Journal of Advances in Biology & Biotechnology

Volume 27, Issue 6, Page 552-562, 2024; Article no.JABB.116799
ISSN: 2394-1081

Performance of Artificially and Naturally Inseminated Honey Bee Queens (*Apis cerana indica*)

Pooja Borah [a*], Ataur Rahman [a] and M.K. Deka [a]

[a] Assam Agricultural University, Jorhat, Assam, 785013, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i6915

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/116799

Original Research Article

Received: 07/03/2024
Accepted: 14/05/2024
Published: 19/05/2024

ABSTRACT

Comparative performance of honey bee, *A. c. indica* colonies, developed from artificially and naturally inseminated queens was recorded in the apiary and under laboratory condition of Department of Entomology, Assam Agricultural University, Jorhat from September'2015 to March'2018.The selection studies were carried out from April, 2015 to January, 2016. Ten viable colonies were selected from 20 colonies of *A. c. indica* for the study. During the study between Artificially Inseminated(AI) and Naturally Inseminated(NI) colonies of *A. c. indica* from April'2017 to March'2018, significant difference were found in terms of brood area, pollen area and nectar area in all the months between AI and NI colonies. Pollination efficiency revealed significant difference between AI and NI colonies in April, May, June, October, December, February and March. Honey yield revealed significant difference between AI and NI colonies in May, July, December, January, February and March. And the comparative performance between AI and NI colonies of *A. c. indica* revealed that performance of AI queens was significantly better than the NI queens.

**Corresponding author: E-mail: pooja.borah@aau.ac.in;*

Cite as: *Borah, P., Rahman, A., & Deka, M. (2024). Performance of Artificially and Naturally Inseminated Honey Bee Queens (Apis cerana indica). Journal of Advances in Biology & Biotechnology, 27(6), 552–562. https://doi.org/10.9734/jabb/2024/v27i6915*

Keywords: Selective breeding; honey bee queens; artificially inseminated; naturally inseminated; comparative performance.

1. INTRODUCTION

The importance of honey bees for the welfare of mankind is fast increasing not only as a source of honey and wax, but also as chief pollinating agent. For this, concerted efforts are being made to improve the honey bee stock for increased honey yield, pollination efficiency and disease resistance. Natural Fertilization (NF) of honey bees results in vigour losses as both good and bad characters and the poor brood patterns from homozygous sex alleles are carried with the progenies .In conventional breeding, they mate within the same colony where the strongest male goesfor nuptial flight but the female size, vigour etc. is not selected. Whereas, Artificial Insemination (AI) gives control over mating and also can reduce the risk of spreading pathogen agents and pests by passing the semen instead of live honeybees.AI is important in order to control and improve genetic breeds, for the preservation and improvement of local breeds and to create disease resistant lines with high productivity. Therefore, selective breeding is needed to help prevent future colony collapse of hives and helps to produce stronger bees that have a resistance to mites and diseases. Wongsiri and Lekprayoon [1] studied artificial insemination between Chinese honey bees, *A. c. indica* and Thai honey bees, *A. c. indica* where the Chinese strain was used as the male parent and the Thai strain as the female parent to produce F_1 hybrid queens and they concluded that the egg producing ability of F_1 hybrid queens was significantly higher than Thai queens. Keeping these views in mind, the present investigation was undertaken to see the comparative performance between AI and NI colonies of *A. c. indica.*

2. MATERIALS AND METHODS

The research works on selective breeding of *A. c. indica* were carried out in the apiary and laboratory conditions at Department of Entomology, Assam Agricultural University, from September, 2015 to March, 2018.

Selection of viable colonies of *A. c. indica*: The selection of viable colony is an important prerequisite in order to get a successful artificial insemination as we will select the viable drone and queen cells from these selected colonies .The selection of viable colonies was carried out from Sep., 2015 to Jan., 2016. Screening of ten viable colonies was done based on parameters *viz*., colony strength, brood area, pollen area, nectar area, pollination efficiency and honey yield. The strength of each experimental colony was estimated by counting the number of frames with bees and expressed in the number of frames. Total brood area per colony of *A. c. indica*. was expressed in sq.cm by measuring the area under eggs, larvae and sealed brood. Similarly, total pollen area and nectar area of *A. c. indica* per colony was expressed in sq.cm by measuring the area under pollen and nectar. Three combs were selected randomly in each of the ten colonies of *A. c. indica*. A paper grid of size 10 x 10 cm was fixed successively on both sides of each of the selected comb in a colony and the area occupied by eggs, larvae, sealed brood, pollen and nectar were measured. The average egg, larval, sealed brood area, pollen and nectar area of the three sample combs were then converted for full colony. The observations were repeated at fourteen days interval. Pollination efficiency of the colonies of *A. c. indica*. was measured in terms of number of pollen loads entering the hive by determining this number at 1000, 1400 and 1700 hours. These studies were repeated three times a month on all the experimental colonies of *A. c. indica*. Number of pollen gatherers per five minute was recorded and the pollen load (milligram) per five minutes was taken as an indicator of pollination efficiency [2].The honey of *A. c. indica*. colonies were extracted and expressed in kilograms per hive.

