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Fazlah Wahid
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Braj Kishor Prasad Singh
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Siva Nand Singh
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Corresponding Author:
Fazlah Wahid
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, Bihar, India

Different artificial methods for rearing queen of *Apis mellifera*

Fazlah Wahid, Braj Kishor Prasad Singh and Siva Nand Singh

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Abstract
Artificial honeybee queen bee rearing is an important part of beekeeping because it helps to re-queen bee colonies on a regular basis, reduces swarming, increases honey production, increases number of colony. The study aim to determine the success rate of emergence of queen bee from artificially grafted larvae in various queen cup sizes, grafting of larvae in concentrated and diluted condition and grafting of larvae in the colonies with and without queen bee. The success of grafted larvae in general, sealing of larvae and emergence of queen bee was 70-73%, 53-61% and 37-41% respectively. The rate of successful grafted larvae, sealing of larvae and emergence of queen bee in dry condition was 58.8%, 46.8% and 44.3% respectively. In wet grafting 76.4%, 70.9% and 51.7% acceptance, sealing and emergence of queen bee respectively. Similarly, more queen bee were emerged from colonies without queen bee than colonies with queen bee conditions. It was found that the success of grafting did not affected by different grafting queen bee cup sizes. Emergence of queen bee was more in wet grafting and grafting of larvae in queen bee less colonies.

Keywords: *Apis mellifera*, queen bee cell cup, grafting, queen bee rearing

Introduction
The honeybee *Apis mellifera* is an important eusocial pollinator for the ecology. A *A. mellifera colony* typically has a queen bee, and the success of colony is highly dependent on this single individual. The in charge of egg laying and production of brood inside the colony is generally a single queen. A queen bee is also important for survival and extension of colony.
Artificially rearing of honeybee queen is an important part of beekeeping because it helps to re-queen bee colonies on a regular basis, reduces swarming, increase colony size and honey production, 1994; Laidlaw and Page, 1997) [18, 16, 17]. A queen bee is an important member of a colony since it is a critical variables determining a colony's output (Laidlaw, 1979; Ruttner, 1983) [16, 17, 22]. Furthermore, several necessary colony qualities, such as disease resistance and gentleness, are determined by the queen bee quality (Ratnieks and Nowogrodzski, 1988) [20], indicating the relevance of queen bee raising artificialiy. A honeybee colony usually has a single queen bee, and colony success is determined by her. Indeed, several queen bee rearing procedure have been practiced over many decades to grow numerous queen bee s from a particular colony (Harry and Laidlaw, 1981) [14]. However, the investigations were restricted to specific races and were largely conducted in temperate climate zones.
The reactions of colonies to different queen bee rearing strategies vary substantially depending on ecology and race of honeybees. Response variations towards alternative queen bee raising procedures have been widely documented because to variances in environmental, biological and behavioural aspects (Nuru and Dereje, 1999; Crailsheim *et al*., 2013) [19, 9]. Furthermore, plants with rich pollen source, relative humidity and temperature have been identified as critical determinants in regulating the artificially raise queen bee in their adoption and quality (Zhadanova, 1967; Cengiz *et al*., 2009) [27, 6]. The importance of environmental elements and the prevalence of response variations among different queen bee raising approaches has been extensively documented (Wen and Chong, 1985; Morse, 1994; Cengiz *et al*., 2009; Crailsheim *et al*., 2013) [24, 6, 18, 9].
Furthermore, more successful grafting in *A. mellifera* queen bee cups with diameters of 7.8 - 9.0 mm than in 10-12 mm cups demonstrated by Skowronek and Skubida (1988) [23].
Apis cerana acceptance differences towards different dimensions of artificial queen bee cup cell have been reported (Abrol *et al*., 2005) [2].

This may emphasise the relevance of evaluating the relation between grafting of larvae in different cup sizes and their acceptance.

