGOING WORKERS	0.43
TIME X Workers	1.14

Mating studies

During the month of October 2013, bumble bee colonies started rearing drone and queen brood. Drone cells were round in shape having dimensions (19.26±.032mm) and were found to be overlapped on other cells. Drones emerged before the emergence of queens and took 26.41 days for their emergence. These bear yellow or white pubescence on their head (vertex), thorax (lateral sides) and sometimes on legs also, bigger in size than workers (18.03±0.30mm) and smaller as compared to queen (26.42±0.24mm); have third antennal segment longer than workers and queen. Drones do not sting as these do not bear sting apparatus. Queens emerged later. The queen to drone ratio in a colony was recorded to be 1:5. Mating studies revealed that drones mate after 2±0.67 days of their emergence and the queens started mating after 3-8 days of their emergence with an average of 6.62±0.79 days. Mating observed was least in the queens after 9 days of their emergence and the queens of age of 6 days showed more mating success.

The mating was observed during late morning to early noon hours from 1100h-1400h. Bumble bees exhibit both single mating (single drone) and multiple mating (2-3 drones). In single mating, the mating time was found to be ranged from 15-42 minutes and average time was recorded to be 36.63 min while in the multiple mating, different drones and queen mate for different times. In first mating, the average time was found to be 13.41 min. While for the second and third mating, it was observed to be 20.08 and 5.75 min, respectively. Thus in single mating, queen bumble bee took 36.63 minutes under laboratory conditions in open or in vials (6cm x 4cm). While in multiple matings, she mated with 2-3 drones and took 39.24 minutes (Table 5).

Chauhan (2011) also recorded the mating time under natural conditions which was found to be 7-9 minutes at a single time with a single drone. Earlier, Duvosin *et al*. (2002) have also reported that the mating lasted for 10-30 minutes and occurs in the day time. *B. hyponorum* queens shows multiple mating and mates 2-3 times (Rosler, 1973).

Diapause in *B. haemorrhoidalis*

After mating, the queens were put in the separate wooden cages for brooding. After 25-31 days of their caging, out of twelve daughter queens, ten (83.33 per cent) queens started wax secretion while the remaining two queens died after 6 and 8 days of their captivity. In these colonies 1-3 workers emerged after 28.4±1.02 days of wax secretion. Three queens of bumble bee died in the month of January due to infection with *Nosema* spores (Fig.3) and Conopid fly larvae/pupa (Fig. 4). CO_2 narcotized bees did not started wax secretion and died after 5-7 days of their narcotization because of unknown reasons. By the end of February, only one queen raised the progeny successfully and reared the population more than 140-180 workers and still the colony is working well in the field conditions. Tropical bumble bee, *B. atratus* also reared the brood year round and make perennial colonies under suitable environmental conditions (Amir and Michener, 1977).

While the remaining colonies also alive but the population is low. This may be because of impartial mating. Field established queens foraged till mid-December and left their nests in the last week of December to First week of January, 2014. None of the marked queens were found in the mother nests in the late January, 2014 which reflects that *B. haemorhoidalis* do not hibernate in the mother nest and search new places for hibernation. Different pests/disease were also recorded along with their incidence during the development time (Fig.5).

Table 5. Mating behaviour of bumble bee, *B. haemorrhoidalis* under laboratory conditions

Queen number	Duration of Single mating	Queen number	Multiple mating Duration (min) of			
			First mating	Second mating	Third mating	Total time
S1	37.42	M1	17.13	18.41	0.00	35.54
S2	42.16	M2	21.46	17.42	6.12	45.00
S3	34.00	M3	7.2	22.16	12.16	41.52
S4	32.14	M4	9.06	29.16	0.00	38.22
S5	37.45	M5	12.21	13.29	10.47	35.97
Mean	36.63±1.53		13.41±2.36	20.08±2.23	5.75±2.27	39.25±1.59

The pests were Conopid flies (*Physocephala tibialis*), Bee moth (*Aphomia socialla*), Nematode (*Sphaereularia bombi*), Nosema (*Nosema bombi*) and mites (*Parasitus fucorum*).

