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First record of successful rearing of *Bombus tunicatus* Smith : a lab setting in Himachal Pradesh, India

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ABSTRACT

Nowadays, due to agricultural modernization, the habitats crucial for the survival of animals and plants have significantly deteriorated. Meadows and pastures have largely disappeared, replaced by covered drains instead of ditches. Bumble bees are crucial pollinators for the agriculture sector and wild plants, and provide pollination supports. Bumble bees need confined access to good inhabiting and slumber locations as well as to alternate substenance sources during times when crop plants are not in bloom for them to be effective, though. Bumble bees are efficient pollinators with high pollination efficiency, and the idea of commercializing these pollinators has gained attention due to the rising demand for pollination services. By taking into account, the present study was conducted to explore the suitable rearing method of the new bumble bee species, *Bombus tunicatus* Smith under laboratory conditions at a temperature of 27±1°C and relative humidity of 65-70 per cent. Spring overwintered bumble bee queens were collected from Nankheri, Shimla (Elevation: 2086 m), and were reared by continuously feeding them with a 50% sucrose solution and fresh pollen pellets. The average period for wax secretion was 19.3±1 days and the mean development period from egg to adult in workers of *B. tunicatus* was recorded as 31-33 days. This is the first study of *B. tunicatus* rearing under laboratory conditions for which further development is needed. To establish a commercial source of pollination in the future, it is required to corroborate their potential or identify suitable species and procedures.

Key words: *Agricultural modernization, Bumble bees, Conservation, Food security, Pollinators*

Introduction

Bumble bees (*Bombus* sp.) belong to the Hymenoptera order and Bombidae family are the most important pollinators under greenhouse and open field conditions and are considered a bio-diversity conservator (Svensson *et al.*, 2000; Amala and Shivalingaswamy, 2017). They live in the temperate zone and have three castes: the queen, the workers, and the drones (Amsalem *et al.*, 2015; Sharma *et al.*, 2022). Bumble bees include approx. 250 species globally, amongst them 48 species are found in India.Under conrolled conditions, bumble bees are seen to be the more effective and dependable pollinators because they perform well in cold, unfavorable weather conditions where honey bee activity is minimal (Thakur, 2012). Additionally, bumble bees can be raised in controlled environments and utilized to pollinate crops cultivated under protected agriculture, such strawberries, capsicum, etc. They

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are well acclimated to the cramped greenhouse circumstances because, despite the windows being opened, they do not fly against them. Workers and males pass away in the late summer and early fall, while queens are the only caste to overwinter (go through diapause), (Sharma *et al*., 2022). Universally five species of bumble bees are used for commercial pollination *viz., Bombu sterrestris*, *B. lucorum*, *B. occidentalis*, *B. ignitus,* and *B. impatiens* (Khalifa *et al*., 2021). Of these, two bumble bee species (*B. terrestris* and *B.impatiens*) have the most prominent role. Numerous native bumble bee species are present in India, *viz*., *Bombu swaltoni* (C.), *B. haemorrhoidalis* (S.), *B. kereinsis*, *B. himalayanus* (S.), *B.asiaticus* (M.), *B. personatus* (S.), *B. rufofaciaticus* (S.), *B. trifaciatus* (S.) and *B. tunicatus* (Sharma *et al.,* 2021). One of these, *B. haemorrhoidalis*, is a widely distributed species found in the Himalayas' lowlands and higher elevations (Saini and Ghattor, 2015). *B. haemorrhoidalis*, a natural bee species, was first domesticated in India in 1997–1998 in a lab setting (Thakur, 2002) in the Apiculture laboratory of Department of Entomology, Dr. YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh. However, Dayal and Rana (2004) were the first to successfully domesticate the bumble bee (*B. haemorrhoidalis*) in captivity. According to past practices, they raised over wintered queens in two-chambered wooden boxes with controlled humidity and temperature levels while feeding them pollen (Ono *et al*., 1994). In an effort to better understand the biology and life cycle of *B. haemorrhoidalis*, many workers have improved nesting materials and rearing habitats (Dayal and Rana, 2004; Thakur *et al.* 2008; Thakur and Kashyap, 2008; Chauhan *et al*., 2013; Yankit, 2018; Sharma *et al*. 2018). Today, efforts are made to choose appropriate natural species and create techniques for its commercialized rearing (Padilla *et al*., 2017). So far in India, attempts were made in the last decade to rear *B. haemorrhoidalis* native bumble bee species and success has been achieved up to small-scale rearing. In this study, our aim was to rear new bumble bee species (*Bombus tunicatus*) other than *B. haemorrhoidalis* under laboratory conditions and to study its developmental biology.

