The Pharma Innovation Journal 2022; SP-11(9): 1745-1750

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(9): 1745-1750

www.thepharmajournal.com
Received: 28-06-2022
Accepted: 30-07-2022

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Comparative studies on domiciles for bumblebee (*Bombus haemorrhoidalis* Smith) rearing

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Abstract
Bumblebees contribute to the pollination of various agricultural and horticultural crops. The use of bumblebees in pollination is highly effective even in protected cultivation. Bumblebees are reared in artificial conditions for their use in fields for pollination. Here we have used different domiciles to test out the colony performance of bumblebees in laboratory rearing. *Bombus haemorrhoidalis* queens were captured from *Caryopteris bicolor*, *Brassica juncea*, *Salvia moorcroftiana*, *Adhatoda vasica*, *Matricaria chamomilla*, *Lupinus mutabilis*, *Papaver rhoeas*, *Trigonella foenum graecum* and *Delphinium ajacis* in nauni area during 2019 and 2020. Among these plants, *A. vasica* and *C. bicolor* were the most important flowering plants from which maximum bumblebee queens were collected from February to April. Three types of nesting materials (wood, cardboard, and polypropylene) were used to rear the bumblebee queens *viz*. wooden boxes, Cardboard boxes, and Polypropylene boxes. Two types of feeding methods were used *i.e*., feeding in bottle caps and automized feeding. The queens that were kept in standard wooden boxes with automized feeding had a shorter pre-oviposition period of 11.25 days and first worker emergence took place 26.25 days after wax secretion and the highest number of workers in the first brood was 5.50 workers/colony. The next best nesting material was a cardboard box having a wooden floor with feeding in bottle caps and automized feeding in which queens had a pre- oviposition period of 33.75 days, first worker emergence took place 46.33 days after wax secretion and the number of workers in the first brood was 3.25. Polypropylene box with feeding in bottle caps and automized feeding was not accepted by the bumblebee queens due to moisture retention.

Keywords: Bumblebee, rearing, domiciles, pollinator, *Bombus haemorrhoidalis*

1. Introduction
Several attempts have been made in order to improve crop production in different crops by introducing bumblebees into the field. For this purpose, bumblebees are reared artificially in the laboratories and the colonies are utilized for the pollination of agricultural crops as well as for scientific studies. Studies on nest architecture helps in creating unique designs of artificial domiciles for laboratory rearing of bumblebees which are useful for pollination of various fruit and vegetable crops grown under open and protected conditions (Jat and Rolania, 2014 and Sowmya *et al*., 2015) [4, 8]. Bumblebees are usually used in greenhouses or caged conditions for pollinating various crops. Successful rearing of bumblebees is being carried out in several countries like Japan, China, Israel, Turkey, etc. Five species of bumblebees *viz*., *Bombus terrestris* (L.), *Bombus impatiens* (C.), *Bombus occidentalis* (G.), *Bombus lucorum* (L.) and *Bombus ignites* (S.) are reared globally for the pollination purpose. *B. impatiens* (C.) and *B. occidentalis* (G.) are reared in North America and are used for pollination (Velthuis and Doorn, 2006) [10]. In India, *B. haemorrhoidalis* Smith was successfully reared for the first time by Dayal and Rana (2004) +. Its rearing has been made for the last one decade (Thakur, 2006; Chauhan, 2011; Yankit, 2016; Nayak, 2018) [9, 1, 11, 5]. The methods for rearing colonies of this social bee have been developed and refined from time to time. Each year innovations are done and applied for betterment in the artificial rearing of bumblebees. Bumblebee queens are collected in the spring season from diverse flora and are utilized for rearing under laboratory conditions. After the emergence of the first worker, the artificial rearing boxes are shifted to the field to allow bees to forage in the field and pollinate the crops.

2. Materials and Methods
2.1 Collection of bumblebee queens
Foraging bumblebee (*B. haemorrhoidalis*) queens were collected from the fields at Nauni

during spring in the early morning and evening. Queens were captured while foraging for pollen and nectar on different plants or hovering over soil to find a spot for nesting. Foraging queens were caught using an insect net, transferred into plastic vials having perforated lids and brought to the laboratory for rearing.

