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Studies on Comparative Performance of Grafted Queen Colony and Naturally Reared Queen Colony of Italian Bee, *Apis mellifera* L. (Hymenoptera: Apidae) at Tiruchirapalli condition

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ABSTRACT: The performance of grafted queens in comparison with the naturally reared queens was determined on (*Apis mellifera* L.) colonies. The results revealed that the grafted queen colony was significantly superior to the naturally reared queen colony. The maximum brood area of egg was 52.29 cm^2, larva was 74.50 cm^2 and pupa was 94.46 cm^2 was observed in the grafted Queen colony. The honey stores and pollen reserves in the grafted Queen colony were 276.92 cm^2 and 41.98 cm^2 respectively. The queen weight and egg laying capability of the grafted Queen was 184.90 mgs and 1829.01 eggs per day respectively were significant over the naturally reared Queen. While the naturally reared Queen (T_1) has a minimum brood area of egg was 39.42 cm^2, larva was 58.17 cm^2, pupa was 94.46 cm^2. The honey stores was 253.13 cm^2 and pollen reserves was 37.13 cm^2 in the natural queen colony. The naturally reared Queen has a minimum mean weight of 171.40 mg among all treatments and egg laying capability of natural queen has 1660.00 eggs per day was lesser than the grafted Queen colony. The grafted queen colony has a significant difference (P>0.05) than the naturally reared queen colony among all the treatments. It is to fact that queens of Italian bee is not well exploited in tropical ecosystems, however in this study various techniques were identified and adopted to rear the mass queen rearing of Italian bees in tropical ecosystem for commercial exploitation.

Keywords: Artificially Grafted Queen, Naturally reared Queen, Brood area, Queen weight, Egg laying capability.

INTRODUCTION

Artificial queen rearing is a specified process for a commercial bee keeping practices. Queen bee is a vital working individual in a both commercial and non-commercial bee colony (Kumar and Singh 2004). It is only the main factor responsible for the stabilization and multiplication of the colony. Usually certain biotic and abiotic factors are responsible for the artificial queen rearing (Mahbobi *et al*., 2012). Abiotic factors include climatic factors and experimental factors such as queen cell cups etc. normalize the larval acceptance and provisioning of diet to the young larvae (Weiss, 1967). Queen supplementary is an essential process for the optimum production of brood and honey cells. Queen pheromones will mark the colony behaviour and its performance. The most important parameter is to recognize the features that influence the feeding responses of grafting larvae. All the premature larvae normally feeds on the royal jelly, on the basis of the extent, heavily fed upon a royal jelly the queen has to be emerged (Coby, 2007). The young one which fed less extent upon royal jelly, those individuals will become a worker population. Royal jelly is a milky white protein rich secretion from the pharyngeal gland of the worker honey bees (Eissa, 2007). Honey is the natural substance that was collected by *A. mellifera* foragers from the nearby floral sources and stored it in the comb to ripen and mature (Al Khalifa and Al-Arify 1999). The constituents of royal jelly will play a major role in the acceptance of royal jelly feed *i.e*., it is usually consists of 52 % of royal jelly primed with 5% of both honey and pollen supplements (Vuillaume, 1956). Various procedures of queen bee rearing have been adopted to stabilize the commercial bee keepers to interchange the older or fragile queen into a fresh queen to initiate the newer colonies (Shah, 2000). Queen bees

are responsible for the placing of fertilized or unfertilized eggs according to the width of the cell (Laidlaw 1997). The queen lays single egg on the cell and also it placed the egg in a proper positioned manner while, a worker lays improper egg laying on the single cell and also the presence of queen will only provide proper coordination of activities in the colony (Mattila and Seeley 2007). A fully mated queen will lays about 2000 eggs/day throughout on flow season (Root and Root 1980). The present study meant at rearing of artificial queen through the certain adopted methods and evaluation of its performances with a naturally reared queen colony. Honey bee contains sufficient nutrients such as amino acids, vitamins and other phenol compounds (Guler *et al.,* 2007).