Diverse studies (Ratnieks and Nowogrodzski, 1988; Buchler *et al*., 2013) [20, 5] examined grafting with diverse ways such as dry and wet grafting and demonstrated that when royal jelly was used as substrate, the adoption and emergence rate of queen bee became high. Furthermore, wet grafted queen bee s had significantly higher morphological qualities than dry grafted queen bee s (Kamel *et al*., 2013) [15]. However, Cushman (2013) [11] observed that dry grafting had a high acceptance rate.

Furthermore, Büchler *et al*. (2013) [5] discovered that the presence or absence of queen bee, as well as the techniques of raising, have a significant impact on the rate of adoption of grafted larvae. Grafting procedures in the colonies with and without queen bee were also investigated, and different queen bee quality metrics were reported (Büchler *et al*., 2013) [5]. In general, rate of adoption of grafted larvae varied in different queen rearing procedures. Apart from different strategies, queen bee rearing is also influenced by density of nursing bees in a colony and presence of food resources in abundance (Wilkinson and Brown, 2002) [25].

We compared in the present study (i) successful grafting, sealing, and their emergence rate of *A. mellifera* queen bees raised using queen bee cells cup of different sizes; (ii) between diluted and concentrated grafting; and (iii) also under the conditions of colonies with and without queen bee an average dry weather conditions.

Materials and Methods

The honeybee *Apis mellifera* colonies considered in this study.The study was conducted in local farm at Rohuwa village, Muzaffarpur district, Bihar during Apri-june 2022. Rearing equipments, queen bee cell cup of different sizes, queen cage, a grafting tool, *A. mellifera* wax, larvae, pollen, honey, water, a wooden frame, and a double jacket pan were all used in this study.

Role of different queen cell cup sizes on grafting success, sealing and emergence of queens

First, 20 naturally occurring queen cells of *A. mellifera* from different colonies were taken. They were cut carefully at height of 0.7-0.9 cm above base and the diameter at the rim were recorded. The diameter of cell cup were ranged between 0.65 cm to 0.85 cm. Artificial queen cell cups were prepared of three different sizes *viz*, 0.7, 0.75, 0.8 cm by dipping wooden stick of corresponding sizes in molten wax. In addition to those three a queen cell cup of 0.85cm (standard) were also used.

Three batches of 3 colonies (3 colonies per batch), total 9 colonies were installed. In each colony received 18-20 queen bee cups (4-5 cups of each size) were fixed on two wooden bar (9-10 cups on each bar) that were suspended from Langstroth frame. All four types of cups were alternatively positioned to get equal chance. 14-16 hour before grafting, the frames containing artificial queen cup cell were placed inside the de-queen colonies for polishing of cups. Before grafting of larvae to the queen cup inside experimental colonies, frames having eggs and young larvae were removed to avoid rearing of emergency queen by worker bees. An average of 54 larvae were grafted for each cup size category, for 216 (four type of cups) larvae. Data on number of successful grafting, larvae sealed, and queen bee emerged were collected and compared with different cup sizes.

Effects of diluted and concentrated grafting on grafting success, sealing and emergence of queens

The experiment was conducted in three batches; each batch consists of triplicate of colonies (total 9 colonies) to examine the influence of dilute and concentrated grating on grafting success, sealing and emergence of queen. Each colony receive 24 grafted larvae, total 246 queen cups were used in whole observations. 14-16 hour before grafting, the frames containing artificial queen cup cell were placed inside the de-queen colonies for polishing of cups. Half of the cups were primed with 4µl concentrated and half with 4µl diluted (1royal jelly: 1 distilled water ratio) royal jelly. The queen bee cups were arranged alternatively for dilute and concentrated grafting, to be equally dispersed in different positions for equal possibilities. 24 hour old larvae were choose for grafting. The experimental colonies were monitored regularly. Data for successful graft were recorded on third to fifth days of grafting. The data for number of graft sealed were taken and lastly number of newly emerged queens were counted.

