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Anjali JS
M.Sc. Student, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Dr. Amritha VS
Professor and Principal Investigator, AICRP on Honeybees and Pollinators, Department of Entomology, College of Agriculture, Vellayani. Thiruvananthapuram, Kerala, India

Dr. Anitha N
Professor and Head, Department of Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Dr. Vijayasree V
Assistant Professor, AICRP on Honey bees and Pollinators, Department of Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Dr. Pratheesh P Gopinath
Assistant Professor and Head, Department of Agricultural statistics, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Corresponding Author:
Anjali JS
M.Sc. Student, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Evaluation of first-generation Indian bee, *Apis cerana indica* colonies raised from breeder colonies by grafting method

Anjali JS, Dr. Amritha VS, Dr. Anitha N, Dr. Vijayasree V and Dr. Pratheesh P Gopinath

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Abstract
Ten best performing Indian bee colonies maintained at AICRP on Honey bees and Pollinators, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala were selected and observations on their development and hygienic traits were recorded from Oct 2022 to Aug 2023. Characters which showed significant variation was subjected to monthly analysis. Monthwise analysis of the parameters revealed that highest honey area was recorded during March (90 cm^2), while the pollen area was at its peak during May to June which ranged from 32 cm^2 to 33 cm^2. Brood area was found highest during August (525 cm^2) followed by March (482.50 cm^2). A significant negative correlation of parameters was observed with rainfall and relative humidity whereas positive correlation was found with sunshine hours. In case of the pollen area, a significant positive correlation was recorded with temperature. Based on the selection index calculation, five breeder colonies were marked and the respective daughter colonies were raised by Doolittle method of grafting. No significant difference was observed in the traits between the breeder colony and first-generation colonies except in the pollen area. Thus, the study revealed that all the traits from the mother colonies got transferred to daughter colonies by grafting method.

Keywords: *Apis cerana indica*, honey area, pollen area, selection index, queen rearing, Doolittle

Introduction
Honey bees are eusocial insects living in communities of 10,000 to 20,000 individuals (Hider, 1988) [4] with remarkable degree of social instincts and division of labour. Bees contribute significantly to biodiversity by pollinating wild plants, thereby, sustaining various ecosystems. They are primary pollinators for many flowering plants and food crops including fruits, vegetables, nuts, and oilseeds. Pollination aided by Indian bee (*Apis cerana indica*) resulted a yield increase of 25 per cent in cucumber and also improves the quality parameters of the crop (Premila *et al*., 2014) [8]. For getting higher honey yield and better pollination, a good population of industrious worker bees is necessary. Maintaining colony's strength heavily relies on the presence of a high-quality queen which is often regarded as the repository of the colony's inherited traits. The queen plays a pivotal role in shaping various traits of the colony, including industriousness, temperament, tendencies towards swarming or absconding, disease resistance, as well as pollen and nectar hoarding ability (Wakjira *et al*., 2019) [18].
Under natural conditions, queens are reared under three primary impulses: swarming, supersedure, and emergency queen rearing. Due to the need of constant monitoring, unpredictable timing for multiplication and limited number of queens, relying simply on natural method is not a good option for queen rearing (Suryanarayana *et al*., 1998) [15]. Artificial queen rearing serves multiple vital purposes in beekeeping where it improves colony strength and population, facilitates the replacement of defective or unproductive queens within colonies, ensures the supply of new queens to queen-less colonies, and enables the replacement of aging queens, specifically those aged two years or older. This comprehensive process optimizes the efficiency, productivity, and overall health of colonies, thereby sustaining the vitality of the beekeeping operation. Thus, mass queen rearing is an essential practice in modern beekeeping for ensuring resilient, productive, and genetically diverse honey bee colonies (Jonstone, 2008) [5].

Materials and Methods

Ten superior Indian bee colonies (C1 to C10) based on the previous records was selected for the study from the Indian bee apiaries maintained at AICRP on Honey bees & Pollinators, Vellayani center. The hives of the selected colonies were maintained with six frames in the brood chamber and artificial feed (sugar solution 50% @250 g per colony) were provided in feeders at weekly intervals during the dearth season (May to Jul) and brood rearing season (Aug to Oct).