Mass rearing of queen bees: After selection of the ten viable colonies in *A. c. indica*, they were used for two purposes. First five colonies were used for production of queens and the rest five colonies were used for the drones. In *A. c. indica*, the queens were prepared by making the colonies queen less. When the colonies were made queen less during breeding season, the strong bee colonies made upto twelve queen cells within one week. Each colony had eight frames, so after queen cell formation, all the eight frames with queen cells were transferred to nucleus boxes. Thus, a single nucleus box had two full frames and one frame with queen cells. One frame may have single or more than two number of queen cells. But once the queen emerges, it was observed to damage the rest queen cells. Therefore, from one nucleus bee box we get one queen and from one selected

bee colony, we get eight queens for artificial insemination. Therefore, in February, a total of forty queens were produced using the five selected colonies and out of which twenty healthy queens were selected for AI.

Preparation of drones for artificial insemination: The mature drones (14 day old) were collected from the selected five colonies of *A. c. indica* in "drone flight cage", and brought to the laboratory for semen collection (Fig 1B). Czekonska *et.al.* [3] studied the influence of *A. mellifera* drone age on volume of semen and viability of spermatozoa and concluded that the mean volume of semen significantly decreased with drone age and sperm viability increased significantly with drone age. To expose semen, the endophallus of the mature drones were everted by hand in two-steps: partial eversion (Fig 1D) and full eversion (Fig 1E). Then the semen from the endophallous was drawn inside the Schelly's syringe (Fig 1F), and 8µl semen was injected per queen. As per the observation of Woyke [4], *A. c. indica* drones produces on average by 0.16µl semen and therefore for a good insemination, *A. c. indica* queen required semen from about forty to sixty drones.

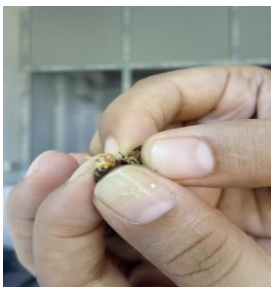
2.1 Preparing the Queen for artificial insemination

Preparing the Anaesthetic: The reduction valve of the carbon dioxide cylinder was adjusted to a delivery pressure of about 5 pounds per square inch and the flow of gas to the queen holder stopper adjusted to a very small stream. It should be just enough to keep the queen quiet. The flow was adjusted with experience by dipping the stopper in water until it gives 2 to 3 bubbles per second [5].

Preparing the Queen: The virgin queens were inseminated between 5 and 12 days post-emergence. After emergence, queens were kept in drone less nucleus colonies with several hundred adult workers. The entrances of the hive were covered with queen excluder material to prevent unwanted natural mating flights [5].The equipment used to perform the insemination operation was P. Schely's instrumental insemination device (Fig 2). The P. Schely's equipment was designed for AI of *Apis mellifera*. Since, the queen size of *A. c. indica* was smaller than *A. mellifera,* and therefore the queen holder (Fig 5B) of the apparatus was redesigned to fit *A. c. indica* queen to perform AI. The syringe and queen holder were aligned on the

A. Drone flight cage

B. Collection of drones



C. Everting drones

D. Partial eversion

E. Full eversion

F. Collection of semen

Fig.1. (A-F): Preparation of drones for artificial insemination of queen bee

instrument stand at 30° to 45° angle to facilitate bypassing the valvefold. The queen was allowed to move into a tube similar to the queen holder. When she reaches the constricted end, she moves in the tube in a to and fro movement. Then the queen holder was placed on the upper side of the tube so that the queen moves to the queen holder in such a way that the abdomen of the queen was facing towards the tapering end of the queen holder (Fig 5C). Thus, the queen was placed in the queen holder with her abdomen protruding last three segments (Fig 5D) and then a slow continuous flow of CO_2 was administered. Mackensen [6] used carbon dioxide as an anesthetic agent to keep honeybee queen calm during insemination process. Then the abdominal plates were separated to expose the vaginal orifice using ventral hook to the left and sting hook to the right. Then the syringe tip was dorsally positioned above the "V", defining the vaginal orifice (Fig 4). Then the tip was inserted into the vaginal orifice 0.5 to 1.0 mm, slightly forward of the apex of the "V". Then the tip was inserted further, another 0.5 to 1.0mm, while using the tip to lift the valvefold ventrally. The valvefold covering the median oviduct was bypassed so as to prevent back-flow of semen from the vaginal orifice. Then 8µl of semen was delivered directly into the median oviduct (Fig 5F). After insemination, syringe tip was removed. Then again, a small air space and small drop of saline, (~0.5µl) was collected to precede the next insemination. Then, the queens were released from the holder and placed in push-in cage(Fig 5G), and returned her to her nucleus colony. Then, on the next day, the queens were released from the push-in-cage to the nucleus colony [5].