Rearing of queen in colonies with or without queen bee

Twelve colonies (10 frame) of approximately uniform strength were employed to test the feasibility of producing queen bee in colony with queen and colony without queen bee. Four colonies are used for this test and the test was repeated three times. In each batch two colony having queen bee and two colony do not have queen bee. Colonies without queen bees were dequeened approx. 15 hour before grating. In both condition eggs and open brood were removed. 24 hour old larvae were choose for grafting. Each colonies receives 24 grafted larvae. The successful graft, sealed pupae and emerging queen bee were then recorded and compared.

Statistical analysis

Using ANOVA techniques, the recorded data was examined for the existence of significant differences in colony performance across treatment groups. Microsoft Excel 2016 was used for statistical analysis. Analysis of Variance (ANOVA) at 5% was employed to test the significance of mean difference.

Results and Discussion

Role of different queen cell cup sizes on grafting success, sealing and emergence of queens.

The rate of successful graft among 216 grafted larvae in different cup sizes were ranged from 70-73% (mean 154 larvae). Guler and Alpay (2005) [13] observed an average 75.83±1.41% successful graft in different *A. mellifera* races. 79.1 - 95.8% successful grafting were reported by Koç and Karacaoğlu (2004) [28]. In addition, Wilkinson and Brown (2002) [25] found 81% successful grafting. Current data was lower than above finding.

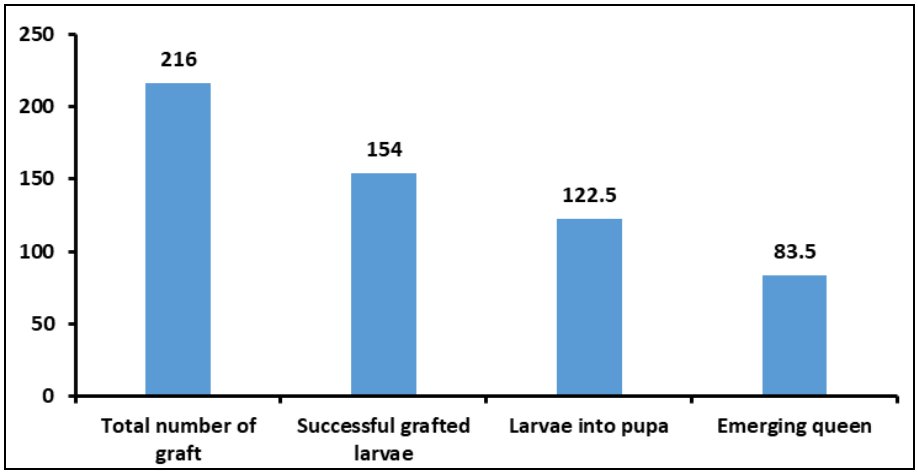

Fig 1: No. of successful graft, sealing of larvae and emergence of queen bee.

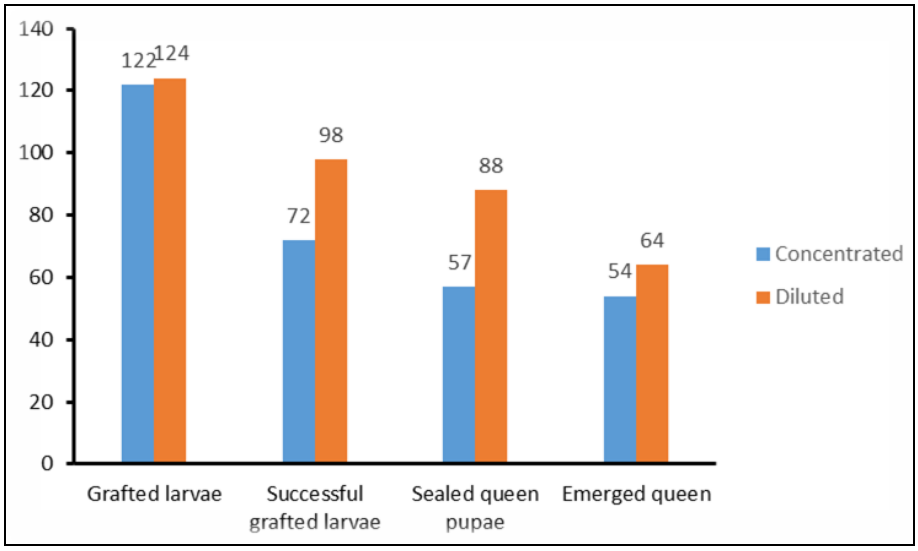

Fig 2: No. of successful graft, sealing and queen bee emergence when larvae was grafted in diluted and concentrated condition.