The economic, brood development and hygienic traits of these ten colonies were recorded for the selection of best five breeder colonies based on the selection index ranking. The following parameters were evaluated:

Honey area (cm^2): The honey/artificial feed storage area on either side of the frame in the brood chamber was calculated with the help of a square grid.

Pollen area (cm^2): The pollen storage area on either side of the central four frames in the brood chamber was calculated with the help of a square grid.

Brood area (cm^2): The brood area on either side of the central four frames in the brood chamber was calculated with the help of a square grid.

Number of combs covered with bees: Visual scoring was given to the combs based on the area covered by the bees on either side of the frames (bees covered fully on both sides of the frame: 1, 75 per cent as a total on both sides of the frame: 0.75, 50 per cent as a total on both sides of the frame: 0.50 and 25 per cent as a total on both sides of the frame: 0.25).

No. of eggs hatched: A comb from the centre of the hive was replaced with a brood (egg, larva and pupa) free frame and the number of eggs laid in the marked area (25 sq.cm) was recorded after three days. Four replications were maintained per hive. After three days, the number of eggs hatched in the marked area was recorded. The number of surviving larvae was also noted in the next day. The percentage of eggs hatched was calculated as follows:

$$\text{Eggs hatched (\%)} = \frac{\text{No. of eggs hatched in an area of 25 sq.cm}}{\text{No. of eggs laid in an area of 25 sq.cm}} \times 100$$

Number of pin-killed pupae removed: For assessing the hygienic activity, about 25 capped pupae was killed by piercing the caps with a needle. The pierced cells were observed for the removal of the killed pupae by the worker bees after two days. The percentage removal of killed pupae was calculated by

$$\text{Percentage removal} = \frac{\text{Number of pin-killed pupae removed} \times 100}{25}$$

Monthwise analysis of the parameters as well as the correlation analysis of the parameters with weather parameters were also assessed.

Calculation of selection index

A standard score known as Z-score was calculated for honey area, pollen area, brood survival rate, brood area and bee population, number of pin-killed pupae removed in all the ten selected colonies. Then selection index (I_{sel}) was calculated by taking the grand total of Z scores for each trait in the respective ten colonies, after that the colonies were ranked according to selection index.

Raising of first-generation queens from the selected stock:

The best performing five colonies (Plate 1.) were selected as breeder colony based on the selection index ranking to raise the next generation using the Doolittle method of grafting. The queen was caged for six days before grafting to prevent egg laying. For raising grafted larvae, another five colonies (cell builder colonies) with sealed brood combs along with abundant honey and pollen stores were selected. The presence of substantial population of nurse bees aged between 5-10 days to facilitate the production of royal jelly was also ensured. This colony was dequeened one day before grafting to create a queen-rearing impulse. Grafting (Plate 2.) was done in morning time at suitable temperature (around 25^0C) and humidity (around 50%). Larvae less than one day age old was removed from the comb of the breeder colony (Plate 3.), one at a time with grafting needle and was transferred into the queen cell cups of the grafting frame, one in each cell cup in the cell builder colony. The best queen cell developed in ten days after larval grafting (Plate 4.) was only retained in the colony and was observed for the queen emergence and egg laying. Observations on various parameters of daughter colonies were recorded from fourth week after brood development and was compared with the mother colony.

Statistical analysis

The data recorded was significantly analyzed by using MS−Excel and software GRAPES Version 1.1.0 (Gopinath *et al*., 2021) [3]. The mean value of data was subjected to statistical analysis using ANOVA. Simple correlation analysis was done to analyse the influence of weather parameters in the colony performance. Two-tailed Student's *t*-test was used to compare the means of two groups of mother colony and daughter colony.

Results

Performance of colonies over the entire seasons

Observations on the economic trait, honey area indicated that the mean area covered by honey ranged from 29.28 cm^2 to 67.00 cm^2. The honey area covered by colony number C1, C4, C7, C8, C10, C2, C9, C6, C3, C5 in descending order was 67, 66.43, 65.50, 63.37, 55.15, 52.75, 49.84, 47.56, 39.81, 29.28 cm^2 respectively. No significant difference was found in pollen area among the colonies. The highest brood area was recorded in C1 (445.83 cm^2) which was on par with C7 (432.00 cm^2), C4 (420.33 cm^2), C8 (411.50 cm^2) and C6 (408.93 cm^2) during all the seasons. Highest bee population was recorded in C7 (4.90) while the lowest was in C2 (3.81). With regard to the brood survival rate, C1 had the highest brood survival rate which was on par with C4, C8, C6 and C7. During the entire season, all colonies had more than 90 per cent removal of pin killed pupae. C4 (99.83) which was on par with C1(99.50%), C8 (99.50%), C6 (99.33) and C7 (99.00%) had the maximum removal of dead larvae.