2.2. Maintenance of bumblebee queens under laboratory conditions

Collected bumblebee queens were kept in different types of nesting boxes (domiciles) and maintained in the B.O.D. incubator at 25 ± 1 °C temperature and 65-70 per cent relative humidity under laboratory conditions. Bumblebee queens were provided with freshly prepared 50 percent sucrose solution and fresh collected corbicular pollen from honeybee colonies as per the requirement of the bumblebee queen or colony. The queen bees were fed daily and the domiciles were cleaned under dark conditions using red light daily. The date of collection of bumblebee queens from the field was noted. Domiciles were observed daily and data was recorded regularly for the time taken for the secretion of wax and the emergence of the first worker after wax secretion in the bumblebee colony.

2.3 Nesting material

Three types of nesting materials (wood, cardboard, and polypropylene) were used to rear the bumblebee queens *viz.* wooden boxes (standard size 16 × 11 × 8 cm), Cardboard boxes and Polypropylene boxes having dimensions of 24 × 17 × 12 cm. Two types of feeding methods were used i.e., feeding in bottle caps and automized feeding. Plastic pipes were arranged in a manner to insert the pollen and sucrose into the nesting box without opening the lid of the box, to ensure minimum disturbance to the bees. Three nesting materials and two types of feedings were combined into six treatments. Each treatment was replicated four times.

2.4 Details of treatments

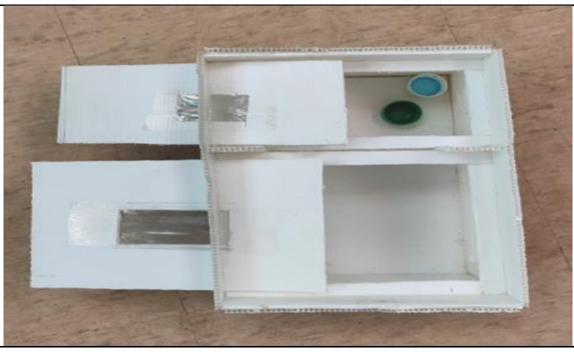
The details of different rearing domiciles are enlisted below:

a) T_1 (Polypropylene box + feeding in bottle caps)
b) T_2 (Polypropylene box + automized feeding)
c) T_3 (Cardboard boxes + wooden floor + feeding in bottle caps)
d) T_4 (Cardboard boxes + polypropylene floor + feeding in bottle caps)
e) T_5 (Cardboard boxes + wooden floor + automized feeding)
f) T_6 (Cardboard boxes + polypropylene floor + automized feeding)
g) T_7 (Wooden boxes + automized feeding)/control

2.5 Observations recorded

a) Date of collection of bumblebee queens
b) Egg laying/wax secretion after queens are placed in individual nest boxes (colony initiation)
c) Day of the emergence of the first worker
d) Number of workers in the first brood

The data were analyzed using MS Excel and OPSTAT. The mean value of data was subjected to analysis of variance as described by Panse and Sukhatme (2002) [6] for Randomized Block Design.



T_1- Polypropylene box + feeding in bottle caps

T_2- Polypropylene box + automized feeding

T_3- Cardboard boxes + wooden floor + feeding in bottle caps

T_4- Cardboard boxes + polypropylene floor + feeding in bottle caps

T₅- Cardboard boxes + wooden floor + automized feeding

T₆- Cardboard boxes + polypropylene floor + automized feeding

T₇- Wooden boxes + automized feeding (control)

Fig 1: Different domiciles used for the rearing of *B. haemorrhoidalis*

3. Results

3.1 Collection of *B. haemorrhoidalis* queens

B. haemorrhoidalis queens were collected with the onset of spring in the early morning and evening hours. Queens were trapped while foraging on the flowers for pollen and nectar from different bee flora of the Nauni area. The list of important bee flora from where the queens were collected in different months is presented in Table 1. It was observed that during February the *B. haemorrhoidalis* flora was Bluebeard, mustard, sage, basuti and chamomile, during March basuti, lupin, and golden poppy, whereas in April, the flora was basuti, methi and rocket larkspur. It is clear from the studies that Bluebeard, mustard, sage, chamomile, basuti, lupin, golden poppy, methi, and rocket larkspur was the available flora for *B. haemorrhoidalis* queen subsistence.