Total number of larva pupate in different cup sizes were ranged from 53% - 61% (mean 122.5pupae) and rate of emerging queens were also ranged from 37- 41% (mean 83.5 queen bees). There were no significant variation observed in success of graft, sealing and emergence of queen from grafting in different cup sizes. This due to the nature of worker bee, which can alter cup sizes, according to their need. The present finding disagree with the result of Skowronek and Skubida (1988) [23] who found that success of graft in case of smaller cup size 0.78-0.9 cm is more than the grafting in 0.10-0.12 cm diameter of cup. Wilkinson & Brown (2002) [25] in Africa reported very low (33%) successful grafting in two races of *A. mellifera*. Furthermore, success of graft and emergence of queen negatively correlated with the plants having short flowering period and ambient climatic condition in which rearing was performed (Zhadanova, 1967; Abdellatif *et al*., 1970) [27, 1]. In addition, Ruttner (1983) [22] eported several in colony factors such as strength and age of nurse bees, how old grafted larvae is, colony with or without queen bee and the period without queen in a colony.

Effects of diluted and concentrated grafting on grafting success, sealing and emergence of queens

Only 72 (58.8%) out of 122 concentrated grafted larvae were accepted, but 98 (76.4%) out of the 124 diluted grafted larvae were successful (Table 1). The variance in successful grafting rate was significantly different between the two techniques (N= 246, *df* = 1, $p< 0.0006$). In terms of sealing rate, dry grafting resulted in only 57 (46.8%) out of 122 grafted larvae sealing, but moist grafting resulted in 88 (70.9%) out of 124 grafted larvae sealing. The sealing rate of grafted larvae between two techniques was significantly different (N= 246, df = 1, $p< 0.0001$). The sealing rate in case of diluted grafting is more than concentrated grafting due to dry and relatively low humidity weather condition of during observation, dilution prevent from desiccation.

The difference in the rate of sealing of grafted larvae between the two techniques was significant (N= 246, df = 1, $p< 0.0001$). The significantly more acceptance and sealing rate of wet grafting versus dry grafting could be attributed to the benefits of wet grafting in reducing desiccation of the grafted larvae due to the area's low humidity conditions. Similarly, El-Din (1999) [12] reported success of grafting was high when royal jelly used during grafting.

In both cases, after sealing of larvae emergence of queen was low with only 54 queen (44.3%) and 64 queen (51.7%) out of 122 concentrated and 124 diluted grafting respectively. The difference in rate of queen emergence in both cases were not significant. The substantial decline in queen emergence rate linked with abiotic factors along with in colony factor that interfered in incubation of pupae.

Table 1: Comparisons in grafting success rate, sealing and emergence of queen bee between concentrated and diluted grafting.

Response	No. (%)			Test	
	concentrated	Diluted	*df*	X^2-value	p-value
Successful grafted larvae	72(58.8)	98(76.4)	1	11.540	0.000
Sealed queen pupae	57(46.8)	88(70.9)	1	14.939	0.000
Emerged queen	54(44.3)	59(51.7)	1	1.331	0.248

Rearing of queen in colonies with or without queen bee

Only 52 larvae (36.1%) Out of 144 grafted larvae provided to colonies with queen bee were successful (Table 2).

Out of 156 grafted larvae, only 111 larvae (71.2%) were successful in case of colonies without queen bee. The difference in successful grafting rate between colonies with and without queen bee was statistically significant ($p<$ 0.0001). Only 39 (27.1%) out of 144 grafted larvae in the colonies with queen bee were pupate, whereas 103 (65.9%) of 156 grafted larvae in colonies without queen bee were pupate less. The difference in the rate of pupation between colonies with and without queen bee was statistically significant ($p<$ 0.0001).

