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RESEARCH ARTICLE

Standardization of Mass Queen Rearing Techniques in Indian Honey Bees

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ABSTRACT

Indian honey bee (*Apis cerana indica* F.) is present throughout our country except in the plains of north India. The queen bee is the mother of the colony, which rules over workers. As it is the source of all the hereditary characters, the colony can be improved by producing a good-quality queen. Even though biologically similar to *A. mellifera,* there were no ample studies on queen rearing in Indian bees than Italian bees. To unveil artificial queen rearing technique, queen cell cup size, type, and quality of the priming material were optimized based on acceptance and adult emergence. Queen cell cups of different sizes *viz*., 4 mm, 5 mm, 6 mm, and 7 mm in diameter, were used. Among them, 7 mm diameter cup showed the highest larval acceptance (28.33%) and adult emergence (25%). Priming media *viz*., water priming, royal jelly priming, diluted royal jelly priming, honey priming, and honey + royal jelly priming were fed to the colonies. Among them, diluted royal jelly showed the highest acceptance with 29.2% and adult emergence of 27.1% followed by, royal jelly (16.7% and 12.5%), honey + royal jelly (10.4% and 6.5%), honey (8.3% and 4.2%), and water (2.1% and 2.1%) respectively. The queen cell cups were prepared artificially from plastic cups too. But they were not readily accepted by worker bees. Hence, it is important to standardize queen rearing methodology in *A. cerana indica* in order to multiply a large number of queens and supply superior-character queens to the beekeepers in India.

Keywords: *Indian honey bee; Queen rearing; Cell cup size; Priming medium; Acceptance.*

INTRODUCTION

The honey bee is one of the social insects that evolved more than 70 mya ago (Linksvayer *et al*., 2012). *Apis cerana* F. is distributed in south and southeastern Asia and is also found in Japan, Afghanistan, northeastern Australia, New Guinea, and Solomon Islands (Koetz, 2013). *Apis cerana indica* F. is present throughout India except in plains of north India. Its life cycle is very similar to that of *A. mellifera*. The queen and drone are the sources of hereditary characters in the colony. But it is impossible to control parentage, and drones from the vicinity can take part in fertilising a queen. So the heredity characters can be improved by producing a good-quality queen. The performance of the honey bee colonies depends on the inherited qualities of the queen. The queen bee is considered an egg-laying machine, and a colony is not productive unless the queen can lay eggs. In natural conditions, the queens are reared only under the three major impulses: swarming, emergency and supersedure (Engels, 1986; Mishra, 1995). A planned program of queen-rearing utilizes the swarming impulse and ensures large-scale queen rearing and the multiplication of colonies.

Beekeepers generally focus on higher honey production, but it is related to the amount of brood rearing as well as the presence of a good-quality queen. Morse and Hooper (1985) stated that the quality of the queen could be judged on the basis of egg-laying capacity, less swarming, resistance to diseases, and the ability to produce more honey. Mass rearing of honey bee queens is important to (a) increase colony strength and numbers, (b) replace defective and unproductive queens from a colony, and (c) provide a new queen to a queen-less colony, (d) replace a two-year-old or older queen before honey flow season to improve colony strength and industriousness, and (e) select and breed colonies with desirable characters.

Queen rearing is the major factor in successful beekeeping (Morse, 1994). Among several methods of queen rearing (including Miller, Alley, Demaree, Hopkins, Barbeau, Smith, and Doolittle), the Doolittle method of queen rearing is famous among beekeepers (Suryanarayana and Rao, 1998). But there were no ample studies on queen rearing in Indian honey bees compared to Italian bees. So, there is a need to standardize queen rearing methodology in *A. cerana indica* in order to multiply a large number of queens and supply colonies of superior character to beekeepers in India.