Table 1: Important bee flora for the collection of *B. haemorrhoidalis* queens in the nauni area

Month	Common name	Botanical name	Trapping time (hr)	No. of queens collected
February	Bluebeard	*Caryopteris bicolor* (M.)	Morning, Evening	8
	Mustard	*Brassica juncea* (L.)	Evening	2
	Sage	*Salvia moorcroftiana* (L.)	Morning, Evening	1
	Basuti	*Adhatoda vasica* (L.)	Morning	1
	Chamomile	*Matricaria chamomilla* (L.)	Evening	2
March	Basuti	*Adhatoda vasica* (L.)	Evening	4
	Lupin	*Lupinus mutabilis* (L.)	Morning	1
	Golden poppy	*Papaver rhoeas* (L.)	Morning	3
April	Basuti	*Adhatoda vasica* (L.)	Evening	5
	Methi	*Trigonella foenum-graecum* (L.)	Evening	1
	Rocket larkspur	*Delphinium ajacis* (L.)	Morning	1

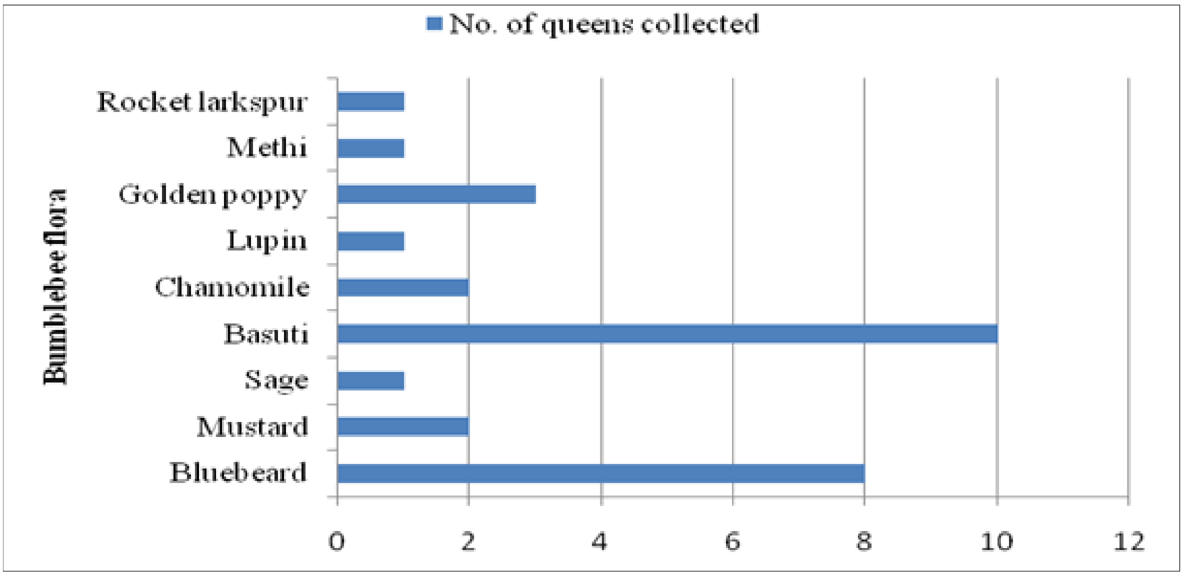


Fig 2: Important bee flora for the collection of *B. haemorrhoidalis* queens in the Nauni area

The major flowering plant for the collection of bumblebee queens in the Nauni area was reported to be *Adhatoda vasica* by earlier workers, Yankit (2016) [11], Singh (2017) [7], Thakur (2006) [9], Nayak (2018) [5] and Devi (2019). In addition to this, Bluebeard (*Caryopteris bicolor* M.) was reported as a major flowering plant, whereas sage (*Salvia moorcroftiana*) and chamomile (*Matricaria chamomilla*) were reported as minor flowering plants for the first time in the Nauni area for collection of bumblebee queens in the present studies (Fig. 2).

3.2 Maintenance of *B. haemorrhoidalis* queens under laboratory conditions

B. haemorrhoidalis queens collected from different flora were maintained under laboratory conditions (25 ± 1 °C and 65-70 % RH) in different types of domiciles. The date of collection of bumblebee queens from the field was noted. Domiciles were observed daily and data was recorded regularly on the time taken for the secretion of wax (colony initiation) and the emergence of the first worker after wax secretion in the bumblebee colony.

3.3 Effect of different types of nesting materials on colony initiation of *B. haemorrhoidalis*

B. haemorrhoidalis queens were reared in three different types of nesting materials under laboratory conditions namely wooden, cardboard, and polypropylene (Fig. 1). The internal floor of the cardboard boxes was made up of wooden and polypropylene material to prevent the deterioration of cardboard with moisture and wax secretion by bumblebee queens during colony initiation. There were seven treatments and each was replicated four times. Therefore 28 queens were used in this experiment.