Materials and Methods

Study area

Survey and collections for rearing new bumble bee species were done in Nankheri (Shimla) and Chitkul valley (Kinnaur) at altitude of 2086 and 3450 m amsl, with geo-coordinates 31.30^0 N, 77.58^0 E and 31.35^0 N, 78.43^0 E, respectively (Fig. 1). Attempts were made to rear bumble bees collected from Chitkul valley (Kinnaur) and Nankheri (Shimla). Queens and workers of bumble bees, were collected for rearing. The rearing was done during 2021 in the apiculture laboratory of the department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India at an altitude 1250 mamsl.

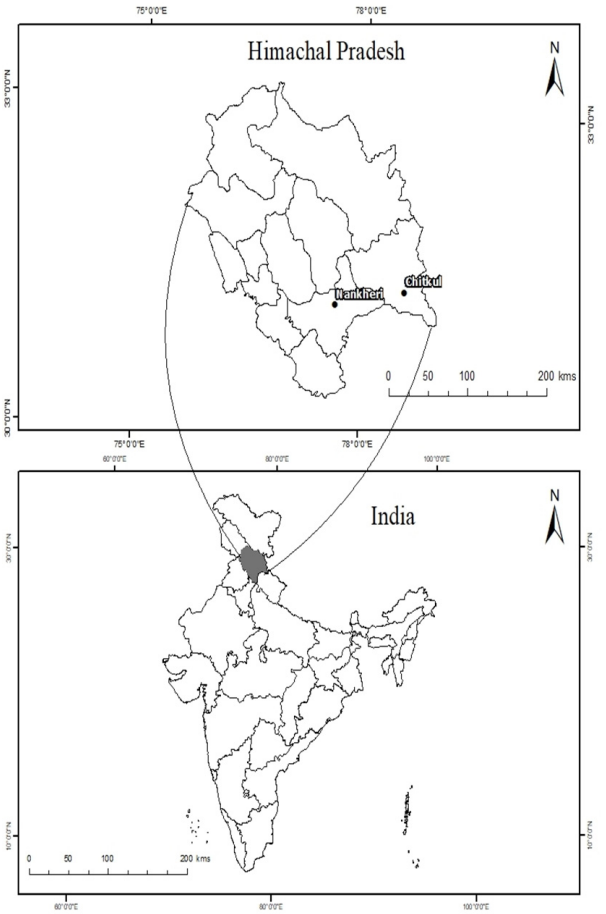


Fig. 1. Map showing the study area for sampling of bumble bee specimens for rearing

Sample Collection

The collection, which included several ecosystems related to horticulture, agriculture, and forests, was based on a random sampling methodology. The bumble bees sampling was done by sweeping hand nylon insect catching net. Bumble bees were collected in the small plastic vials having ventilation holes and brought to Apiculture laboratory, Nauni.

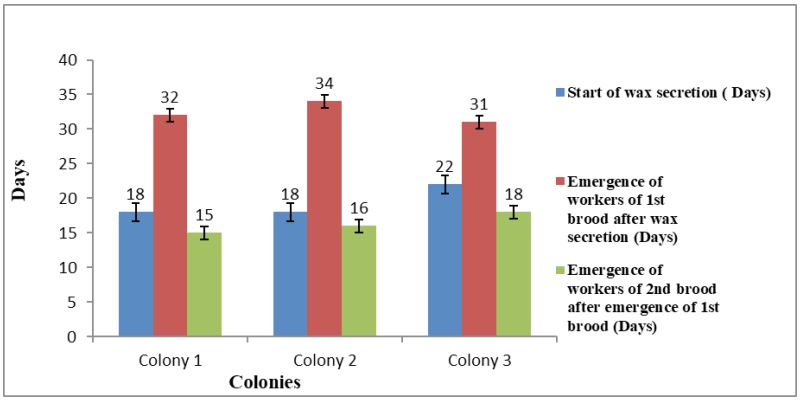
Collected bees were provided with cotton balls dipped insucrose solution(1:1) at the time of transportation. Dead specimens were removed and pinned for species identification. The live specimens (Queens and workers) of same species were kept in two chambered wooden domiciles (16cm x11 cm x 8 cm) with perforated side walls, earlier used for rearing *B. haemorrhoidalis*. These domiciles were shifted to culture room of Department of Entomology, which was maintained at 25-28 ^{0}C temperature and 60-70 per cent relative. During survey, pictures of the bumble bee species, surroundings, habitats, and host plants were obtained. Later, the specimens were identified using the literature that was at hand and information obtained from the Jodhpur branch of the Zoological Survey of India.

