Journal of Advances in Biology & Biotechnology

Volume 27, Issue 9, Page 724-735, 2024; Article no.JABB.120906
ISSN: 2394-1081

Performance of *Apis mellifera* L. Colonies Developed from Artificially and Naturally Inseminated Queens

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i91345

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/120906

Original Research Article

Received: 28/06/2024
Accepted: 31/08/2024
Published: 05/09/2024

ABSTRACT

A comparative study between artificially and naturally inseminated queens of *Apis mellifera* L. colonies 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 from 99 colonies of *A. mellifera* L. and ten viable colonies were selected which were later used for preparation of queen and collection of drones. Comparative performance between artificially inseminated (AI) and naturally inseminated (NI) colonies of *A. mellifera* L. in the year 2016-17 revealed that brood area (3151.1±86.3 sq.cm in AI and 2966.8±89.3 sq.cm in NI), pollen area (798.4±75.2sq.cm in AI and 622.8±40.4sq.cm in NI) and nectar area (1626.9±131.8sq.cm in AI and 1421.7±126.9sq.cm in NI) have significant difference in all the months. Similarly, in the year 2017-18, brood area (3155.4±92.1sq.cm in AI and 3015.08±86.9 sq.cm in NI), pollen area (742.3±42.03sq.cm in AI and 651.4±40.4sq.cm in NI) and nectar area (1589.3±130.0sq.cm in AI and 1471.3±130.6sq.cm in NI) have significant difference in

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Cite as: *Borah, Pooja, and Ataur Rahman. 2024. "Performance of Apis Mellifera L. Colonies Developed from Artificially and Naturally Inseminated Queens". Journal of Advances in Biology & Biotechnology 27 (9):724-35. https://doi.org/10.9734/jabb/2024/v27i91345.*

all the months. In 2016-17, the pollination efficiency (9.2±0.4mg and 7.1±0.4mg pollen load in AI and NI, respectively) has significant difference between AI and NI colonies in all the months except for May, December, January and February. Whereas, in 2017-18, pollination efficiency (9.1±0.5mg and 7.5±0.4mg pollen load in AI and NI, respectively) has significant difference in all the months except for October and March. Honey yield (3.75±.11 sq.cm in AI and 3.27±.10sq.cm in NI) showed significant difference in April, May, June, December, January and February during 2016-17 and in 2017-18, honey yield (3.89±.11 sq.cm in AI and 3.38±.10 sq.cm in NI) revealed significant difference between AI and NI colonies in May, June, December, January and February. Therefore, in both 2016-17 and 2017-18, the performance of AI colonies was significantly higher than NI colonies.

Keywords: Apis mellifera L.; artificially inseminated queens; colonies; honey yield; naturally inseminated queens and 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, disease resistance and geographical adaptability. On the basis of morphological characteristics, honey bees are classified into 4 species; the European honeybees, *Apis mellif*era L., the Eastern honey bees, *A. cerana* F., the gaint honey bee, *A. dorsata* F., and the dwarf honey bee, *A. florea* F. *Apis mellifera* L. is the western bee species introduced in India during 1960s, first in Kangra Valley of Himachal Pradesh. In Assam, *A. mellifera* was introduced during late 1990, for the first time at Assam Agricultural University, Jorhat.

Natural fertilization of honey bees results in various problems like stock looses vigour due to inbreeding. In natural fertilization 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 go for nuptial flight but the female size, vigour etc. is not selected. Therefore, selective breeding is needed to help prevent future colony collapse of hives. Selective breeding also helps to produce stronger bees that have a resistance to mites and diseases in bees. It also helps to increase honey production. 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. In 1920's the development of artificial insemination technique was started. The method was improved over time and now instrumental insemination is a success and reliable technique.

Artificial insemination is important in order to control and improve genetic breeds, for the preservation and improvement of local breeds and to create disease resistant lines and lines with high productivity. Keeping these views in mind, the present investigation has been undertaken.

2. MATERIALS AND METHODS

The research works on selective breeding of *A. mellifera* L. have been carried out in the apiary and laboratory conditions of Department of Entomology, Assam Agricultural University, from September 2015 to March' 2018.

Selection of viable colonies of *Apis mellifera*: The selection of viable colony is an important prerequisite in order to get a successful artificial insemination as we select the viable drone and queen cells from these selected colonies. The selection of viable colonies was carried out from September, 2015 to January, 2016 from 99 colonies of *Apis mellifera* L. Colonies were selected by visual observation and the colonies having good strength were screened out. Then 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. To measure brood, pollen and nectar area, three combs were selected randomly in each of the ten colonies. 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 and expressed in sq.cm. 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. mellifera*L. 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. 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 [1]. The honey of *A. mellifera*L. colonies were extracted and expressed in kilograms per hive.

Mass rearing of queen bees: After selection of the ten viable colonies in *A. mellifera* L., 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. The method for queen raising was followed as per Doolittle method developed by G. M. Doolittle (1846). In both the year 2016 and 2017, twenty numbers of *A. mellifera* L. queens were raised in the month of February for artificial insemination. The steps followed for queen production were discussed below:

Making queen cell cups: For preparation of queen cell cups, the dripping sticks were first dipped into a weak solution of honey mixed with water. The excess liquid was shaken off from the dripping stick and then was dipped into molten wax to a depth of 6-8mm. Then the dripping stick was removed and exposed to air until the wax solidifies. (Fig. 1A).