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Materials and Methods

Experiments were conducted on artificial queen rearing of *A. cerana indica* in the insectary unit of the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, during the months of September 2017 to April 2018. A total of 24 colonies of *A. cerana indica* were selected for this study. The colonies had six frames of strength and sufficient pollen and honey stores. Four colonies that served as replications were tried for each treatment and 12 queen cell cups were used per treatment. The experiments included the optimization of queen cell cup size, type, and priming material. In the present investigation, the Doolittle method of queen rearing was followed, which included the following steps:

Preparation of cell-builder colonies

A cell-builder colony was developed by selecting a strong colony of six brood frames and seven super frames. The frame includes a fully sealed brood, honey, and pollen. For queen rearing, the cell builder colony was dequeened in the morning, and the frame fitted with grafted queen cell cups was placed in the centre of the colony.

Preparation of artificial queen cell cups

The queen cell cups were prepared artificially by immersing a dipping tool (of appropriate thickness, prepared out of wood or multi-purpose sealant-M-Seal®, and measured using a vernier caliper) initially into cold water for wetting and then into melted bee wax for about 2 or 3 times to get about 0.5 cm height. After the first dip, the tool was dipped a lesser depth each time to give a thin top and thick bottom to the cell cup (Plate 1). Then the dipping tool with a wax cup was immediately immersed in the cold water. So the wax cup should harden for easy detachment (Plate 2). The queen cell cups were prepared for different sizes, *viz*., 4 mm, 5 mm, 6 mm, and 7 mm.

Plastic queen cell cup preparation

For the first time, 3D printing technology was used to prepare plastic queen cell cups suitable for *A. cerana indica* with polycarbonate material. This 3D printing technology is a very viable technology for small-scale design and testing of polycarbonate materials in biological research. Hence, this technology was resorted to with the objective of standardizing the cell size and recommending large-scale production of plastic cups for commercial mass queen rearing. Two different sizes: i) base: 4 mm, height: 5 mm, top: 7 mm; and ii) base: 5 mm, height: 5 mm, top: 7 mm (Plate 3).

Collection of royal jelly and preparation of priming material

A strong colony with abundant nectar and pollen stores and young brood was dequeened and allowed to raise queen cells. The collection was done just before sealing; the queen cells were cut, and the grafted larvae were removed from the cells. Royal jelly was collected into a sterilized polypropylene tube with a small spatula from the newly formed queen cells. The collected royal jelly was stored in a freezer to avoid any biochemical changes that would deteriorate the sample. The royal jelly collected was used as priming material in various ways. While grafting, royal jelly was diluted with some water and placed at the bottom of the cell with the help of a micropipette. Immediately, a day-old larva was grafted to the queen cell over the royal jelly priming medium.

Grafting of a larva to the queen cells

Artificially prepared queen cell cups were mounted on a cell holding wax pieces with a little molten wax. The grafting frame was placed in the cell-builder colony and left for about 30 minutes so that the worker bees could clean and polish the cups. Then the grafting frame was removed from the cell-builder colony and primed with diluted royal jelly. One-day-old larvae selected from a breeder colony (another colony with a good pedigree) were transferred to cell cups by using a grafting needle. At the time of grafting, the tip of the grafting needle was gently passed underneath the one-day-old larva present in the cell (Plate 4). The tiny larva that floated on the flattened, curved end of the needle was lifted and carefully transferred into the bottom of the primed cup. Soon after grafting all the cell cups, the grafting frame was placed back into the cell builder colony.

Effect of queen cell cup size on larval acceptance

Queen cell cups of different sizes, *viz*., 4 mm, 5 mm, 6 mm, and 7 mm in diameter, were used in the study. All of these cups were prepared by dipping tools with their external dimensions equivalent to the above cup sizes at the tip. Queen rearing was carried out with the above cups with the objective of producing more queens. Five colonies were selected for each treatment, which served as replications. All the selected colonies have six frames of strength and sufficient honey and pollen stores. In each colony, one grafting frame was kept, and twelve queen cell cups were grafted (Plate 5). The number of queen cells accepted out of twelve cups was recorded and expressed either as a number of cups per 12 cups or as a percentage acceptance.