Fig. 2. Schely's instrumental insemination device

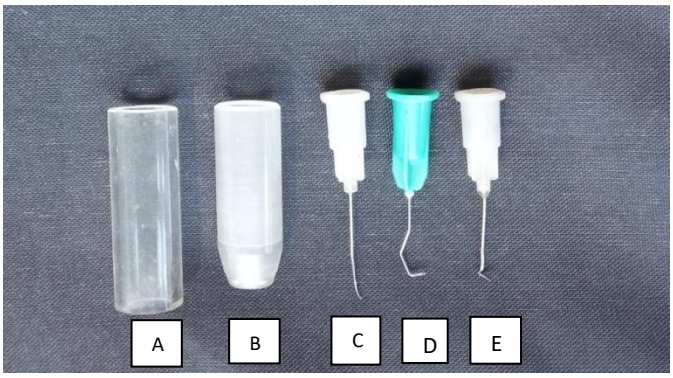


Fig. 3. (A) Plastic tube, (B) Queen holder, (C) Holding hook, (D) Sting hook and (E) Ventral hook

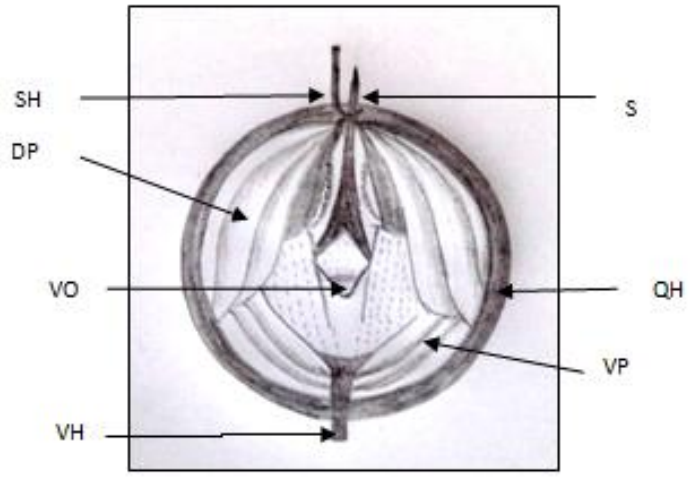
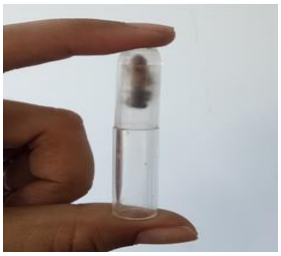


Fig. 4. Sting chamber of queen (SH-Sting hook; DP- Dorsal plate; VO- Vaginal orifice; VH-Ventral hook; VP-Ventral plate; QH- Queen holder and S- Sting)

A. Queen cages

B. Passing queen to plastic tube



C. Passing queen to holder *via* plastic tube

D. Queen placed on queen holder

E. Opening of vaginal orifice

F. Injection of semen

G.*A. c. indica*F. push-in cage

Fig. 5. (A-G): Preparation of virgin queen for artificial insemination

3. RESULTS AND DISCUSSION

3.1 Selection of viable colonies of *A. c. indica*

The selection of viable colonies was also worked out for *A. c. indica* colonies from Sep., 2015 to Jan., 2016. The results in table 2 revealed that there was significant difference among the *A. c. indica* colonies with regard to colony strength, pollen and nectar area. The strength of the colonies ranged between 6.5±0.1 to 6.8±0.1 numbers of frames. The brood area of the colonies was recorded between 920.6±189.8 to 1203.5±179.6 sq.cm, whereas, the pollen area was recorded between 527.2±53.7 to 598.8±52.3 sq.cm. The nectar area of the ten selected viable colonies of *A. c. indica* ranged between 170.4±14.4 to 288.1±13.2sq.cm.and pollination efficiency was recorded with 3.8±1.07 to 5.9±1.04 milligram pollen load per five minute. The honey yield of the colonies was found to have ranged between 1.09±0.4 to 2.4±0.4 kg. In the selected colonies of *A. c. indica***,** no incidence of diseases and pests were observed and moreover, swarming and absconding behaviour were also found to be negligible during the study periods. The colonies were found to be calm and hygienic in nature. Cale and Gowen [7] reported that significant positive correlation among egg laying rate, colony population and honey production. Again, Cale [8] reported positive correlation between pollen stores and honey stores. Similar studies were reported by other workers [9,10] and for brood rearing and honey yield [11]. Rosenthal *et al*. [12] and Hussein [13] reported significant positive correlation between brood reared and pollen stored in his studies. Singh *et al*. [14] reported that stronger colonies possessed more brood, stored more honey and raised more queen cells.

