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Effect of Different Types of Boxes on Rearing of Bumble Bee, *Bombus terrestris*

Muhammad Imran*, Munir Ahmad, Muhammad Naeem, Muhammad Farooq Nasir, Imran Bodlah, Muhammad Nasir and Umer Ayyaz Aslam Sheikh

Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University, Murree Road, Rawalpindi, Pakistan

ABSTRACT

Life history parameters of bumblebee *Bombus terrestris* (L.) (Hymenoptera: Apidae) were investigated by using rearing boxes of wood, cardboard and polycarbonate materials on three developmental stages *i.e*., colony initiation, development and maturation stages under controlled laboratory conditions. Queen oviposition rate was 1.4-fold higher in polycarbonate box compared to wooden and cardboard materials. Early start of egg-laying (first oviposition period) and earlier emergence of first worker were found in polycarbonate box as compared to others. Similarly, colony foundation stage in polycarbonate box took 58.3 days which was about 7-10 days shorter than others box types. Early emergence of sexual were observed in polycarbonate box with maximum numbers of daughter queens and males emerged 1.7-4.0 fold higher than from cardboard and wooden material boxes. Foundation queen (mother queen) survival duration was the lowest in cardboard box (64.6 days) while highest in polycarbonate box (109.5 days). Mortality of workers and males was 2.1-fold less in polycarbonate as compare to cardboard and wooden materials. Such important abiotic affect of rearing environment suggests polycarbonate boxes the most suitable on all three developmental stages of bumblebees for efficient commercial mass rearing.

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Authors' Contributions
MI and MA planned and executed the experimental work. MI, MA and IB wrote the article. MN and MFN analyzed the data. M. Nasir and UAAS reared the insects.

Key words
***Bombus terrestris*, Abiotic factor, Box materials, Life history, Mass rearing environment.**

INTRODUCTION

Bumblebees are considered the most important pollinators and very effective in greenhouse crops pollination due to their morphological characters (Velthuis and van Doorn, 2006; Sheikh *et al*., 2014; Imran *et al*., 2015). First attempt for rearing of bumblebee was made by Sladen (1912). Present commercial utilization about more than one million colonies of bumblebees made them tremendously successful (Beekman and van Stratum, 2000). Their wide use in crop pollination services reduce labor costs for manual pollination and produce good crops yield both in quality and quantity (Velthuis and van Doorn, 2006). Although commercial scale rearing started since 1987 but problems still exist for quality and quantity of bumblebee rearing on commercial scale (Hughes, 1996). Significant variation has been observed in three main colony stages which made many researchers to work out procedures to get maximum number of colonies (Velthuis and van Doorn, 2006).

Bumblebee rearing industry faces many problems of high labor cost, low colony success rate in initiation and hibernation limiting economic benefits (Velthuis and van Doorn, 2006). Three major colony stages depend mainly on colony initiation stage which is estimated as not more than 50% (Gosterit and Gurel, 2005). Other major factor affecting of bumblebees are queen health, nectar, quality pollen and favorable environmental conditions. These factors influence the egg laying rate of foundation queen and production of sexual (Beekman and van Stratum, 2000; Yeninar *et al*., 2000; Velthuis and van Doorn, 2006).

Since 1991 in Japan, European bumblebees are being used in greenhouse on large scale for crops pollination, especially used for tomato crop pollination because they are consider best pollination for tomato crop. About 70,000 colonies were imported for pollination purpose in 2003 (Goka, 2003; Goka *et al*., 2006; Kunitake and Goka, 2006). The important stage in rearing of bumblebee on commercial scale is the stimulation of mother queen for egg laying. There are several methods for stimulation of queen since the earliest reports of domestication (Sladen, 1912; Yoon *et al*., 2002; Kwon *et al*., 2006; Velthuis and van Doorn, 2006). One important method is introduction of two queens in single rearing box, this method is effective way of queen stimulation for colony initiation and this was used for rearing of *Bombus ternarius* and *B. terricola* (Plowright and Jay, 1966). A number of considerations have to make while raising a bumblebee on commercial scale, including infection by disease, pest attack, use of pollinator for the pollination of specific plants and easy way of management (MacFarlane *et al*., 1994).

