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EFFECT OF DIFFERENT STIMULATION METHODS ON COLONY SURVIVAL AND DEVELOPMENT OF *BOMBUS HAEMORRHOIDALIS* SMITH IN HIMACHAL PRADESH

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ABSTRACT

One of the key steps to facilitate year-round rearing of bumblebees is to break diapause period. In the present investigations, different stimulation methods were evaluated viz., cold temperature and CO_2 narcosis, and their effects on colony parameters were studied. The daughter queens collected during winters were exposed to low temperatures i.e. 4± 0.5 ˚C for two months. But this did not show any results as all the queens died after two months. Another method used was exposing the queens with single and double CO_2 narcosis. The present study concluded that bumblebee workers kept along with double narcotized queens was one of the best stimulation methods followed by single narcotized queens kept along with bumblebee workers.

Key words: bumblebees, rearing, diapause, stimulation methods, CO_2, narcosis, temperature, colony, parameters, daughter queens, bumblebee workers

Bumblebees are one of the most important groups of pollinators and due to higher pollination efficiency; their use in pollination of crops grown under protected cultivation has been increased widely. Following this, their industrial rearing techniques have been developed extensively in many countries all over the world (Pinchinat et al., 1979) and if it can be performed on an economic scale, year-round laboratory rearing appears to be one of the best long-term methods for mass production of bumblebees (Abak et al., 1995). Thus, several laboratories have been established all over the world including China for rearing bumblebees and a large number of scientific studies have been carried out on social behaviour, biology, pollinating application and others since 1996, which have made remarkable progress (An et al., 2001; Peng et al., 2003; Guo et al., 2003). For all these purposes, it is desirable to culture bumblebees throughout the year but due to its annual life cycle, it becomes more difficult and is the major criterion to be kept in mind while rearing bumblebees all over the year.

B. haemorrhoidalis, an indigenous bumblebee of India, starts its life cycle at the onset of spring under natural conditions when they emerge from hibernation and spend maximum time searching for suitable sites for nesting. After nesting, they start brooding and developing a new colony but during winters the daughter queens emerged will enter diapause (Chauhan et al., 2013; Owen, 2016). Diapause is an intrinsic characteristic of queens and is independent of external factors such as temperature and light (Alford, 1969).

The long hibernation period and annual life cycle, the social structure of the colonies and the lack of productive shelf life are major hindrances in the year round rearing of bumblebees (Hughes, 1996). The storage of hibernating queens at low temperature for several months to mimic diapause has been widely used for commercial rearing purposes. Previous studies revealed that a CO_2 treatment could also be used to inhibit diapause as well as to activate overwintered queens (Roseler, 1985). In India, there has been meagre research on the effects of CO_2 narcosis on the onset of oviposition and colony development in post-diapausing *B. haemorrhoidalis* queens. In this study, we worked out the effect of cold temperature and CO_2 narcosis on colony development and the survival rate of *B. haemorrhoidalis* colonies.

MATERIALS AND METHODS

Fecundated *B. haemorrhoidalis* queens were collected while foraging during the month of March-May (2018-19) and then reared under laboratory conditions (25-30 ^{0}C temperature and 60-65% RH) until first brood emergence. These queens were provided with 50% sucrose solution and fresh pollens on a daily basis and proper hygienic conditions were maintained by cleaning the hives using cotton with water and disinfectant. Once first brood emerged the well-developed colonies were brought under natural field conditions during the month of May-July and kept under the shade net house for providing favourable conditions. These colonies were observed daily up to October-December for emergence of drones and daughter queens. The newly emerged daughter queens were collected using pointed forceps and brought to the laboratory for further studies.

The cold treatment was given to daughter queens by inserting into plastic vials having holes on their top which was later stored in the refrigerator at 4±0.5 ^{o}C for two months. The hibernated queens that emerged during spring were collected in the month of February and March. These queens were given a single (treatment of carbon dioxide for 30 minutes) and double (treatment of carbon dioxide for 30 minutes in two consecutive days) CO_2 narcosis treatment as described below. For this, the queens collected were transferred to glass jar and were exposed to the mild stream of carbon dioxide i.e. one bubble/ sec for 30 minutes.

A total of six treatments were taken under the present study viz., T1: Single narcosis treated queen with three bumblebee workers, T2: Double narcosis treated queen with three bumblebee workers, T3: Single narcosis treated queen with three honey bee workers, T4: Double narcosis treated queen with three honey bee workers, T5: Control with three bumblebee workers, T6: Control with three honey bee workers. Honey bee workers were taken in alteration with bumblebee workers to check which one of them would contribute more to the stimulation of bumblebee queens and whether they are compatible with bumblebee queens or not. After narcotization, these queens were reared as per standard method of bumblebee rearing (Devi, 2019) and kept along with three bumblebee workers or three honey bee workers (used for stimulation of queens to lay eggs) and their effect on survival as well as life history parameters were studied using Completely Randomized Block Design.

RESULTS AND DISCUSSION

A total of nine daughter queens were harvested from field established colonies during the months of October-November and were brought to the laboratory.

Cold treatment

Out of total nine harvested queens, six queens were kept under refrigerated conditions at a temperature of 4± 0.5 ^{0}C for two months. However, remaining three queens were kept in BOD at 25-30 ^{0}C temperature and 60-65% RH. All the cold treated queens died due to exposure of low temperature while only one queen under control initiated wax secretion which also died within 5-6 days of wax secretion.