3.2 Comparative Performance of *A. c. indica* in Artificially Inseminated and Naturally Inseminated Colonies

The comparative performance of *A. c. indica* was carried out between April, 2017 to March, 2018. A total twenty number of *A. c. indica* queens were raised for AI in Feb., 2017 and after artificial insemination five queens survived. Similarly, number of NI colonies were maintained for comparison. The possibility of instrumental insemination of *A. c. indica* queens was studied by Woyke [4], where he found that semen was difficult to be separated from mucus and average semen was only 0.16µl per drone. He also reported that 40-60 drones are necessary for effective insemination of *A. c. indica* queen. The average values of the five AI and NI colonies of *A. c. indica* were discussed in Table 2.

Table 1. Selection of viable *A. c. indica* colonies

Month of observation	Colony number	Strength (No. of frames with bees)	Brood area (sq.cm)	Pollen area(sq.cm)	Nectar area(sq.cm)	Pollination efficiency (mg)	Honey yield (Transformed) (kg)
Sept'2015 - Jan'2016	colony 1	6.5±0.1	1184.8±183.9	598.8±52.3	288.1±13.2	5.2±0.9	2.4±0.4
Sept'2015 - Jan'2016	colony 2	6.5±0.1	944.0±189.9	530.6±56.4	193.0±14.6	3.8±1.07	1.2±0.4
Sept'2015 - Jan'2016	colony 3	6.6±0.1	1203.5±179.6	535.8±54.4	182.0±11.06	5.0±0.9	1.1±0.4
Sept'2015 - Jan'2016	colony 4	6.6±0.1	965.0±187.8	527.2±53.7	170.4±14.4	4.4±1.0	1.2±0.4
Sept'2015 –Jan' 2016	colony 5	6.6±0.2	940.0±188.6	536.8±55.07	188.0±14.6	4.5±0.9	1.1±0.4
Sept'2015 - Jan'2016	colony 6	6.7±0.1	928.8±188.07	527.6±53.3	174.6±15.1	4.0±0.9	1.09±0.4
Sept'2015 - Jan'2016	colony 7	6.7±0.1	920.6±189.8	533.6±53.4	185.4±12.3	3.8±0.9	1.1±0.4
Sept'2015 - Jan'2016	colony 8	6.7±0.1	971.2±180.8	543.6±56.6	194.6±15.9	4.2±1.05	1.2±0.4
Sept'2015 - Jan'2016	colony 9	6.8±0.1	926.8±181.5	540.01±53.9	172.4±9.2	4.6±0.9	1.2±0.4
Sept'2015 - Jan'2016	colony 10	6.7±0.1	925.0±189.1	528.8±53.8	179.4±13.7	5.9±1.04	1.1±0.4
Sept'2015 - Jan'2016	C.D. 5%	0.07	NS	9.8	10.7	NS	NS

Data based on mean of 10 colonies

Table 2. Comparative performance between artificially and naturally inseminated colonies of *A. c. indica*