Previous studies on colony initiation of bumblebee showed that success percentage is very low either working with laboratory reared bumblebee queens or wild queens

* Corresponding author: imranmalikvirgo@gmail.com
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(Kwon *et al*., 2006; Velthuis and van Doorn, 2006). Kwon *et al*. (2006) determined that out of 24 mated daughter queens of *B. terrestris* introduced into starter box only 6 queens (25%) produced their first batch of worker. Mah *et al*. (2001) reported that 50% and 25% successful colony initiation was observed from three different wild caught Korean bumblebee species (*B. ignitus*, *B. ardens ardens*, *B. hypocrita sapporoensis*). To maximize colony initiation percentage, many methods have been tried by using different species of bumblebee as test subjects. Many researchers worked on its environmental condition including rearing box material. Yoon *et al*. (2004) in their experiment used two type of box materials (cardboard box and plastic box) to identify which is best for rearing and found plastic box with maximum colony initiation percentage than cardboard box.

Importance of rearing box material highlight their importance and made us to compared the effect of rearing box made from three different materials (cardboard, wood and polycarbonate) for different colony development stages of colony initiation, foundation and maturation for efficient mass rearing environment.

MATERIALS AND METHODS

Experimental insects

Study was conducted to investigate the effect of box materials on the developmental stages of the European bumblebee (*Bombus terrestris* L.) under standard laboratory conditions in Non-Apis Bee Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan. Experimental daughter queens and males of *B. terrestris* were obtained from bumblebee hives imported from Koppert Biological Systems, Netherlands and reared on fresh and frozen honeybees collected pollen with commercial grade sucrose sugar at 50% concentration. These bumblebee hives were assigned numbers for identification. Daughter queens were mated with the males of same species belonging to different bumblebee hives to avoid inbreeding problems. Mated queens were kept for one week for preparing themselves for next stage of hibernation. These were kept at 2.5°C for 2.5 months for artificial hibernation requirement (Tasei and Aupinel, 2008). After hibernation, nacrosis with CO_2 gas as previously suggested was performed (Velthuis and van Doorn, 2006). These were then used for colony development through rearing box materials.

Climatic conditions in rearing room

We followed the basic colony rearing technique as described by Yoon and Kim (2002). The experimental groups were reared in three size of boxes made of three different types of materials cardboard (0.7 cm thick), wood (0.7 cm thick), polycarbonate (0.5 cm thick)). Climatic conditions were kept at 25±2°C and 60±10% relative humidity as per used by Duchateau and Velthuis (1988).

Comparative study of rearing boxes

Different types of rearing boxes were used at different stages. Starter boxes were used for colony initiation and when first worker emerged, each colony was shifted in a foundation box (medium size box). As soon as colony started to produce males and daughter queens, it was shifted in a maturation box (Velthuis and van Doorn, 2006). Size of colony boxes were 10.5×14.5×6.5 cm^3 at initiation stage, 21.0×21.0×15.0 cm^3 at colony foundation stage and 24.0×27.0×18.0 cm^3 at colony maturation stage. The shapes of the boxes were similar in structure except in the difference size at different stages of colony development. In all types of nest boxes, small holes were made at front wall of the chamber and at both sides of the walls to have environmental conditions as maintained. To minimize the risk of fungus insides, rearing boxes were checked and cleaned everyday with a piece of cotton or weekly cleaning with it soaked with 75% ethyl alcohol. Before weekly cleaning, bumblebees were removed to avoid any hazards of alcohol. Glass plates were used as lid to observe them without disturbing normal activities and later remained covered with black sheet.

Data analysis

Means of different life history parameters were subjected to statistical method using means ± SD (standard deviation) and compared with ANOVA (analysis of variance) at 5% probability for comparison of percentage values. Chi-squares analysis was performed using SPSS software (Norus, 2006) with graphs prepared by using Microsoft Office Excel 2007.

RESULTS

At colony initiation stage, observed parameters were pre-oviposition period and first worker emergence days. At colony foundation stage, we observed period at which colony reached at colony foundation stage (when workers strength reached at 50). For the third stage of colony maturation, first male and first queen emergence day, total emerged males and new daughter queens, total life span of foundation queen in the hive and mortally rate were observed.