MATERIALS AND METHODS

The proportional culture of artificial and natural queen rearing of Italian bee colony was evaluated based on the presence of brood area of immature stages such as egg, larva and mature stage such as worker, drone populations at the count of seven days interval at bee garden at ADAC & RI, Tiruchirapalli (Latitude 10°45′N and 78°36′E) during the year 2021-2022. Six bee colonies of equal brood strength of Italian bee populations were selected for the experimentation.

Treatment (T_1) – Natural colony. This method comprised of a naturally formed and potent queen in the bee colonies. The queen was formed by the worker bees in the colonies by building one to many queen cells in the colony itself at regular intervals by built a specialized queen cell at a corner of the frames in that colony and lay the eggs on it. By feeding the royal jelly comparatively higher levels to the immature stages found in those naturally constructed cells, the natural queen was emerged. It was considered as treatment (T_1).

Natural colony consist of either it will be the superseded queen or emergency queen or swarming reared queen. The colony consists of six frames with optimum population. The performance of both the colonies was evaluated based on the presence of brood area i.e., number of egg cells, pupal cells, larval cells on them by measuring the brood area on both the colonies by using 25 cm^2 grid scale. The separate count was taken to reveal the food store capacity of both the colonies by measuring the number of pollen cells and honey cells by using 25 cm^2 grid scale. The weight of both queens *i.e.,* natural queen and grafted queen was taken at regular intervals to evaluate the efficiency of both the colonies. These are different parameters to be studied for evaluation of these two different colonies.

Artificially reared queen. The artificially reared queen or grafted queen was carried as treatment (T_2).

Artificial queen rearing procedure (Doolittle, 1915).

(Treatment – T_2) – Grafted queen colony or artificial reared queen colony

Materials requisite

— Chinese needle
— Melted bees wax
— Cell starter colony
— Nucleus colony
— Artificial queen rearing frame. (consist of larval cell cups, cup holder and rotary frame cell

For a grafting process we need an Italian bee colony with an optimum population with 7-8 frames and presence of adequate brood strength. For a grafting purpose we need only queen less colonies. Insert the queen rearing frame (as shown in the Fig. 1) into queen less bee hive for about 24 hours to make the components sterile and moulding of bees wax around the cup. Select one or two frames from the colony with good genetic structure and brood strength for selecting the early in star larvae from the population. Grafting 12-24 hours old worker cell larvae from the selected frame & placed it in a queen rearing cup by using Chinese grafting needle. After completion of grafting, insert the queen rearing frame into a Queen less colony for about 5-10 days. After 5-12 days of frame insertion, check the Queen emergence in the queen rearing cup and take the mature pupal Queen cells separately (as shown in the Fig. 2) and placed it in a plastic cage box with mesh like structure (as shown in the Fig 2). After hatching of queen cells from the wire mesh cage, placed the cages into the separated brood frame and then pinned into the queen less colony (one day old queen). After a day later releases the queen from the cage into the colony for allow to build brood in colony. The experimentation was analysed through AGRES software with CRD with two treatments with six replications.

RESULTS AND DISCUSSIONS

Brood area of the colony. The brood area comprises of the presence of area of eggs, larval cell (uncapped cells) and the presence of pupal cells (capped cells) were measured by using a solid rectangular grid of 25 cm^2. The brood area of natural queen colony and larval grafted queen colony was interpreted below. The area of egg laying at grafted Queen was 52.29 cm^2. In naturally reared queen, the presence of area of eggs was 39.42 cm^2. By these aspects the egg laying capability was maximum at grafted queen when compared with naturally reared Queen (Table 1). By relating the brood emergence viz., presence of larvae (uncapped), pupa (capped) cells. The grafted queen has the maximum efficiency with 74.50 cm^2 of larva (uncapped cells). The natural queen has the uncapped cells of 58.17 cm^2. In these aspects the grafted queen has a superior quality than the natural formed queen (Table 1). By comparing the another important brood character was the presence of capped cells in the brood area of the colony, The capped cells was found maximum at the grafted queen at the 35^{th} day count at 102.33 cm^2 when compared with naturally grafted queen colony has the capped cells at the final day count at 94.46 cm^2 (Table 1).