MONTH	Strength (No. offrames with bees)			Brood area (sq.cm)			Pollen area (sq.cm)			Nectar area (sq.cm)			Pollination efficiency (mg)			Honey yield (Transformed) (sq.cm)		
	AI	NI	C.D. 5%	AI	NI	C.D. 5%	AI	NI	C.D. 5%	AI	NI	C.D. 5%	AI	NI	C.D. 5%	AI	NI	C.D. 5%
Apr'17	8.0±0.2	8.0±0.2	NS	2971.8±10.4	2837.2±24.2	86.06	1055.2±20.8	834.0±19.2	110.6	1328.8±10.5	1194.8±13.0	68.1	12.0±0.3	10.4±0.5	0.6	2.2±0.01(4.02)	2.1±0.05(3.5)	NS
May'17	8.0±0.2	8.0±0.2	NS	3337.0±12.7	3250.4±12.6	81.2	839.8±8.4	645.0±8.5	28.8	1370.2±6.5	1257.2±9.4	29.5	10.0±0.2	7.2±0.3	1.03	2.07±0.01(4.4)	2.0±0.02(3.9)	0.04
Jun'17	8.0±0.2	7.8±0.2	NS	2850.4±8.1	2717.4±8.3	38.3	774.4±12.5	571.4±10.04	23.7	829.4±8.8	773.2±5.8	45.3	9.8±0.2	7.0±0.2	1.6	1.0±0.01(3.3)	1.0±0.02 (3.1)	NS
July'17	7.0±0.2	6.8±0.2	NS	1671.6±12.6	1559.4±9.0	27.3	569.8±9.4	515.8±8.9	47.1	548.8±10.5	451.2±5.1	19.8	4.4±0.2	3.4±0.2	NS	1.0±0.01(2.7)	1.0±0.02(1.7)	0.1
Aug'17	6.8±0.2	6.6±0.2	NS	1443.6±11.8	1324.2±19.1	51.9	529.4±8.9	472.6±9.8	10.1	455.8±6.5	418.0±6.5	9.01	4.0±0.2	3.4±0.4	NS	1.0±0.01(0.0)	1.0±0.02(0.0)	NS
Sept'17	6.6±0.1	6.4±0.1	NS	1309.0±12.1	983.2±23.9	62.8	522.6±10.7	452.8±18.0	32.3	388.2±8.1	295.2±16.9	29.6	3.4±0.4	2.8±0.3	NS	2.34±0.01(0.0)	2.2±0.26(0.0)	NS
Oct'17	6.0±0.24	6.0±0.2	NS	735.0±13.9	607.8±20.6	53.4	464.4±10.6	306.2±12.7	49.5	265.4±8.1	105.2±8.7	10.7	3.4±0.2	2.4±0.2	0.8	1.0±0.01(0.0)	1.0±0.02(0.0)	NS
Nov'17	6.0±0.2	6.0±0.2	NS	622.8±11.5	466.8±16.2	33.5	285.6±12.5	254.8±7.2	9.8	228.4±14.0	79.8±10.1	20.3	2.4±0.2	1.4±0.3	NS	2.1±0.05(0.0)	1.9±0.05(0.0)	NS
Dec'17	6.0±0.2	6.0±0.2	NS	539.0±15.1	427.2±15.8	15.4	352.2±14.3	302.4±11.7	10.06	179.0±13.4	48.0±9.6	29.3	3.4±0.4	1.4±0.3	0.8	2.1±0.03(0.0)	1.9±0.03(.00)	0.1
Jan'18	6.8±0.2	6.4±0.2	NS	1421.2±21.5	1122.8±30.0	47.9	827.4±6.1	593.2±9.9	52.3	461.2±5.4	299.0±7.9	54.5	7.4±0.3	6.2±0.3	NS	2.2±0.03(3.8)	2.0±0.02(2.6)	NS
Febr'18	7.4±0.2	7.4±0.2	NS	2368.2±10.6	2153.6±17.1	91.1	966.6±5.6	881.2±8.9	29.3	679.8±11.5	484.2±11.5	34.5	13.0±0.3	8±0.2	0.8	1.0±0.03(4.1)	1.0±0.02(3.1)	0.1
Mar'18	7.6±0.2	7.6±0.2	NS	2632.6±9.9	2319.2±14.6	55.01	1233.8±9.3	1099.2±10.3	43.07	1158.6±8.8	958.4±11.4	51.2	14.4±0.3	11.2±0.2	1.3	1.9±0.01(3.6)	1.6±0.04(2.8)	0.1
Mean	7.01±.1	6.9±10	NS	1825.1±121.7	1647.4±122.6	13.2	701.76±36.7	579.9±31.6	9.6	657.8±52.8	530.3±52.7	7.9	7.3±0.5	5.4±0.4	0.2	2.1±0.07	1.7±.06	0.02

Data based on mean of 5 colonies

3.3 Strength of Colony

The data on strength of colony revealed that there was no significant difference between AI and NI colonies of *A. c. indica*.

3.4 Brood Area

The data on brood area revealed that highly significant difference between AI and NI colonies in April, 2017 to March, 2018. Brood area was 2971.8±10.4 sq.cm in April which reached 3337.0±12.7sq.cm in May. While, gradual decrease in brood area was observed from June to November (2850.4±8.1, 1671.6±12.6, 1443.6±11.8, 1309.0±12.1, 735.0±13.9 and 622.8±11.5 sq.cm, respectively) and lowest was recorded in December (539.0±15.1 sq.cm). Then again in the month of January, February and March (1421.2±21.5, 2368.2±10.6 and 2632.6±9.9 sq.cm, respectively), an increasing trend of brood area was observed in AI colonies. The Fig.3B revealed that brood rearing activity was having fluctuating trend depending upon climatic variation and floral conditions.

Similarly, brood area in the NI colonies was 2837.2±14.2 sq.cm in April. The highest brood area was recorded in May (3250.4±12.6 sq.cm) which further showed a gradual decrease from June to November (2717.4±8.3, 1559.4±9.0, 1324.2±19.1, 983.2±23.9, 607.8±20.6 and 466.8±16.2 sq.cm, respectively and lowest was found in December (427.2±15.8 sq.cm). Then again, brood area was found to be increasing in January, February and March (1122.8±30.0, 2153.6±17.1 and 2319.2 ±14.6) sq.cm, respectively in NI colonies. Hence, due to high fecundity of AI queen, in all the months, the brood area was higher in AI colonies than NI colonies. The results were in agreement with the findings of Wongsiri and Lekprayoon [1].

3.5 Pollen Area

The data on pollen area revealed highly significant difference between AI and NI colonies April, 2017 to March, 2018. Pollen area was 1055.2±20.8 sq.cm in the month of April. There was a gradual decrease in pollen area from May to October (839.8±8.4, 774.4±12.5, 569.8±9.4, 529.4±8.9, 522.6±10.7 and 464.4±10.6 sq.cm, respectively) and lowest was recorded in November (285.6±12.5 sq. cm). Then again increase in pollen area was observed from December towards February (352.2±14.3, 827.4±6.1 and 966.6±5.6 sq.cm, respectively) and reached its peak in March (1233.8±9.3 sq.cm) in AI colonies. The Fig.1C revealed that pollen was gathered throughout the year in variable quantity depending on the availability of bee flora.