Colony initiation stage

Insignificant differences were observed among three different box materials on the pre-oviposition of

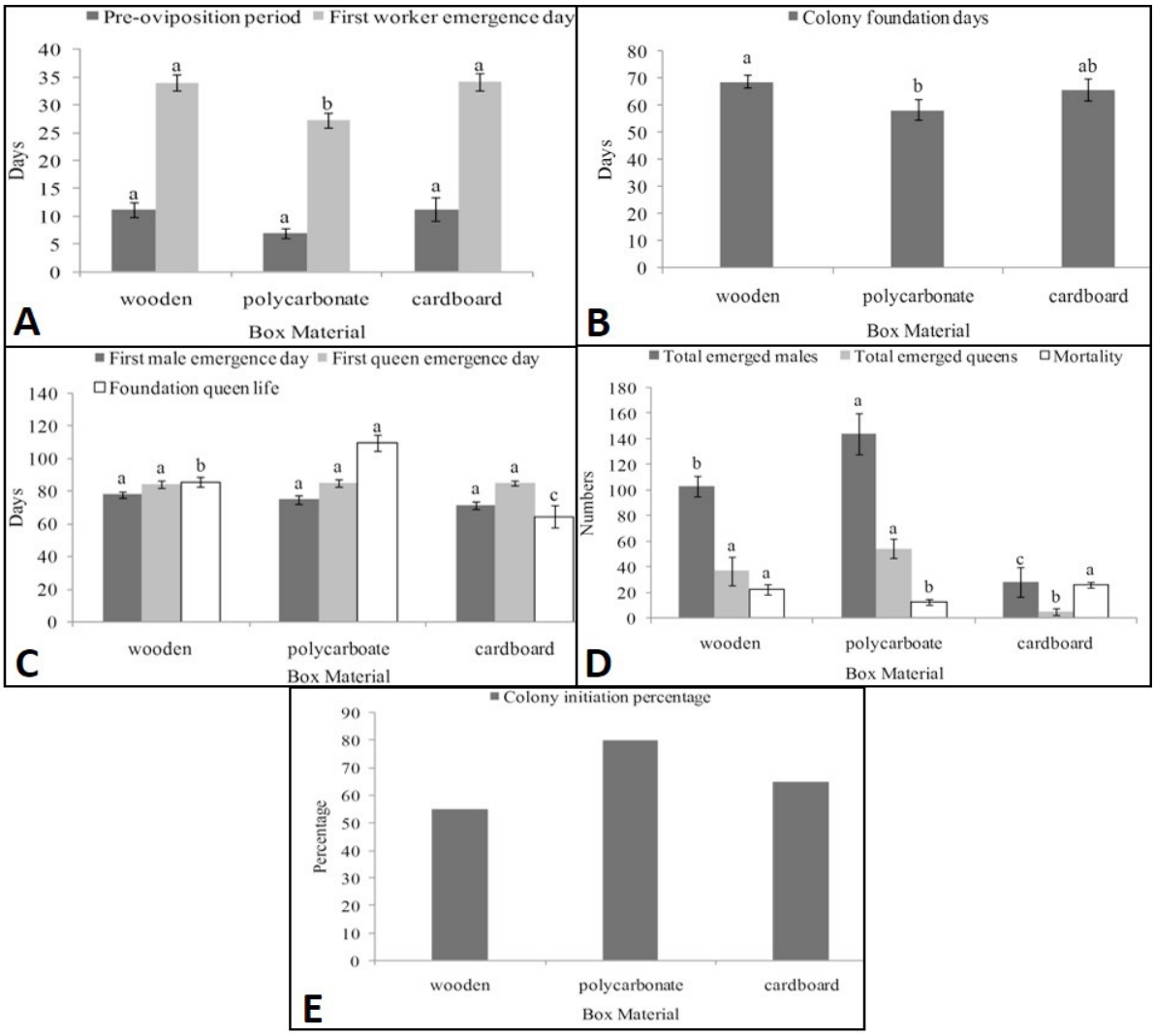

Fig. 1. Effect of starter box material on pre-oviposition period and emergence of first worker day in the colony (A), colony foundation days (B), first emergence of male, queen and foundation queen life in the colony (C), emergence of total males, daughter queens and mortality in the colony (D), and colony initiation percentage (E) of *Bombus terrestris* under controlled laboratory condition.

bumblebee. Minimum oviposition period (6.9±0.97 days) was observed from the colonies reared in polycarbonate box and maximum (11.2±2.1 days) was observed in cardboard box. Significant difference was observed for first emergence day of worker with minimum (27.3±1.26 days) in polycarbonate boxes and maximum (34.2±1.54 days) from colonies reared in cardboard boxes (Fig. 1A). Polycarbonate box was better than others box materials for pre-oviposition period and day of first worker emergence (Fig. 1A).