3.4 Pre-oviposition period

In *B. haemorrhoidalis,* the pre-oviposition period is described as the number of days from the date of queen collection to the date of wax mound formation by the queen (Fig 2). The data presented in Table 2 revealed that the pre-oviposition period was significantly minimum (11.25 days) in queens kept in standard wooden domicile with automized feeding followed by those kept in cardboard boxes having a wooden floor with feeding in bottle caps (33.75 days), cardboard box having a wooden floor with automized feeding (34.25 days), polypropylene box with automized feeding (43.50 days), cardboard box having polypropylene floor with automized feeding (53.00 days), cardboard box having polypropylene floor with feeding in bottle caps (55.33 days, whereas maximum pre-oviposition period was recorded in queens kept in polypropylene box with feeding in bottle caps (63.00 days). Data recorded on the pre-oviposition period of *B. haemorrhoidalis* showed that the queens kept in a standard wooden box with automized feeding initiated early wax secretion and wax mound formation as compared to all other treatments.

The data further showed that *B. haemorrhoidalis* queens preferred cardboard boxes having a wooden floor with feeding in bottle caps, cardboard box having a wooden floor with automized feeding and standard wooden box with automized feeding for egg laying as all the queens (100%) were recorded to lay eggs in these treatments. Seventy-five percent acceptance for egg laying was recorded in two treatments namely cardboard box having polypropylene floor with automized feeding and cardboard box having polypropylene floor with feeding in bottle caps. Whereas, the polypropylene box with feeding in bottle caps and polypropylene box with automized feeding treatments were least preferred by *B. haemorrhoidalis* queens as only 50 percent of queens started egg laying in these nesting boxes. The present studies indicate that a standard wooden box with automized feeding was most preferred (100% acceptance) with the minimum pre-oviposition period (11.25 days). The next best treatment is the cardboard box having a wooden floor with feeding in bottle caps and the cardboard box having a wooden floor with automized feeding with 75 % acceptance.

Table 2: Effect of different types of nesting boxes on the pre-oviposition period of *B. haemorrhoidalis* queens

Treatment code	Pre-oviposition period (days)					
	Q1	Q2	Q3	Q4	Mean	Queen laid eggs (%)
T1	78	48	-	-	63.00	50
T2	49	38	-	-	43.50	50
T3	28	42	30	35	33.75	100
T4	35	31	41	30	34.25	100
T5	52	49	65	-	55.33	75
T6	59	51	49	-	53.00	75
T7	10	15	12	8	11.25	100
CD (0.05)	T1, T2=15.10; T1, T3=13.07; T1, T4=13.07; T1, T5=13.78; T1, T6=13.78; T1, T7=13.07; T2, T3=13.07; T2, T4=13.07; T2, T5=13.78; T2, T6=13.78; T2, T7=13.07; T3, T4=10.67; T3, T5=11.53; T3, T6=11.53; T3, T7=10.67; T4, T5=11.53; T4, T6=11.53; T4, T7=10.67; T5, T6=12.33; T5, T7=11.53; T6, T7=11.53					

3.5 First worker emergence

In *B. haemorrhoidalis*, the number of days taken from the pre-oviposition period to the emergence of the first worker was counted to determine the effectiveness of the nesting material in the development of the *B. haemorrhoidalis* colony (Fig 3). The lesser the number of days taken by the *B. haemorrhoidalis* queens from wax secretion to the emergence of the first worker, the more acceptable the nesting material was found. The data on the effect of different types of nesting material on the emergence of the first worker after wax secretion is presented in Table 3. The data showed that 100 percent of queens produced workers successfully when kept in a standard wooden box with automized feeding followed by 75 percent of queens in cardboard box having a wooden floor with feeding in bottle caps and 50 percent in cardboard box having a wooden floor with automized feeding, cardboard box having polypropylene floor with feeding in bottle caps and cardboard box having polypropylene floor with automized feeding.

The data presented in Table 3 showed that a significantly minimum number of days (26.25 days) was recorded for first worker emergence after wax secretion in *B. haemorrhoidalis* queens kept in a standard wooden box with automized feeding. It was followed by cardboard box having a wooden floor with automized feeding (45.00 days), cardboard box having a wooden floor with feeding in bottle caps (46.33 days), cardboard box having a polypropylene floor with automized feeding (53.50 days) and cardboard box having polypropylene floor with feeding in bottle caps (55.00).