CO_2 treatment

a) Pre-oviposition period

The pre-oviposition period was found minimum (3.80 days) in double narcotized queens kept with bumblebee workers followed by single narcotized queens with bumblebee workers (4.75 days) which were statistically at par. A significantly longer pre-oviposition period (19.30 days) was observed in single narcotized queens kept with honey bee workers which were statistically at par with control along with honey bee workers (18.00 days). The data in Table 1 also revealed that 100% egg laying was observed in

Table 1. Effect of different stimulation methods on pre-oviposition period of *B. haemorrhoidalis*

Stimulation methods	Mean	Queens laid egg (%)
Single narcosis + bumblebee workers (A)	4.75	80
Double narcosis + bumblebee workers (B)	3.80	100
Single narcosis + honey bee workers (C)	19.30	60
Double narcosis + honey bee workers (D)	13.00	40
Control + bumblebee workers (E)	15.00	80
Control + honey bee workers (F)	18.00	20
C.D. p=0.05	A,B = 4.39; A,C = 5.00; A,D = 5.66; A,E = 1.52; A,F = 1.86; B,C = 1.57; B,D = 1.79; B,E = 1.44; B,F = 1.79; C,D = 1.96; C,E = 1.64; C,F = 1.96; D,E = 1.86; D,F = 2.14; E,F = 1.86	

Table 2. Effect of different stimulation methods on emergence of first brood and mortality rate (%) under laboratory conditions

Stimulation methods	First worker emergence (days)	Workers emerged (number)	Mortality rate (%)
Single narcosis + bumblebee workers (A)	17.00	1.00	40
Double narcosis + bumblebee workers (B)	23.33	5.00	20
Single narcosis + honey bee workers (C)	Nil	Nil	60
Double narcosis + honey bee workers (D)	Nil	Nil	100
Control + bumblebee workers (E)	Nil	Nil	20
Control + honey bee workers (F)	Nil	Nil	60

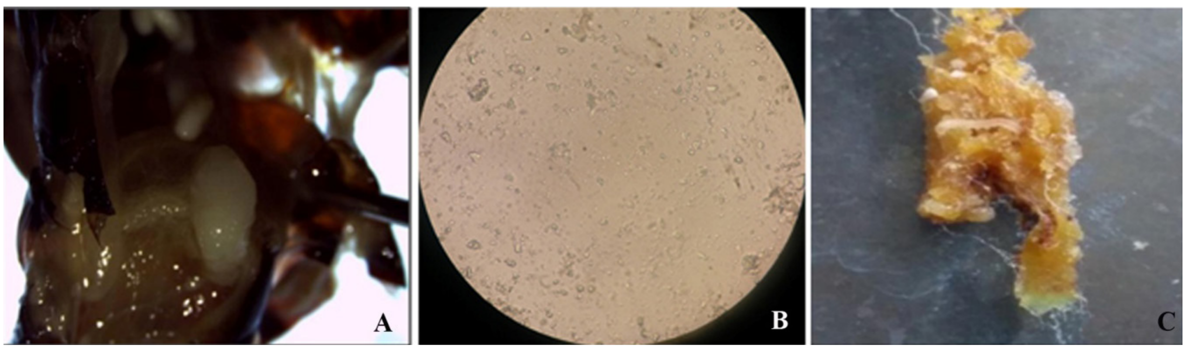

Fig. 1. Pests and disease infestation in *B. haemorrhoidalis* (A. Conopid fly larva in abdomen of *B. haemorrhoidalis* queens; B. Microscopic view of nosema spores; C. Wax moth larva)

double narcotized queens kept with bumblebee workers followed by 80% in single narcotized with bumblebee workers and control with bumblebee workers, 60% in single narcotized queens with honey bee workers, 40% in double narcotized queens with honey bee workers and 20% in control with honey bee workers.

b) First worker emergence and number of workers in first brood

First worker emergence was observed only in single and double narcotized queens kept with bumblebee workers. However, no worker emergence was observed in case of other stimulation methods. It was also observed that double narcotized queens produced higher number of workers (5.00 workers/ colony) in comparison with single narcotized queens (1.00 worker/ colony). While single narcotized queens with bumblebee workers took less duration for worker emergence (17.00 days) as compared to double narcotized queens (23.33 days) (Table 2).

c) Mortality rate

Highest mortality rate (100%) was observed in double narcotized queens kept with honey bee workers followed by single narcotized queens with honey bee workers and control with honey bee workers (80%), single narcotized queens with bumblebee workers (40%) and double narcotized queens with bumblebee workers and control with bumblebee workers (20%) (Table 2).

The higher mortality of *B. haemorrhoidalis* queens was observed due to infestation with nosema (66.67%), bee moth larva (6.67%) and conopid fly larva (26.67%) (Fig. 1).

The present findings are supported by the findings of previous researchers (Gurel and Gosterit, 2008; Roseler, 1985; Kwon et al., 2003; Eijnde et al., 1991). Gurel and Gosterit (2008) reported that *B. terrestris* queens kept with bumblebee workers is one of the effective methods for year-round rearing of bumblebees. Roseler (1985) also reported that the single and double narcotized queens produced eggs earlier as compared to non narcotized queens. He also reported that single CO_2 narcotized queens showed best results over post diapason queens. Eijnde et al. (1991) also treated mated queens with CO_2 twice for 30 minutes following Roseler's study. However, Kwon et al. (2003) found the addition of young male pupa to single queens was one of the best tool for production of good colonies. Karsli and Gurel (2013) also observed that single (6.69±0.28 days) and double narcotized queens (6.06±0.23 days) initiate colony earlier as compared to non narcotized queens (11.22±1.29 days).

Thereafter, 80% single and double narcotized queens laid eggs while only 45% queens laid eggs in case of non narcotized queens.

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