Month wise analysis of the parameters revealed that the highest honey storage was found during March (90.50 cm^2) and lowest in July (35.10 cm^2) (Fig 1). During August (brood rearing season), a sudden increase in honey area is observed followed by subsequent decrease in the rest of the two months. In contrast to honey area, pollen area was found higher during May to June which ranged from 32.00 cm^2 to 33.00 cm^2 (Fig 2). Brood area was recorded to be highest in August (525.00 cm^2) followed by March (482.50 cm^2) (Fig 3). A significant negative correlation of parameters was observed with rainfall and relative humidity whereas positive

correlation was found with sunshine hours. In case of pollen area, a significant positive correlation was recorded with temperature. No significant relationship was found with wind velocity.

From the grand total of Z scores, selection index was calculated and ranking of colonies were done. The colony, C1 (212.57) is the best colony followed by C7 (178.69), C4 (174.49), C6 (163.81) and C8 (149.61), hence these colonies were selected for raising the next generation.

Evaluation of the performance of first-generation colonies

Observations on economic traits, colony development behaviour and hygienic behaviour of the daughter (first generation) were recorded in the brood rearing season. No significant difference was found in the honey area which ranged from 48.33 cm^2 to 61.67 cm^2. The pollen area varied from 25.83 cm^2 - 51cm^2. C15 (51.00 cm^2) with highest pollen area was on par with C13 (50.00 cm^2) and C14 (41.00cm^2). Colony development traits *viz*., brood area, bee population and number of eggs hatched showed no significant difference among colonies. In case of pin-killed pupae removed, all colonies had more than 95 per cent of the dead pupae removed, however the results were found non-significant.

Comparative evaluation of the parameters between the mother colony and daughter colony revealed that there was no significant difference between the respective parameters except that of pollen area.

Discussion

In the present study, selection of colonies was made based on certain traits which are in conformity with Thakur (1994) [16], where the selection of superior honey-producing breeder colonies was done based on biological (brood survival rate, pin-killed pupae removed and bee population) and economic traits (honey stores and pollen stores). Sharma *et al*., (2017) [12] highlighted notable distinctions among colony parameters, such as pollen stores, colony strength, brood area, and honey stores specifically in Nauni conditions.

Highest honey area was recorded in March during honey flow season while highest pollen storage was observed during June – July in the dearth season; brood area was highest in August during the brood rearing season. Studies conducted by Reddy (1980) [9] at Bangalore revealed that higher honey and pollen storage was found in February and April while at Dharwad, Karnataka, brood rearing activity was greatest in January to March with a minimum in July followed by a sharp increase in August (Sattigi and Lingappa, 1994) [11]. In northern parts of India, maximum pollen and honey stores was observed in October as reported by Kumar and Wakode (1997) [6] in their studies conducted at Pune. But in Jeolikot, Uttar Pradesh maximum brood area and pollen area was recorded in October (Verma, 1988) [17]. Thus, studies conducted by different researchers showed that different traits like honey area, pollen area and brood area varies in different regions depending upon climatic conditions prevailing and foraging sources in that area.

The traits like honey, pollen and brood area are dependent on weather parameters like temperature, relative humidity and rainfall as proved by the correlation analysis. All the parameters showed a significant negative correlation with rainfall, relative humidity whereas significant positive correlation was observed with sunshine hours. This can be explained in a context that during high humid rainy conditions, foraging activity decreases which may affect the honey and pollen storage. This is in accordance with the studies by Pastagia and Patel (2014) [7] where they reported a significant negative correlation in brood area, pollen area, and nectar area with the weather factors like minimum temperature, relative humidity, and rainfall. Conversely, they found a positive association with sunshine hours. Similarly, Das and Rahman (2000) [1] reported findings consistent with the present study which collectively emphasize the considerable influence of weather conditions on the behaviour and activity of Indian bees.