Area of food reserves in the colony. The presence of brood area of the colony not only defines the performance of queen in that colony. Apart from that the pollen stores and honey reserves also one of the prominent parameter when we compare the performance of different bee colonies of same species. The honey reserves were found greater at grafted queen colony at 276.92 cm^2 whereas in natural queen colony

has the honey reserves of 253.13 cm^2 (Table 1). The pollen stores in the grafted queen colony were superior value of 41.98 cm^2 than natural colony of 37.13 cm^2 (Table 1).

Fig. 1. Queen rearing frame of Italian bee (*Apis mellifera* L.). It consists of queen rearing cup, cup holder and wooden rotatable frame of sixteen numbers of queen rearing cups.

Fig. 2. Pupal stage of larval grafted Queen of Italian bee and adult queen emerged and kept it at a cage for release into the queen less colony.

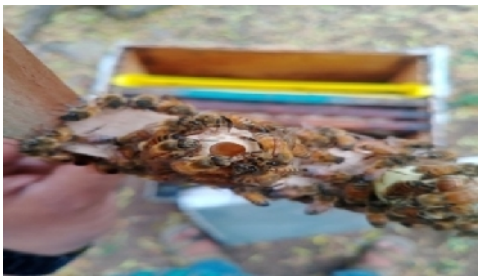

Fig. 3. Grafted Larva nourishing with Royal jelly.

Weight comparison of natural queen and grafted queen colony. The weight of queen was taken to evaluate the performance of the queens of both the colonies, the diet consumption or gravid and flaccid queens will determine the weight comparison results. In grafted queen has weighed at a maximum mean value of 184.9 mgs compared to naturally found queen has a mean value of 171.4 mgs (Table 2).

Comparison of egg laying capability of natural queen and grafted queen colony. By comparing the egg laying capability of the two types of Italian bee colonies in our bee flora, Grafted Queen has the maximum capacity of being 1829.01 eggs/day followed by the Natural Queen has the maximum capability of 1660.0 eggs/day (Table 3). It can be assessed by measuring the eggs using 25 cm^2 grids on the frame.

The experimentation was carried out in a mean of six observations based on the measurement of pollen store, honey store, brood area (eggs, larva, pupa). The experiment was carried out in a CRD Analysis in AGRES software. The differences in queen weight was analysed with SPSS software in a paired t-test with six treatments and four replications.

Table 1: Comparison of brood area performance of Italian bee colonies of both natural and artificial queens.

Day of observation	Queen source		Brood (no.)*			Honey cells (no.)*	Pollen cells (no.)*
			Uncapped		Capped		
			Egg	Larva	Pupa		
0^{th} day	**Natural queen** (T_1)	1	40.50	71.00	61.75	201.50	44.50
7^{th} day		2	22.50	31.50	58.50	298.75	28.50
14^{th} day		3	26.75	24.25	64.50	272.25	17.25
21^{st} day		4	39.75	41.25	84.00	257.00	41.00
28^{th} day		5	62.00	69.00	128.00	239.25	42.50
35^{th} day		6	45.00	112.00	170.00	250.00	49.00
Mean			39.42	58.17	94.46	253.13	37.13
0^{th} day	**Grafted queen** (T_2)	1	13.00	21.50	33.50	451.00	34.50
7^{th} day		2	33.50	29.00	18.50	286.00	42.50
14^{th} day		3	70.75	47.75	72.00	210.25	25.25
21^{st} day		4	82.25	41.00	86.75	257.5	43.25
28^{th} day		5	56.75	141.25	159.75	228.5	37.50
35^{th} day		6	57.50	166.5	243.5	228.25	40.00
Mean			52.29	74.50	102.33	276.92	41.98
SEd			0.93	1.66	1.94	2.44	5.57
CD (p=0.05)			2.08	3.71	4.33	1.09	2.49