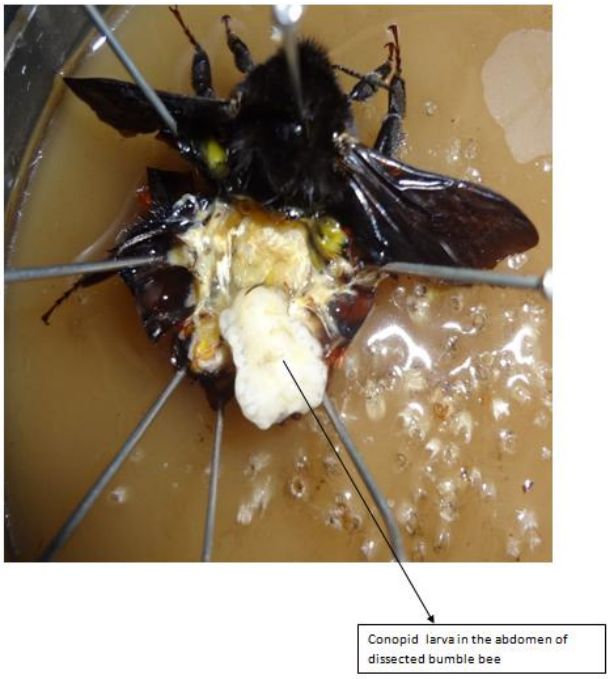

Fig. 3. Conopid fly larvae inside bumble bee abdomen

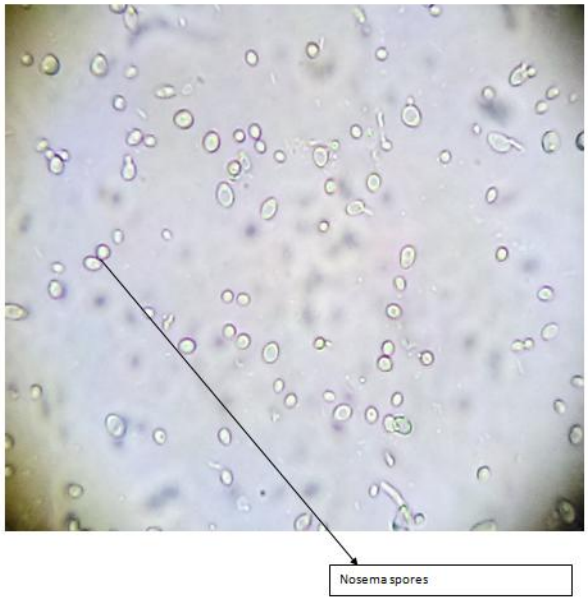

Fig. 4. Nosema spores from bumble bee gut

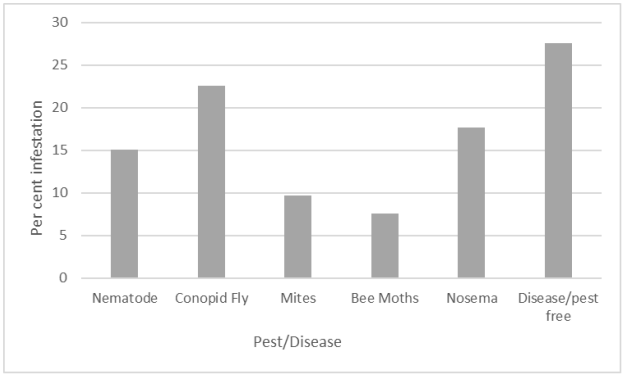

Fig. 5. Per cent infestation of different pests and diseases in bumble bees

Conopid flies, nematodes and mites infest the bumble bee colonies (Chauhan *et al*., 2013) Rearing of brood by the mated daughter queens after mating under incubator conditions revealed that *B. haemorrhoidalis* does not undergo hibernation if congenial conditions of temperature and humidity provided under controlled conditions. The diapause is facultative and can be overcome by providing required brooding environmental conditions of temperature and humidity.

Conclusion

Indian native bumble bee, *B. haemorrhoidalis* can be domesticated by initiating the brooding under laboratory conditions and then transferred to the field conditions for further development. Successful mating can be accomplished under laboratory conditions in the transparent plastic vials or wire cages. *B. haemorrhoidalis* does not undergo obligate hibernation and the diapause is found to be facultative which can be overcome by providing the required conditions of temperature and humidity for brooding thus inferring that this native species can be reared throughout the year. Thus, by capturing naturally mated queens before their desertation or by allowing them mate under controlled conditions, the bumble bee colonies can be available throughout the year for their use for pollination. It was the first successful attempt to rear the bumble bee, *B. haemorrhoidalis* round the year which provides a new base for the establishment of Bombiculture industry in the country to overcome the pollination problems under protected conditions.

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