Effect of queen cell priming medium on larval acceptance and queen emergence

In this study, four types of priming media were compared for their influence on larval acceptance and queen emergence. The media *viz*., water priming, royal jelly, diluted royal jelly, honey and honey + royal jelly priming were used to prime the cups. The comparison of the above media for their effectiveness in queen rearing was made using queen cell cups of 7 mm diameter, and queen rearing was carried out. In each colony, one grafting frame was kept, and twelve queen cell cups were grafted and primed with appropriate priming agents. The number of queen cells accepted out of twelve cups was recorded and expressed either as a number of cups per 12 cups or as a percentage acceptance. Parameters such as larval acceptance and queen emergence were recorded.

Acceptance (%) and Emergence (%)

The number of grafted larva accepted was recorded two days after grafting by observing the covering of nurse bees on cell cups, the elongation of cell cups by worker bees, and the presence of royal larva in the cell. The percentage acceptance was worked out as follows:

$$\text{Acceptance (\%)} = \frac{\text{Number of larva accepted}}{\text{Total number of larva grafted}} \times 100$$

The number of queens that emerged from the queen cells, which were counted, and the emergence were worked out as follows:

$$\text{Emergence (\%)} = \frac{\text{Number ofqueens emerged}}{\text{Total number of larva grafted}} \times 100$$

Successful queen

The success of the queen depends upon her return after mating. The mated queen showed signs of smooth and graceful movement and a swollen abdomen. The queen generally starts egg-laying 3-5 days after mating.

Statistical analysis

The data obtained were subjected to statistical analysis employing complete randomized design (CRD) analysis, and means were compared with a critical difference (CD) using MS excel.

RESULTS AND DISCUSSION

Effect of queen cell cup size on acceptance of grafted larvae and queen emergence

All the colonies in an apiary are not equally superior performers. Hence, the quality of the queen is important for selection. Acceptance of grafted larvae and the emergence of an adult were influenced by queen cell cup size. It was estimated with four different cup sizes of 4 mm, 5 mm, 6 mm, and 7 mm diameter by grafting a 1-day-old larva into queen cells. Each cup size was replicated 12 times per colony. Among them, a 7 mm diameter cup showed the highest larval acceptance of 28.33% (3.4 / 12 cups) with 25 % adult emergence followed by a 6 mm diameter cup with 10% acceptance and 8.33 % adult emergence (Table 1). The cups of 4 mm and 5 mm diameter were not accepted for rearing the queens (Figure 1). Both the acceptance and emergence rates were found to be highest with queen cell cups of size 7 mm, which was significantly different from other sizes of cell cups.

Effect of priming media on acceptance of grafted larvae and queen emergence

The experiment was conducted to find out the influence of priming media on the acceptance of grafted larvae and adult emergence. This study was done using a 7 mm diameter queen cell cup. Five different priming media were used for grafting: water, royal jelly, diluted royal jelly (1:1 with distilled water), honey, and honey with royal jelly. Among them, diluted royal jelly showed the highest acceptance with 29.2% acceptance (3.5/12 cups) and 27.1% adult emergence (3.3/12 cups), followed by royal jelly priming (2.0/12 cups) with 16.7% acceptance and 12.5% adult emergence (1.5/12 cups), honey + royal jelly priming (1.3/12 cups) with 10.4% acceptance and 6.5% adult emergence (0.8/12 cups), honey priming (1.0/12 cups) with 8.3% acceptance and 4.2% adult emergence (0.5/12 cups), and water priming (0.3/12 cups) with 2.1% acceptance and adult emergence each (Table 2). Both the acceptance and emergence rates were found to be maximum with priming media of diluted royal jelly, which was significantly different from other priming media (Figure 2).