Colony foundation stage

Insignificant differences existed between these three types of boxes for colony foundation stage. Colonies reared in polycarbonate boxes took minimum days (58.3±3.84) while reared in wooden boxes with maximum (68.8±2.41 days) (Fig. 1B). Performance of polycarbonate boxes was effective than others box types for this stage.

Colony maturation stage

First emergence day of male and daughter queen: First emergence of male in the colony was insignificant statistically for these type boxes. However, it was minimum (71.5±2.25 days) for emergence of first male in cardboard boxes but maximum (77.9±2.03 days) in wooden

boxes (Fig. 1C). First daughter queen emergence day was also statistically insignificant, however, it was minimum (84.7±1.59 days) from colonies reared in cardboard boxes and maximum (85.0±2.43 days) in polycarbonate boxes (Fig. 1C). Although, there was not much difference for the first sexual emergence day in all three box types yet polycarbonate box performed better by taking longer colony life duration for pollination in greenhouse crops and get sexuals for long colony period (Fig. 1C).

Foundation queen life

Highly significant difference was observed among three different box types on the life of foundation queen in the colony. Minimum life (64.6±6.87 days) was observed from the colonies reared in cardboard boxes while maximum (109.5±4.82 days) from colonies reared in polycarbonate boxes (Fig. 1D). Thus, polycarbonate boxes might help the mother queen life than others due to better maintain environment inside box (Fig. 1C).

Total males and daughter queen emergence

Significant difference existed among three different box material types to total number of male emergence in the colony during the colony life cycle. Out of 30 colonies, 10 on each box material, minimum males (28.2±11.62) emerged from colonies reared in cardboard boxes while maximum (144.3±16.42) from polycarbonate boxes (Fig. 1D). Similarly, it was significant when observed for total daughter queens emerged. It was minimum (5.1±2.55) from colonies reared in cardboard boxes but maximum (54.4±7.76) daughter queens in polycarbonate boxes (Fig. 1D). Maximum new daughter queens and males were emerged in polycarbonate boxes for continuous mass rearing for next generation as compared to others boxes types (Fig. 1D).

Colony initiation percentage and percent mortality

The most important character in colony of *B. terrestris* is considered the laying of first egg batch which shows the reproductive success of foundation queen called as colony initiation. Polycarbonate box was more effective with 80% colony initiation compared to cardboard (65%) and wooden box (55%) (Fig. 1E). Minimum percent mortality (12.7±0.92) was observed in polycarbonate boxes and maximum (26.25±1.27 and 22.5±1.95) in cardboard boxes and wooden boxes, respectively (Fig. 1D).

DISCUSSION

Rearing of bumblebee has very long history many scientists worked to find our best rearing techniques with lowest economic cost. Previous work for environmental conditions to find out best temperature and humidity for rearing of bumblebees and artificial nesting for queen to start colony has been proposed (Beekman and Stratus, 2000; Yoon *et al*., 2002, 2004; Gosterit and Gurel, 2005; Imran *et al*., 2015). Based on our results at colony initiation stage, foundation queens laid eggs earlier in polycarbonate boxes than wooden and cardboard boxes. There are many possible reasons, Yoon *et al*. (2004) worked to compare two materials cardboard and plastic and found that foundation queen laid earlier eggs in plastic box as compare to cardboard box and there is no finding about survival of queens, so our result was similar to their finding, but our result also explain that polycarbonate box was most effective from both cardboard and wooden box materials.