Similarly, pollen area in the NI colonies was recorded (834.0±19.2 sq.cm) in April which showed a decreasing trend from May towards October (645.0±8.5, 571.4±10.04, 515.8±8.9, 472.6±9.8, 452.8±18.0 and 306.2±12.7 sq.cm, respectively) and lowest was recorded in November (254.8±7.2 sq.cm). Then again, a gradual increase in pollen area was observed from December to February (302.4±11.7, 593.2±9.9 and 881.2±8.9 sq.cm) which reached peak in March (1099.2±10.3 sq.cm) in NI colonies. Based on the data we can draw the inference that, in all the months pollen area was significantly higher in AI colonies as compared to NI colonies which was due to high efficiency of the workers.

3.6 Nectar Area

Nectar area data presented in Table 2 showed that highly significant difference was observed in AI and NI colonies from April, 2017 to March,2018. The nectar area was recorded 1328.8±10.5 sq.cm in April which reached peak in May (1370.2±6.5 sq.cm). Data showed a decreasing trend from June towards November (829.4±8.8, 548.8±10.5, 455.8±6.5, 388.2±8.1, 265.4±8.1 and 228.4±14.0sq.cm, respectively) and lowest nectar area was recorded in December (179.0±13.4sq.cm) (Fig. 1D). Thereafter, the nectar area gradually increased in January, February and March (461.2±5.4, 679.8±11.5 and 1158.6±8.8 sq.cm, respectively) in AI colonies. The quantity of nectar area was varying depending upon the availability of bee flora and moreover, nectar area was found significant correlation with maximum relative humidity (Table 3).Such observation was in conformity with the results obtained by Painkra [15].

Again, in NI colonies nectar area was recorded in April (1194.8±13.0 sq.cm) and peak in May (1257.2±9.4 sq.cm). A gradual decrease in nectar area was observed from June to November (773.2±5.8, 451.2±5.1, 418.0±6.5, 295.2±16.9, 105.2±8.7 and 79.8±10.1 sq.cm, respectively) and lowest was recorded in December (48.0±9.6 sq.cm).Nectar area showed gradual increase in January, February and March (299.0±7.9, 484.2±11.5 and 958.4±11.4 sq.cm, respectively) in NI colonies.

Table 3. Correlation of brood area, pollen area and nectar area of *A. c. indica* with different meteorological factors

Month of observation	Brood area		Pollen area		Nectar area		Maximum temperature (°C)		Maximum relative humidity (%)		Total rainfall (mm)		Bright sunshine hours (hr)	
	AI	NI	AI	NI	AI	NI								
April'17	2971.8	2837.2	1055.2	834.0	1328.8	1194.8	29.7		91		111.8		4.5	
May'17	3337.0	3250.4	839.8	645.0	1370.2	1257.2	30.7		92		226.9		4.2	
June'17	2850.4	2717.4	774.4	571.4	829.4	773.2	32.8		95		399.2		3.7	
July'17	1671.6	1559.4	569.8	515.8	548.8	451.2	33.6		94		275.1		4.0	
August'17	1443.6	1324.2	529.4	472.6	455.8	418.0	31.1		94		269.1		3.4	
September'17	1309.0	983.2	522.6	452.8	388.2	295.2	32.7		94		324.4		3.3	
October'17	735.0	607.8	464.4	306.2	265.4	105.2	31.1		96		192.8		5.2	
November'17	622.8	466.8	285.6	254.8	228.4	79.8	28.4		95		14.7		7.4	
December'17	539.0	427.2	352.2	302.4	179.0	48.0	25.8		99		0.0		5.4	
January'18	1421.2	1122.8	827.4	593.2	461.2	299.0	23.9		98		2.7		4.7	
February'18	2368.2	2153.6	966.6	881.2	679.8	484.2	24.5		97		29.1		3.4	
March'18	2632.6	2319.2	1233.8	1099.2	1158.6	958.4	27.3		95		76.0		4.0	
							AI	NI	AI	NI	AI	NI	AI	NI
Correlation with brood area (r=)							-0.13	-0.16	-0.58*	-0.60*	-0.27	-0.29	-0.53	-0.50
Correlation with pollen area (r=)							-0.25	-0.27	0.27	0.25	-0.09	-0.13	-0.49	-0.51
Correlation with nectar area(r=)							-0.09	-0.17	-0.66*	-0.70*	-0.13	-0.23	-0.37	-0.41

**= Significant at 5% level of probability*

Hence, from the data we can conclude that nectar area of AI colonies was significantly higher than NI colonies and this increase in nectar area was due to high vigour of AI colonies.