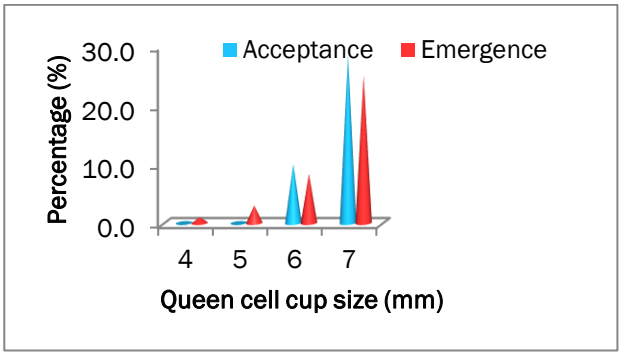


Figure 1. Effect of queen cell cup size on acceptance and emergence of queens

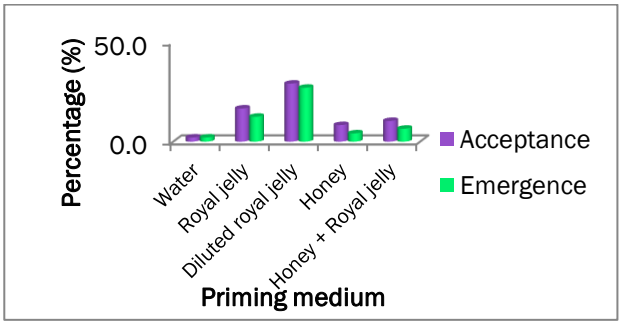


Figure 2. Effect of priming media on acceptance and emergence of queens

Effect of plastic queen cell cups

The 3D-printed plastic queen cell cups were made of polycarbonate material. Two different sizes: i) base: 4 mm, height: 5 mm, top: 7 mm; and ii) base: 5 mm, height: 5 mm, top: 7 mm. Grafted larvae were not accepted for queen rearing by worker bees. There have been many studies with *A. mellifera* on mass queen rearing. However, the technique is not completely successful with *A. cerana indica*. Hence, there is a need to standardize mass queen rearing with this species of honey bee. Even though queen cell cups made of beeswax are being used, plastic cups are also readily accepted by worker bees for queen rearing in *A. mellifera*. Hence, attempts were made to standardize the cup size with *A. cerana indica* so that even plastic cups could be designed and effectively used. The results of the attempts made to fix the appropriate queen cell cup size are discussed below.

The larval acceptance and queen emergence were studied for rearing *A.cerana indica* queens with queen cell cups of 4, 5, 6, and 7 mm diameter by grafting single-day-old worker larvae. It was observed that 7-mm-diameter cups were the most suitable, with the highest acceptance and emergence. Even with a slight decrease in cell cup size of 1 mm, *i.e.,* from 7 mm to 6 mm, there was a decline in the acceptance rate of the queen cell by worker bees. The cup with a 7 mm diameter showed the highest larval acceptance of 28.33% (3.4 queens / 12 cups) (Table 1). and adult emergence of 25% (3 queens / 12 cups), followed by a 6 mm diameter cup that had 10% acceptance (1.2 queens / 12 cups), and 8.33 % adult emergence (1 queen / 12 cups). The cups of 4 mm and 5 mm diameter were not accepted for rearing the queens. Doolittle (1888) evolved a method of transferring very young larvae from worker cells to specially prepared queen cell cups. In the present studies, attempts were made to rear 12 queens in each hive, which is as per Doolittle (1889), who recommended a cell bar with 12 cells as a minimum and 24 cells as a maximum in the Italian bee.

The results of the present studies are in conformity with those of Abrol *et al.* (2005) that *A. cerana* highly prefers queen cell cups with an internal diameter of 8.6 mm at the mouth, 6.2 mm at the base, and 8.8 mm depth (Abrol *et al.*, 2005). Kusuma (2003) reported that the percent acceptance and emergence of queens were high (80.00%) when artificial queen cell cups of 7.5 x 7.0 mm were used, and minimum acceptance and emergence (33.33% and 20.00%, respectively) were observed by using 6.5 x 7.0 mm cups in Indian bees. Kumar (2000) also used 6, 7, 8, and 9 mm diameter queen cell cups for *A. cerana indica* queen rearing. In that, 7 mm diameter queen cell cups showed the highest acceptance (81.11%), emergence (76.23%), and body weight (140.97 mg).