Maintenance of bumble bee queens under laboratory conditions: The wooden domiciles were kept in the dark with the provision of red light so that bumble bees were least disturbed during feeding and cleaning of domiciles. The queens were fed every day. Freshly prepared 50% sucrose solution, freshly collected pollen, or frozen corbicular pollen gathered by honey bee colonies were fed to the queens.In the brood room, the feeding was done in tiny petri dishes or plastic containers/caps with dimensions of 2.85–3.14 cm in diameter and 1.5–2.0 cm in height. Artificial domiciles were cleaned daily. By cleaning the rearing box using hot water every two days, hygienic conditions were maintained. With the help of forceps, cotton was first dipped in the hot water then carefully without disturbing the bumble bees, their excreta was removed and thereafter washing of all feeding and other handling accessories with laboratory reagent and then rinsed with 70 percent ethanol for sanitation. Under controlled laboratory circumstances, the bumble bee's development period was split into two phases. The first brood's appearance after wax secretion, the number of workers in the first brood following the queen's entrapment were all noted during the colony initiation stage. We kept track of the number of workers in the second brood, development time, and the number of days between the appearance of the first and second broods at the colony foundation stage.

Results and Discussion

Rearing of *Bombus tunicatus* under laboratory conditions

At colony initiation stage: The average time from the day the queens were trapped until wax produc-



Graphical representation of *Bombus tunicatus* colony development

Table 1. Development of *Bombus tunicatus* colonies from queens

Colonies	Start of wax secretion (Days)	Emergence of workers of 1st brood after wax secretion (Days)	No. of workers in 1st brood	Emergence of workers of 1st brood after trapping of queen (Days)	Emergence of workers of 2nd brood after emergence of 1st brood (Days)	No. of workers in 2nd brood
Colony 1	18	32	7	46	15	8
Colony 2	18	34	8	47	16	5
Colony 3	22	31	6	53	18	6
Mean	19.3±1	32.3±1.5	7±1	48.6±3.7	16.3±1.5	6.3±1.5

tion began was 19.5 ±3.5 days. First brood cell was noticed after the pre-oviposition period had lasted for 6 days. Similar to this, it was discovered that the first brood's workers typically appeared after 28± 4.2 days of secretion of wax. The average number of workers in the initial batch was 7.

At colony foundation stage: It was discovered that the average time it took for workers from the second brood to emerge after those from the first brood was 42 days. The mean of workers emerged in the 2nd batch, according to the data, was 6. In the current study, it was discovered that *B. tunicatus* takes an average of 89–92 days to development from egg (wax secretion) to adult (first workers emerged). In Nauni, Himachal Pradesh, India, Yankit (2018) raised *B. haemorrhoidasl is* Smith in two chambered wooden boxes that were 1586.5 cm in size. She noticed that the gathered queens in the months of February and March began secreting wax after 14.12± 1.54 days and 11.00 ± 2.66 days respectively, after being trapped. Averaging 25.66±3.64 days after wax secretion (egg laying) in queens trapped and the first group of workers began to emerge.

According to earlier studies conducted in laboratories (Dayal and Rana, 2004; Kashyap, 2007; Yankit *et al*., 2018; Nayak *et al.,* 2020), the average time it took for *B. haemorrhoidalis* to develop from wax secretion to adult (emergence of first worker) was between 18 and 33 days. In 50, 64 and 73 days, respectively, the first male of the three species of *B. ardensardens*, *B. hypocritasapporoensis*, and *B. ignitus* emerged.

The present study covered rearing technique of *Bombus tunicatus*. The *B. tunicatus* colonies were reared up to 2nd brood and survived for 38± 14.03 days after emergence of 1st worker of brood. *B. tunicatus* can be domesticated under laboratory conditions and further development can be done. Bumble bees play a key role in agricultural sector and conservation, being essential pollinators for crops, fruit trees and wildflowers. This was a preliminary study and *B. tunicatus* was reared for the first time so, further development is required. It is required to verify their potential or identify promising species and methodologies in order to build a commercial source of pollination in the future.

Fig 2: *Bombus tunicatus* colony development (a) Queen and worker of *B. tunicatus* kept inside wooden cages (b) Brood cell initiation (c) Brood cell development (d) First brood (e) Second brood

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