3.7 Pollination Efficiency

Pollination efficiency data revealed that significant difference was observed between AI and NI colonies in April, May, June, October, December, February and March. The pollination efficiency during this month's were ranged 12.0±0.3, 10.0±0.2, 9.8±0.2, 3.4±0.2, 3.4±0.4, 13.0±0.3 and 14.4±0.3 milligram of pollen load, respectively in AI colonies and 10.4±0.5, 7.2±0.3, 7.0±0.2, 2.4±0.2, 1.4±0.3, 8.0±0.2, and 11.2±0.2 milligram of pollen load, respectively in NI colonies (Fig. 3E). Whereas, pollination efficiency in July, August, September, November and January were found non significant between AI colonies (4.4±0.2, 4.0±0.2, 3.4±0.4, 2.4±0.2 and 7.4±0.3milligram, respectively) and NI colonies (3.4±0.2, 3.4±0.4, 2.8±0.3, 1.4±0.3 and 6.2±0.3, milligram of pollen load, respectively). Thus, pollination efficiency was found to be higher in all the months in AI colonies due to high efficiency of pollen gatherers of AI colonies than NI colonies of *A. c. indica*.

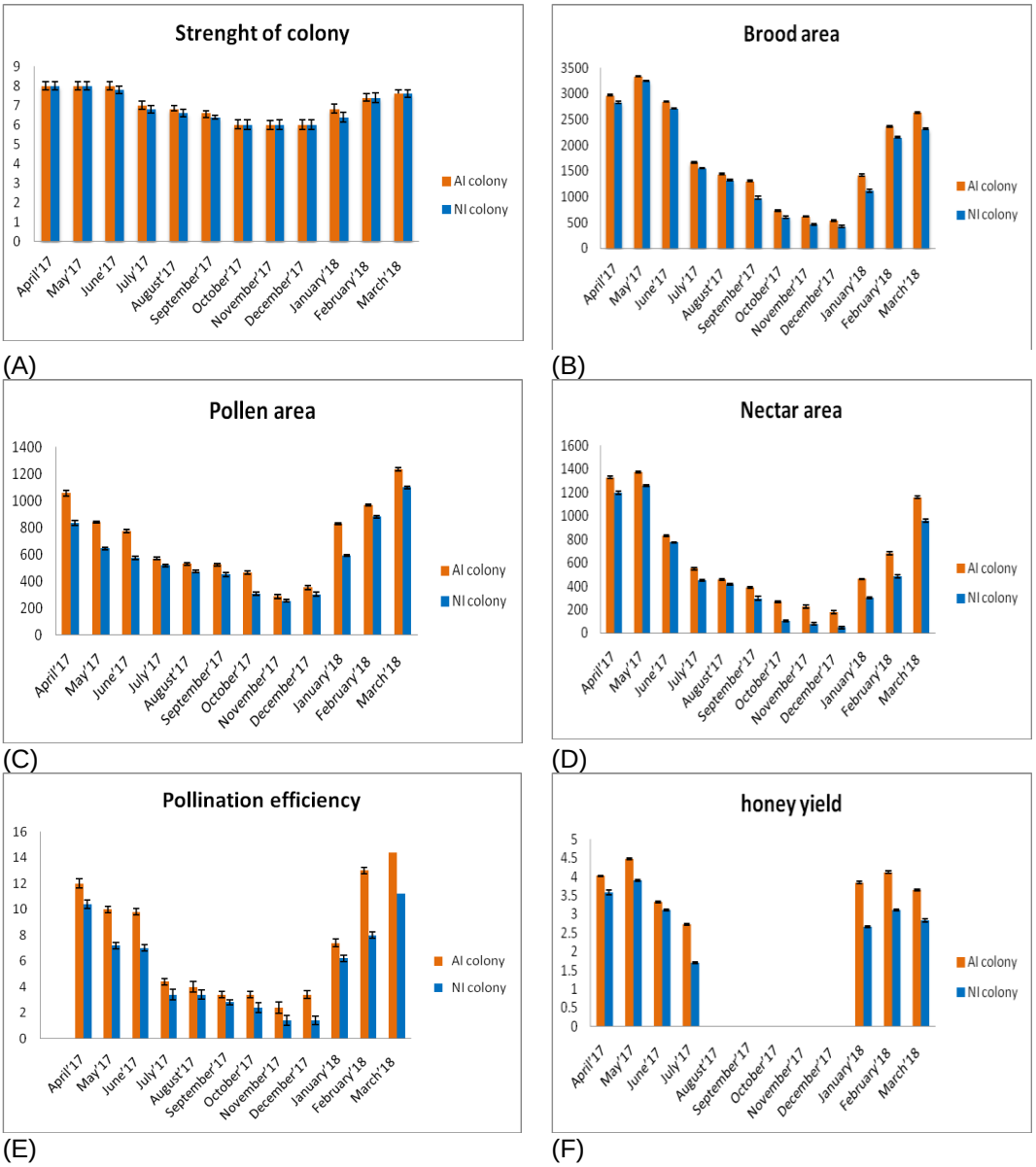


Fig. 6 (A-F). Comparative performance of *A. c. indica* in artificially and naturally inseminated colonies (April'17 to March'18)