Queen cell cup material also influences the acceptance of grafted larvae. For conducting the above-mentioned experiments, wax cups were used. Mishra (1998) reported that mass queen rearing was possible in *A. cerana indica,* and it was basically the same as for *A. mellifera,* but essentially the queen wax cup size should be smaller. Dodologlu and Emsen (2007) reported 93.33 and 95% larval acceptance rates for queen-right and queen-less colonies, respectively, in *A. mellifera* from wax cups. In the present studies, the percentage acceptance of royal larva was very low (28.33%) compared to the results obtained by earlier researchers (more than 80).

The plastic queen cells were prepared for the first time for the Indian honey bees. However, to our disappointment, grafted larvae were not accepted by worker bees with either size of the queen cell cups. These cups were not preferred by worker bees. The probable reason for non-acceptance could be the printing resolution of the 3D printer was so low that it could produce a minimum thickness of 0.4 mm, which was not acceptable to the bees, particularly at the edges of the queen cell cup where about 0.2 to 0.1 mm thickness is needed. Also, the polycarbonate material used might not have been preferred by the honey bees, and a better material should be attempted in the future.

In the case of *A. mellifera,* queen rearing using plastic cell cups has been practised for a long time (Laidlaw and Eckert, 1962). However, Ebadi and Gary (2015) testified that the acceptance of queen was higher in bee wax (86.6%) and cell capping wax (70.0%) than in paraffin wax, which was not accepted. The performance of bee wax cups is better compared to plastic cell cups in which the larvae are grafted (Chang, 1977). A moot point is the necessity of introducing empty cups in advance to precondition the colony. According to some authors, preconditioning positively influences the acceptance of grafted larvae (Delaplane and Harbo, 1988). But Skowronek and Skubida (1988) indicated that conditioning treatments serve no purpose. The current study also concluded that there was no difference between preconditioned and unconditioned grafting.

The suitable medium required for the grafted larvae was studied by using five different priming media *viz*., water, royal jelly, diluted royal jelly, honey, and honey with royal jelly (Table 2). Among them, diluted royal jelly was the best priming medium, which had the highest acceptance of grafted larvae and adult emergence and the lowest in water priming, even though it was on par with that of cups smeared with royal jelly. This study was in near agreement with Kumar (2000), who suggested royal jelly was the best priming medium, which had the highest larval acceptance and queen emergence even though it was on par with diluted royal jelly. El-Gaward and El-Din (1999) reported that the acceptance and emergence of *A. mellifera* queens were the highest while using royal jelly, and the lowest occurred when drone larval juice was used. The other media, like honey, honey with royal jelly, and water, were found to be unsuitable, as they had the lowest acceptance of larvae and adult emergence. In this study, preference was given to queen-less colonies for getting more queens. We have done queen-less in the morning and grafting in the afternoon on the same day to get more acceptance. However, Adam (1987) was convinced that although the highest quality queens could be raised using the queen-right method, there was an average acceptance rate of about 90% with the queen-less method.

Table 1. Effect of queen cell cup size on acceptance of grafted larvae

Sl. No.	Queen cell cup size	Grafts per replication	No. of cells accepted	Acceptance (%)	Emergence (%)
1	4.0 mm	12	0.0 $(0.71)_c$	0	0
2	5.0 mm	12	0.0 $(0.71)_c$	0	0
3	6.0 mm	12	1.2 $(1.23)_b$	10.00	8.33
4	7.0 mm	12	3.4 $(1.97)_a$	28.33	25.00
	CD		0.32		

*Mean of five observations, figures in parentheses is square root transformed values. In a column, means followed by common lower case alphabet (s) are significantly different (p = 0.05).