The comparatively high labor cost and low success rate are the main problem in the rearing of bumblebee on commercial scale, so variation occur in the production of males, workers, new queens and in emerging time and their survival (Beekman and Stratum, 2000). According to statistical analysis, significant difference was seen in the Pre-oviposition period, worker emergence day, sexual emergence and these characteristics are important criteria in commercial rearing of bumblebees. These characters are affected by many factors including rearing food and environmental conditions. Many researchers recommended different range of environmental conditions (Plowright and Jay, 1966; Beekman and Stratum, 2000; Jie *et al*., 2005). Earlier emergence of first worker in the first batch was observed in polycarbonate box as compare to wooden and cardboard box. A possible reason may be due to some existence of environmental conditions variation due to materials response to environment. Under high relative humidity of 65±10% in the rearing room, cardboard and wooden materials might have absorbed certain moisture to become somewhat which resulted increased moisture but somewhat decreased temperature similar to natural environment (Duchateau and Velthuis, 1988; Velthuis, 2002; Velthuis and van Doorn, 2006). Comparison of present results with that of Yoon *et al*. (2004) differ from ours who observed earlier first worker emergence from colonies reared in cardboard boxes than plastic boxes. Late emergence of first worker in cardboard and wooden boxes might be due to increased moisture level. However, there existed more chances of fungal spread in high moisture where two species (*Nosema apis* and *N. bombi*) might attack bumblebees excretory system and make them weak (Fries *et al*., 2001).

Colony development is the stage to decide either colony is saleable for pollination or collect sexuals for next multiplication. However, there exist little comparable data for effect of nest box material to colony development. Colonies reared in cardboard box material were observed earlier for foundation stage than that in plastic box (Yoon *et al*., 2004). However, our observation of colonies reared in polycarbonate boxes showed earlier foundation stage than cardboard boxes. Other possibility may include presence

of pest like Indian meal moth (Caron, 1992). Chance of attack of this pest is much higher at foundation stage than colony initiation stage. Larvae of this moth not only decrease colony development but also damage the pupae of bumblebees (Ruijter *et al*., 1997; Kwon *et al*., 2006).

Emergence of sexual in bumblebee colony is either through mother queen switch for it (Duchateau and Velthuis, 1988) or influence of workers to queens which start laying haploid eggs and results in competition point. It start when density of workers increase or health of mother queen decrease who become unable to control the colony by releasing pheromones or when the environmental conditions of the colony change and the attack of diseased and pest increased (van Honk *et al*., 1980; Röseler *et al*., 1981; Bloch and Hefetz, 1999). Colonies producing earlier sexuals are used for continuity of rearing process while those producing late are good for pollination (Yoon *et al*., 2004). Chances of disease and pest attacked were high in cardboard and wooden boxes than that of polycarbonate boxes. Foundation queen life is very important. If she lives short life and dies earlier than colony also end earlier and vice versa. Colonies with long life cycle are considered most effective not only for pollination but also for mass rearing. We observed short mother queen life in cardboard boxes than those that in polycarbonate boxes. Cardboard boxes might have bit higher relative humidity due to its material for high absorption increasing *N. bombi* which may be possible reason affecting chance of mother queen longevity and causing earlier mortality (Fries *et al*., 2001).

To get maximum numbers of colonies in next generation, it is necessary to obtained higher males and new daughter queens per colony. Maximum numbers of sexual were produced from those colonies reared in cardboard boxes and minimum in plastic boxes materials (Yoon *et al*., 2004). This might differ due to thickness of box, bumblebee species or some other factors as we observed more in polycarbonate boxes than that in cardboard ones. Many scientists worked to apply different methods to get maximum numbers of sexual. Food of bumblebee comprises mainly pollens and nectar or sugar solution (Park *et al*., 2004). Different environmental conditions including temperature and humidity also affect on sexual emergence (Gurel and Gosterit, 2008). Maximum mortality was observed from those colonies reared in cardboard boxes than that of polycarbonate and wooden boxes. Indian meal moth might be possible reason which rear better in high relative humidity which might exist in cardboard boxes (Caron, 1992). Similar was result in contradict for egg-laying (Yoon *et al*., 2004).

It can be concluded that at three developmental stages (colony initiation, colony foundation and colony maturation) of bumblebee colonies, boxes with polycarbonate materials were more effective to get maximum oviposition rate, males, daughter queens, decreased mortality and protection of colonies from the attack of different types of insects pests and diseases.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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