3.8 Honey Yield

The data on honey yield revealed significant difference between AI and NI colonies in May, July, December, January, February and March. The respective values during this month's were 2.0±0.01, 1.0±0.01, 2.1±0.03, 2.2±0.03, 1.0±0.03 and 1.9±0.01 kg honey yield, respectively in AI colonies and 2.0 ±0.02, 1.0±0.02, 1.9±0.03, 2.0±0.02, 1.0±0.02 and 1.6±0.04 kg honey yield, respectively in NI colonies. Whereas, the data in April, June, August September, October and November were found to be non significant between AI colonies (2.2±0.01, 1.0±0.01, 1.0±0.01, 2.3±0.01, 1.0±0.01 and 2.1±0.05 kg, respectively) and NI colonies (2.1±0.05, 1.0±0.02, 1.0±0.02, 2.2±0.2, 1±0.02 and 1.9±0.05 kilogram, respectively).

4. CONCLUSION

From the present study it is well understood that artificial insemination is an important tool in order to control and improve genetic characteristics of honey bee species, for the preservation and improvement of local breeds and to create disease resistant lines and lines with high productivity. The comparative performance of AI queen and NI queen indicated a better performance of the artificially inseminated queens over naturally inseminated queens of *A. c. indica* with respect to brood area(6%), pollen area (21%), nectar area(12%) and pollination efficiency which directly influences honey production. In view of this, it can be concluded that in order to retain the vigour and vitality of the exotic and indigenous honey bee species, the AI approach is essential. Hence, bee breeder can utilize AI tool for improving the honey bee races with respect to yield attributing and disease resistant character and this may also be utilized in commercial venture.

ACKNOWLEDGEMENT

The authors are thankful to Lilacharan Dutta,Owner at C.K. Udyog, Dr. R. N. Sarma Department of Plant Breeding and Genetics and Retd. Dr. D. Das Borah, Department of Agricultural Statistics, Assam Agricultural University, Jorhat for their sincere help during the period of investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wongsir S Lekprayoon,C. Hybridization of chinese honey bee A. c. indica cerana and Thai honey bee A. c. indica indica by artificial insemination. Chulalongkorn Univ., Bangkok (Thailand);1990.
   Available:http://agris.fao.org/agris-search/search
2. Mattu VK, Verma LR. Studies on the annual foraging cycle of *A. c. indica indica* F. in Shimla Hills of the North-West Himalayas. Apidologie. 1985;16:1-18.
3. Czekonska K, Mickiewicz BC, Chorbinski P. The influence of honey bee (*Apis mellifera*) drone age on volume of semen and viability of spermatozoa. Journal of Apicultural Science. 2013;57:1.
4. Woyke J. Instrumental Insemination of *A. c. indica* queens. Journal of Apicultural Research. 1973;12(3):151-58.
5. Cobey SW, Tarpy DR, Wokey J. Standard methods for instrumental insemination of Apis mellifera queens. Journal of Apicultural Research. 2013;52(4).
6. Mackensen O. Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queens. Journal of Economic Entomology. 1947;40(3):344-249.
7. Cale GH, Gowen JW. Heterosis in honeybees (*Apis mellifera* L.).Genetics. 1956;41:292-303.
8. Cale GH. Pollen gathering relationship to honey collections and egg laying in honeybees. American Bee Journal. 1968;108:8-9.
9. Hussein MH. Stimulative feeding of honeybee colonies (*A. mellifera* L.).*In Assiut. Proc.* IV Arab Pest Conference Tanta, Egypt. 1981a;367-375.
10. Nelson DL, Gary NE. Honey productivity of honey bee colonies in relation to body weight, attractiveness and fecundity of the queen. Journal of Apicultural Research. 1983;22:207-213.
11. Kepena L. Breeding studies of regional pedigree bee- breeding, Pol'Nohospodarstvo. 1968;14: 628-637.
12. Rosenthal C, Chiva R, Caragiani S. Observations on the dynamics of the pollen stores in brood nest.*Apic.* Bucuresti. 19712;4: 2-5.
13. Hussein MH. Pollen gathering activity of honeybee workers. In Proc. IV Arab Pest. Conf. Egypt. 1981b;377-385.
14. Singh L, Brar HS, Vashishat CL. Effect of different bee strengths on

seasonal brood rearing, colony stores and development of queen cells in *Apis mellifera* L. at Ludhiana, India. Indian Bee Journal. 19925;4(1-4):68-75.

15. Painkra MK. (). Performance of *A. c. indica indica* F. under different migratory mode in Northern parts of India. Thesis, Indira Gandhi Krishi Vishwavidyalaya, Raipur; 2016.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/116799