*Mean of six observations

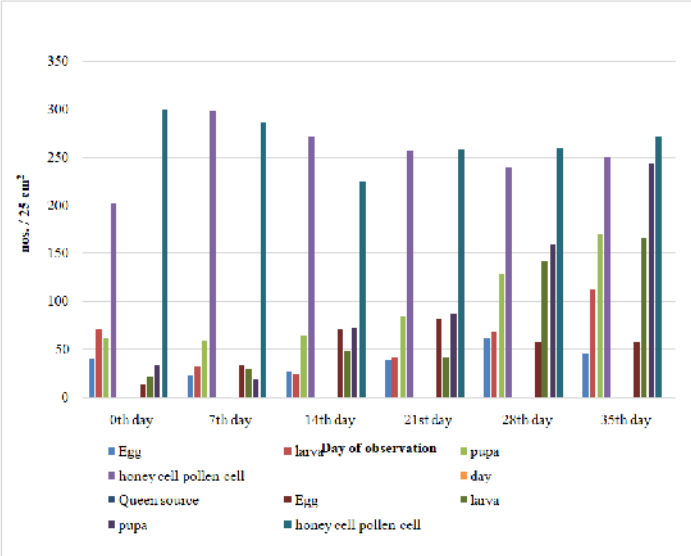

Fig. 4. Brood development studies of Italian bee *A. mellifera* through artificial queen rearing.

Table 2: Comparative Weight of Natural Queen and Artificial queen of Italian bee colonies (mg).

Standard week	Grafted Queen (mg)*	Natural Queen (mg)*
1	188.3 (14.22)	171.8 (13.60)
2	182.5 (14.00)	167.1 (13.42)
3	188.0 (14.21)	172.1 (13.62)
4	182.8 (14.02)	171.1 (13.57)
5	187.0 (14.17)	177.9 (13.83)
6	181.3 (13.96)	168.7 (13.48)
Mean	184.9 (14.10)	171.4 (13.59)
SEm	1.27	1.51
t value (0.05)	2.78	

*Mean of six observations
Figures in the paranthesis are square root transformed values.

Table 3: Comparative egg laying capacity of natural and grafted Italian bee colonies.

Standard Week	Grafted Queen*	Natural Queen*
1	1780.5 (42.69)	1640.5 (41.00)
2	1822.3 (43.18)	1620.4 (40.75)
3	1770.7 (42.57)	1636.6 (40.95)
4	1850.2 (43.51)	1710.0 (41.85)
5	1880.4 (43.86)	1692.5 (41.64)
6	1870.0 (43.74)	1660.0 (41.24)
Mean	1829.01 (43.26)	1660.0 (41.24)
SEm	18.76	14.20
t-value (0.05)	2.570	