From the selected five parent colonies (C1, C4, C6, C7 and C8), corresponding daughter colonies were raised (C11, C12, C13, C14 and C15) using Doolittle method of grafting during brood rearing season, considering the favourable conditions for the improved colony development during this time. No significant difference was found between breeder and first-generation colony except in case of pollen area indicating similar performance (Fig. 4). Dodologlu *et al*. (2004) [2] reported that the breeding value of the queen honey bee depends on its age, emergence weight, rearing period, rearing conditions, number of ovarioles and spermatozoa stored, which in turn was higher for honey bees reared using the Doolittle method compared to those reared through the natural queen cell method. So, queen bees raised through the grafting method (Doolittle method) exhibit superiority over those reared using natural queen cells. But in the present experiment, drone control was not done this may be the reason for the lack of superior characters. In natural queen rearing, it is impossible to find the drone with whom the queen has mated. Richard *et al*. (2007) [10] has reported that in each queen mating nuclei colony at least 10 drone colonies are required in order to obtain best results. As the observations in the present study were taken for one season only, there is a possibility for the improvement in characters in the upcoming seasons.

Plate 1: Breeder colony

Plate 2: Grafting of larvae

Plate 3: Sealed queen cells

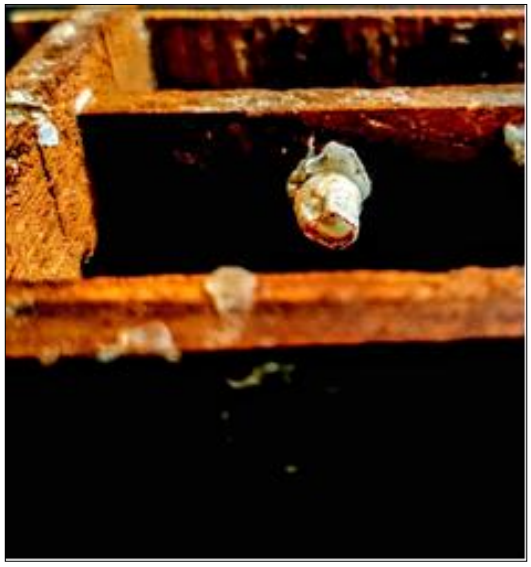

Plate 4: Emerged queen cell

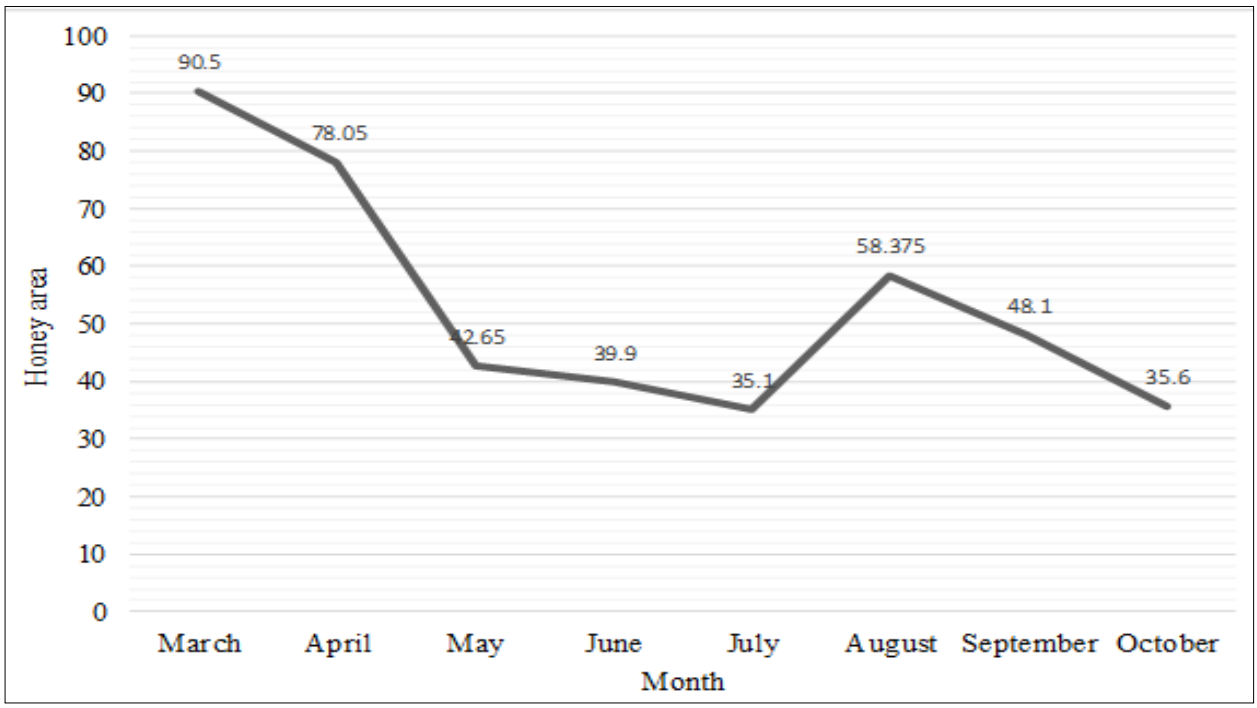

Fig 1: Month wise evaluation of honey area

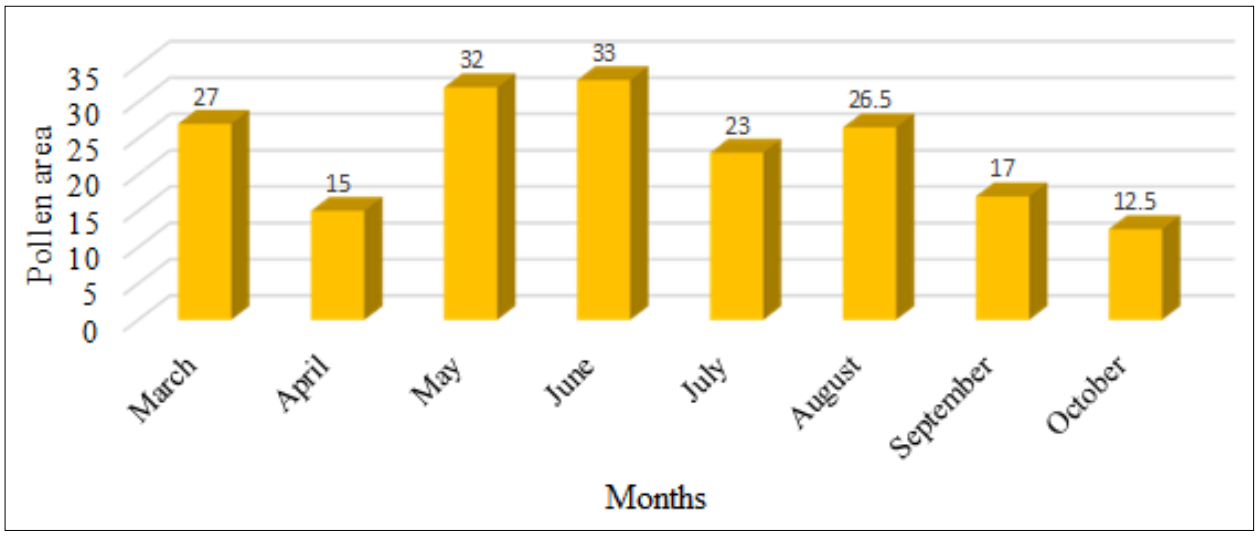

Fig 2: Monthly evaluation of pollen area

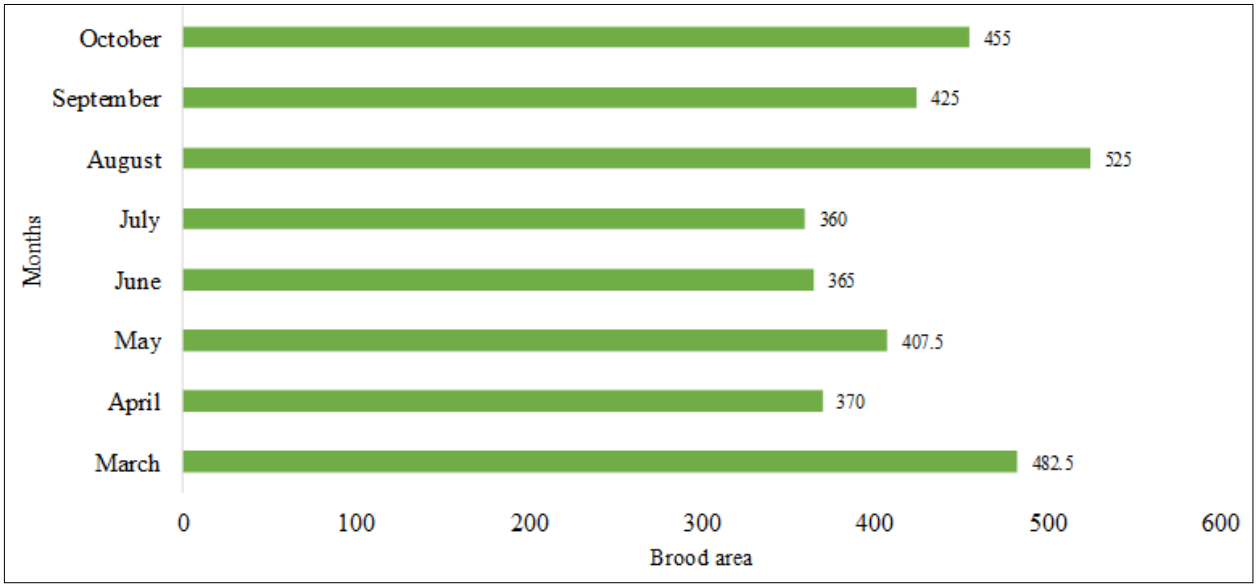

Fig 3: Monthly evaluation of brood area

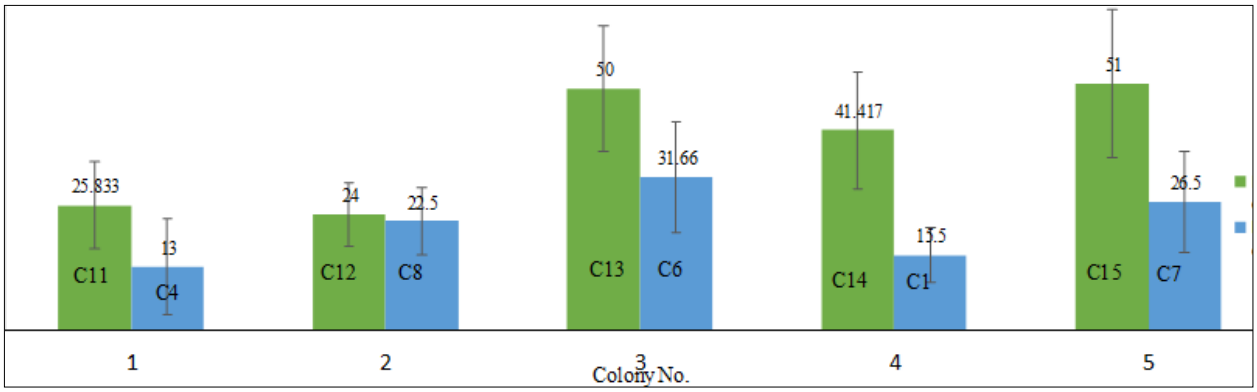

Fig 4: Comparative evaluation of daughter colony and mother colony in terms of pollen area

Daughter colony	C11	C12	C13	C14	C15
Mother colony	C4	C8	C6	C1	C7

* Significant (Student's t-test, $p< 0.05$)