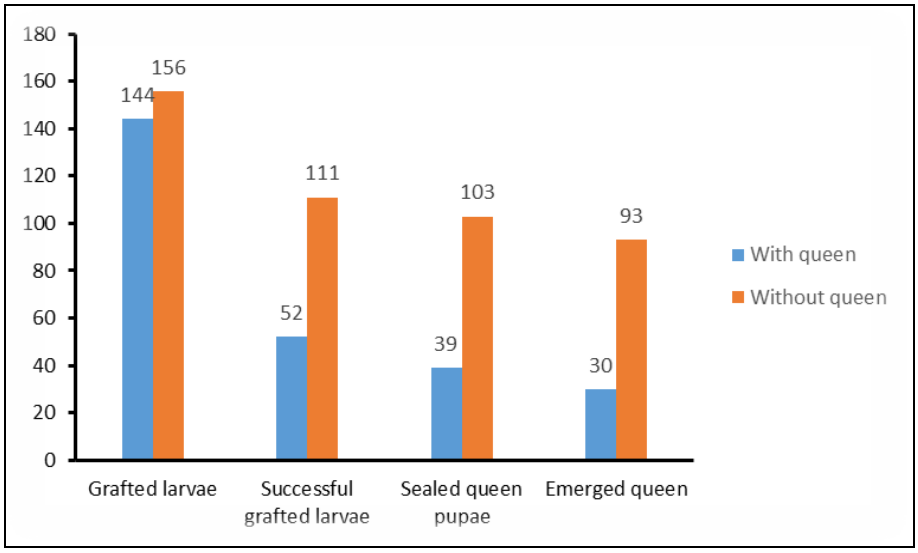

Fig 3: No. of successful grafting, sealing and queen bee emergence, when larvae were grafted in colonies with and without queen bee.

Only 30 queen bee (21%) emerged out from colonies with queen bee, whereas from colonies without queen, 93 queen bee (59.6%) were emerged. The difference rate of emergence of queen between the two conditions was statistically significant (p<0.0001). Ahmad and Dar, 2013 reported similar result that success of grafted larvae is higher in colonies without queen than colonies with queen. Furthermore, Crailsheim *et al*. (2013) [9] found that producing queen bee in colonies without queen was more effective than in colonies with queen bees.

Table 2: Comparisons in successful grafting rate, pupation and queen bee emergence, between colonies with and without queen bee

Response	No. (%)			Test	
	With Queen	Without Queen	*df*	X^2-value	p-value
Successful grafted larvae	52(36.1)	111(71.2)	1	37.059	1.15E-09
Sealed queen pupae	39(27.1)	103(65.9)	1	45.55188	1.15E-09
Emerged queen	30(21)	93(59.6)	1	46.5577	8.9E-12

The small colony size may account for the comparatively low grafting success, pupation, and rate of emergence of queen bee from colonies having queen. The small colony size is well recognised and considered an adaptation to prevent dangers during the region's extended dearth season (Ruttner, 1988) [21]. Spread of queen pheromone and recognition of queen bee presence is easier in case of smaller populated colonies. Rearing of queen bee in a colonies with queen bee, only considerable number of queen bee produced, but mass production of *A. mellifera* queen bee may not achieved (Buchler *et al*., 2013) [5]. Rearing of queen bee without de–queening the existing queen is useful in maintaining colony integrity as well as production of queen for personal colony extension.

Conclusion

From present study, it is concluded that the success of grafting does not depend on the different grafting wax cup sizes because worker bees can alter the wax cup size according to body size. Successful grafting, pupation and emergence of queen from cups in which diluted royal jelly is used as substrate is more than concentred grafting in dry climatic condition. Similarly Successful grafting, pupation and emergence of queen from colonies without queen is high than colonies with queen.

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