Table 2. Effect of priming medium on acceptance of grafted larvae

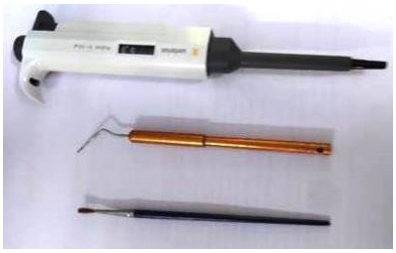
Sl. No.	Priming Medium	No. of grafts per replication	No. of cells accepted	Acceptance (%)	Emergence (%)
1	Water	12	0.3 $(0.84)_c$	2.1	2.1
2	Royal jelly	12	2.0 $(1.56)_{ab}$	16.7	12.5
3	Diluted royal jelly	12	3.5 $(1.99)_a$	29.2	27.1
4	Honey	12	1.0 $(1.0)_c$	8.3	4.2
5	Honey + Royal jelly	12	1.3 $(1.3)_{bc}$	10.4	6.5
	CD		0.47		

*Mean of four observations, figures in parentheses is square root transformed values. In a column, means followed by common lower case alphabet (s) are significantly different (p= 0.05).

Plate 1. Materials required for queen rearing

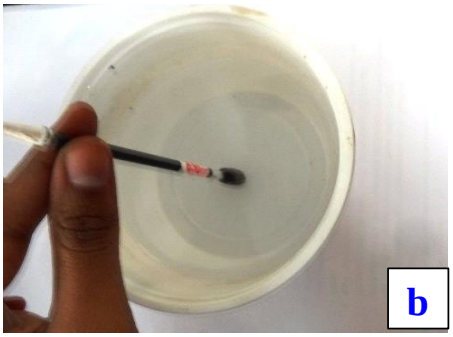
a. Queen cell cup dye

b. Magnifying lens



c. Grafting tool set*
*Micropipette, Grafting needle and Camel hair brush

Plate 2. Preparation of queen cell cups



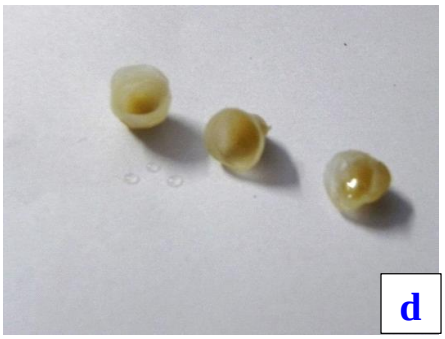


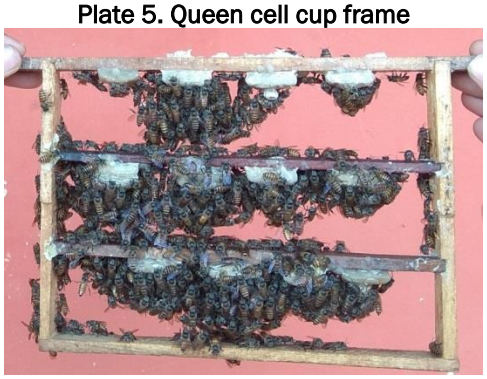
Plate 3. Plastic queen cell cups with different measurements

Base : 5 mm
Top : 7 mm
Height : 5 mm

Base : 4 mm
Top : 7 mm
Height : 5 mm

Plate 4. Larva grafting of Indian honey bee larva

Plate 5. Queen cell cup frame



CONCLUSION

In the experiment to standardize queen rearing with *A. cerana indica, a* queen cell cup size of 7 mm diameter showed the highest larval acceptance (28.33%) and adult emergence (25%), followed by a 6 mm diameter cup with 10% acceptance and 8.33% adult emergence. The remaining cup sizes were not suited for rearing queens. Diluted royal jelly (1:1) as priming medium showed the highest larval acceptance (29.2%) and adult emergence (27.1%), followed by undiluted royal jelly, honey + royal jelly, honey, and water. Compared to *A. mellifera*, queen rearing results were not as satisfactory with *A. cerana indica*. Honey bee behavior and reproductive biology of these two bees vary, which might be the reason behind this change. Further studies need to be carried out to standardize the colony conditions for the rearing of better-quality queens in Indian honey bees.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required.

Author contributions

Research work-SC, Idea conceptualization-SMR, Experiments-SC, Guidance–SMR, SA, Writing original draft –SC, Writing- reviewing &editing-SC,SMR,SA.

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