Table 1: Evaluation of traits in *Apis cerana indica* from Oct 2022-July 2023

Colony Number	Honey area (cm^2)	Pollen area (cm^2)	Brood area (cm^2)	Bee population	Brood survival rate (%)	Pin-killed pupae removed (%)
C1	67.00^{a}	27.37	557.29^{a}	4.45	96.42^{a}	99.50^{a}
C2	52.75^{ab}	23.00	325.52^{c}	3.81	84.03^{c}	92.33^{b}
C3	39.81^{bc}	28.34	264.27^{d}	4.14	80.82^{c}	94.83^{b}
C4	66.43^{a}	21.25	525.41^{a}	4.40	94.63^{a}	99.83^{a}
C5	29.28^{c}	21.87	398.33^{b}	4.20	78.58^{c}	93.83^{b}
C6	47.56^{abc}	27.50	511.17^{a}	4.82	93.67^{a}	99.33^{a}
C7	65.50^{a}	22.56	540.00^{a}	4.90	92.36^{ab}	99.00^{a}
C8	63.37^{a}	17.87	514.37^{a}	4.32	94.32^{a}	99.50^{a}
C9	49.84^{abc}	17.06	304.89^{cd}	4.26	84.20^{c}	92.16^{b}
C10	55.15^{ab}	18.87	272.91^{cd}	4.32	85.57^{bc}	91.67^{b}
CD (0.05)	20.169	NS	2.78	NS	7.856	3.802

Table 2: Selection index of colonies

Colony	Honey area (a)	Pollen area (b)	Brood area (c)	Bee population (d)	Brood survival rate (e)	Pin killed pupae removed (f)	Selection index	Rank
	$Z_a = [(a-a_a)/a_a] \times 100$ a_a – Average of a values of all the ten colonies	$Z_b = [(b-a_b)/a_b] \times 100$ a_b – Average of b values of all the ten colonies	$Z_c = [(c-a_c)/a_c] \times 100$ a_c – Average of c values of all the ten colonies	$Z_d = [(d-a_d)/a_d] \times 100$ a_d– Average of d values of all the ten colonies	$Z_e = [(e-a_e)/a_e] \times 100$ a_e– Average of e values of all the ten colonies	$Z_f = [f-a_f)/a_f] \times 100$ a_f – Average of f values of all the ten colonies	Isel. $=Z_a+ Z_b+ Z_c+ Z_d+ Z_e+ Z_f+ Z_g$	
C1	27.77	29.29	0.32	115.90	9.52	29.77	212.57	1st
C2	0.59	8.62	-0.22	68.91	-4.54	20.42	93.79	6th

C3	-24.07	33.86	-0.37	56.99	-8.19	23.68	81.90	7th
C4	26.70	0.36	0.24	109.48	7.49	30.20	174.49	3rd
C5	-44.15	3.31	-0.05	83.87	-10.73	22.37	54.61	9th
C6	-9.29	29.88	0.21	107.06	6.40	29.55	163.81	4th
C7	24.91	6.55	0.28	112.90	4.91	29.11	178.69	2nd
C8	20.86	-15.57	0.22	107.20	7.13	29.76	149.61	5th
C9	-4.94	-19.41	-0.27	65.24	-4.34	20.20	56.46	10th
C10	5.18	-10.85	-0.35	58.91	-2.79	19.55	69.64	8th

Table 3: Evaluation of first-generation colonies during brood rearing season from Aug 2023 to Oct 2023

Daughter colony	Honey area (cm^2)	Pollen area(cm^2)	Brood area(cm^2)	Bee population	Brood survival rate (%)	Pin-killed pupae removed (%)
C11	58.00	24.00[b]	536.13	4.04	98.00	96.65
C12	49.00	51.00[a]	539.09	4.75	100.00	100.00
C13	61.67	50.00[a]	569.54	4.75	100.00	100.00
C14	50.17	25.83[b]	517.27	4.50	93.50	98.00
C15	48.33	41.00[a]	557.72	5.08	100.00	100.00
CD (0.05)	NS	13.836	NS	NS	NS	NS

Conclusion

Monthwise analysis of economic and development traits of Indian bee colonies revealed that highest honey storage was recorded during March, while the pollen area was at its peak during May-June and brood area was found highest during August which is due to the influence of climatic condition prevailing in the area. A significant negative correlation of parameters was observed with rainfall and relative humidity whereas positive correlation was found with sunshine hours by using correlation analysis. Comparative analysis of traits between the parent and daughter colonies revealed that no significant difference exists among the colonies, thus they exhibited a similar performance. According to the results obtained from this study, it can be concluded that the superior traits from the breeder colony was transferred to daughter colonies by using grafting method of queen rearing. However, further studies have to be conducted regarding this aspect to improve the graft acceptance rate in Indian bees and more innovative methods have to be developed considering the difficulty in identifying the appropriate larvae for grafting and larval death during grafting. Further studies are recommended to determine the important factors that affect the final success in obtaining hatched queen with superior quality.

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