Table 3: Effect of nesting material on the duration of first worker emergence after wax secretion

Treatment code	First worker emergence after wax secretion (days)					
	Q1	Q2	Q3	Q4	Mean	Queens produced workers (%)
T1	-	-	-	-	-	Nil
T2	-	-	-	-	-	Nil
T3	45	56	38	-	46.33	75
T4	34	56	-	-	45.00	50
T5	59	51	-	-	55.00	50
T6	47	60	-	-	53.50	50
T7	24	21	28	32	26.25	100
CD (0.05)	T3, T4=16.75; T3, T5=16.75; T3, T6=16.75; T3, T7=14.01; T4, T5=18.35; T4, T6=18.35;T4, T7=15.89; T5, T6=18.35; T5, T7=15.89; T6, T7=15.89					

3.6 Number of workers in the first brood

The data presented in Table 4 on the number of workers who emerged in the first brood revealed that a significantly maximum number of workers (5.50 workers/colony) in the first brood emerged from queens kept in a standard wooden box with automized feeding followed by those kept in cardboard box having a wooden floor with automized feeding (3.25 workers), cardboard box having a wooden floor with feeding in bottle caps (2.75 workers), cardboard box having polypropylene floor with feeding in bottle caps (0.75 workers), whereas a minimum number of workers emerged in cardboard box having polypropylene floor with automized feeding (0.50 worker). No worker emergence was observed in queens kept in polypropylene boxes with automized feeding and polypropylene box with feeding in bottle caps however, 50 percent of queens started wax mound formation in these boxes.

Table 4: The effect of nesting boxes on the number of workers emerged in the first brood

Treatment code	Number of workers in the first brood				
	Q1	Q2	Q3	Q4	Mean
T1	0	0	0	0	0.00
T2	0	0	0	0	0.00
T3	6	5	0	0	2.75
T4	7	6	0	0	3.25
T5	2	1	0	0	0.75
T6	1	1	0	0	0.50
T7	5	4	7	6	5.50
CD (0.05)	2.70				

The present findings on the effect of nesting materials on *B. haemorrhoidalis* rearing revealed that queens preferred standard wooden boxes with automized feeding for egg laying and colony development as they started egg laying earlier (11.25 days), earlier emergence of the first worker (26.25 days) and produced more mean number of workers (5.50) in first brood as compared to other nesting material and type of feeding combinations.

Fig 2: Wax cell formation by *B. haemorrhoidalis* in a cardboard box having a wooden floor

Fig 3: Worker emergence by *B. haemorrhoidalis* in a standard wooden box with automized feeding

In the present studies, modification of the floor of cardboard boxes with wooden plank and polypropylene sheet (used in both brooding and feeding chambers) was done to prevent the deterioration of the cardboard due to defecation by bumblebees and moisture absorption, and it was observed better. *B. haemorrhoidalis* showed a preference for cardboard boxes having wooden floors with both automized and bottle caps feeding, next to a standard wooden box with automized feeding for colony initiation and colony development. In the current studies of polypropylene domiciles, wax secretion was observed but further development by bumblebees was not observed. This may be due to higher moisture retention and low ventilation.

4. Conclusion

Bombus haemorrhoidalis queens were captured from *Caryopteris bicolor*, *Brassica juncea*, *Salvia moorcroftiana*, *Adhatoda vasica*, *Matricaria chamomilla*, *Lupinus mutabilis*, *Papaver rhoeas*, *Trigonella foenum-graecum* and *Delphinium ajacis* in nauni area during 2019 and 2020. Among these plants, *A. vasica* and *C. bicolor* were the most important flowering plants from which maximum bumblebee queens were collected from February to April. The queens that were kept in standard wooden boxes with automized feeding had a shorter pre-oviposition period of 11.25 days and first worker emergence took place 26.25 days after wax secretion and the highest number of workers in the first brood was 5.50 workers/colony. The next best nesting material was a cardboard box having a wooden floor with feeding in bottle caps and automized feeding in which queens had a pre-oviposition period of 33.75 days, first worker emergence took place 46.33 days after wax secretion and the number of workers in the first brood was 3.25. Polypropylene box with feeding in bottle caps and automized feeding was not accepted by the bumblebee queens.

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