*****Mean of six observations
Figures in the paranthesis are square root transformed values.

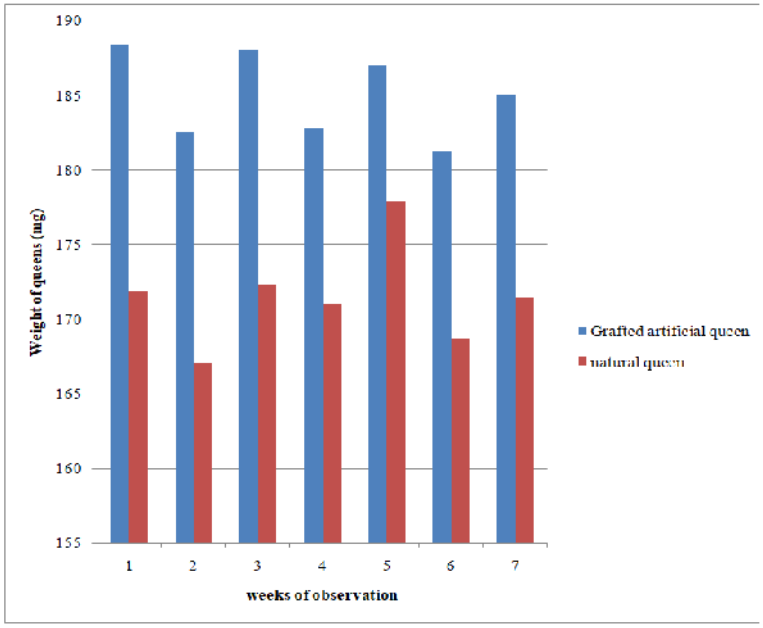

Fig. 5. Comparison of weight of Grafted and natural queen of Italian bees.

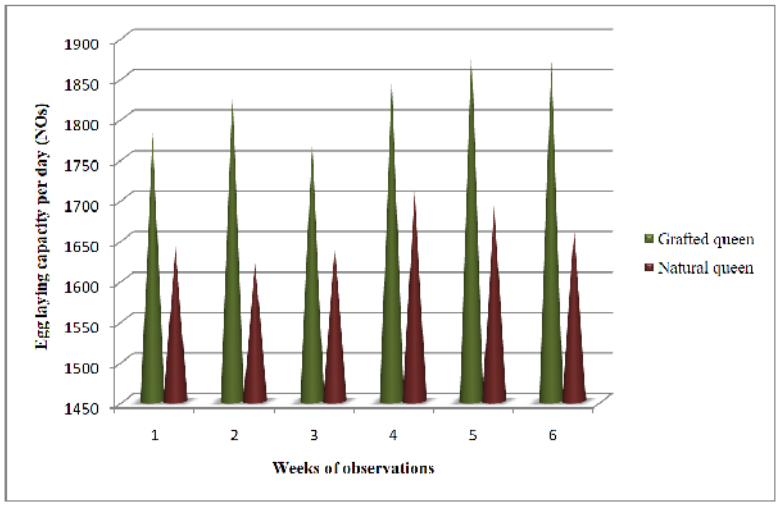

Fig. 6. Egg laying capacity of Grafted Artificial queen and Natural queen of Italian bees.

CONCLUSION

The present findings were consistent with previous research, which found that queen bees created by the grafting procedure performed better than queen bees produced through other methods (Doolittle, 1915; Abrol *et al*., 2005; Abbasi *et al.*, 2015). Suryanarayanan *et al.* (1998) also reported that grafted Queen colonies were found good Quality than the naturally found Queen colonies in terms of brood performance and good structure of organization and coordination among the population. The present studies were coincide with the food consuming ratio of workers and the types of races that will have vigour performance and produce good quality queens was mainly based on the nutrient supplement and heredity characteristics given by Shehata (2009). In this study, the equipments such as grafting needle, cell cup, and grafting cup mounting was sterile with bees wax for easy acceptance of grafted larvae by the worker bees and free from microbial contamination (Dodologlu and Emsen 2007). The larval acceptance ratio was 68% by the worker bees in the queen less colony due to the Tropical conditions at Tiruchirapalli. The above larval acceptance according to the ecological conditions was given by Cengiz *et al.*, (2009). The results proved that both the types of bee colonies are significantly differed under different treatments and the following trends were similar under different parameters of study. It also concluded that grafted queen colonies will provide higher stability due to the significant advantages of brood production and food reserves ability over the naturally found queen colony among all treatments in the study (El Din Haes, 1999). The Italian bee queens are important prospectus of the colony maintenance and development. By this study the commercial apiaries will produce mass reared the queens of Italian bees and will sold separately for the farmers for colony maintenance and for

development in the commercial basis at tropical conditions like Tiruchirapalli regions.

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Conflict of Interest: None.

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