Affixing cell cup: After preparation of queen cell cups, the cell cups were attached to the bar made of wood, resembling top bar of the frame (Fig. 1B).

Grafting: The cell cups were then offered to a colony overnight for the bees to work on the cells and to make them more acceptable and then the cell cups were rinsed with fresh royal jelly (Fig. 1C). Larvae of about 24 hours of age were selected and carefully inserted with the help of grafting needle in the cell cups (Fig. 1D). Then the frame with grafted larvae was given to the cell builder colony (Fig. 1E).

Transplanting queen cells: The sealed queen cells were transferred to nucleus colonies and the young queens emerged out from the cells after twelve days (Fig. 1G and 1H).

Preparation of drones for artificial insemination: The mature drones (14 day old) were collected from the selected five colonies of *A. mellifera* L. in “drone flight cage”, and brought to the laboratory for semen collection (Fig. 2B). To expose semen, the endophallus of the mature drones were everted by hand in two-steps: the partial eversion (Fig. 2D) and the full eversion (Fig. 2E). Then the semen from the endophallous was drawn inside the Schelly’s syringe (Fig. 2F), and 8µl semen was injected per queen. As per the observation of Woyke [2], *A. mellifera* L. drones produces on an average 1.5 µl semen and therefore for a good insemination, queen required semen from about eight to ten drones. During mating, each drone ejaculates about 6 to 12 million sperm [3].

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 [4].

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 [4].

The equipment used to perform the insemination operation was P. Schely’s instrumental insemination device (Fig. 3). The syringe and queen holder were aligned on the instrument stand at a 30° to 45° angle to facilitate bypassing the valvefold. The queen was allowed to move into a tube similar to the queen holder (Fig. 6B). 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. 6C). Thus, the queen was placed in the queen holder with her abdomen protruding last three segments (Fig. 6D) and then a slow continuous flow of CO_2 was administered. 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

A. Making queen cell cups

B. Affixing cell cup

C. Royal jelly for queen cells

D. Grafting

E. Transferring of frame with grafted larvae to cell builder colony

F. Artificial queen cells

G. Nucleus box of *Apis mellifera* L.

H. Nucleus box of *Apiscerana*F.

Fig. 1(A-H). Mass rearing of queen bees

A. Drone flight cage

B. Collection of drones

C. Everting drones

D. Partial eversion

E. Full eversion

F. Collection of semen

Fig. 2(A-F). Preparation of drones for artificial insemination of queen bee

Fig. 3. Schely's instrumental insemination device

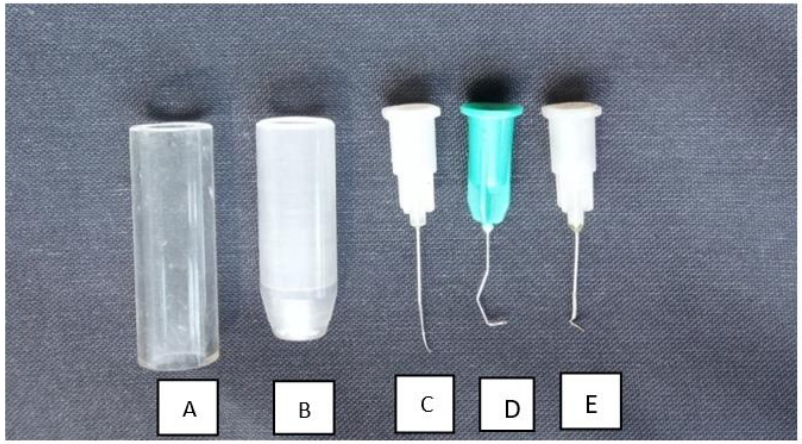


Fig. 4. (A) Plastic tube, (B) Queen holder, (C) Holding hook, (D) Sting hook and (E) Ventral hook

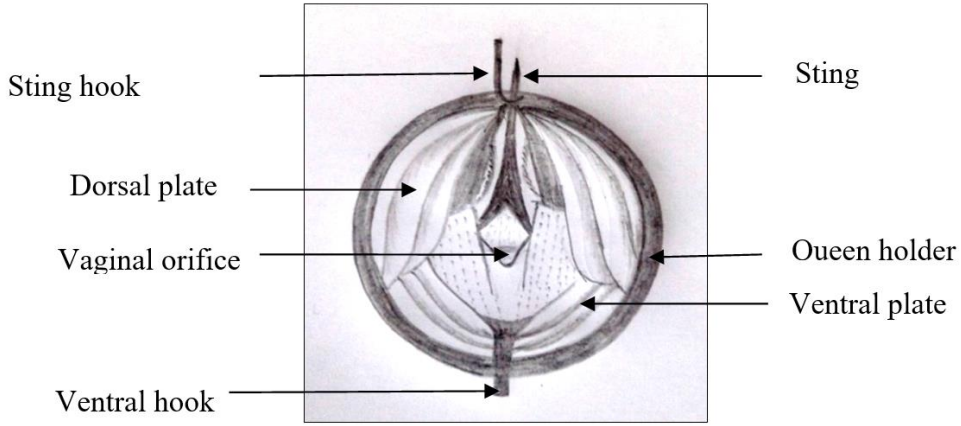
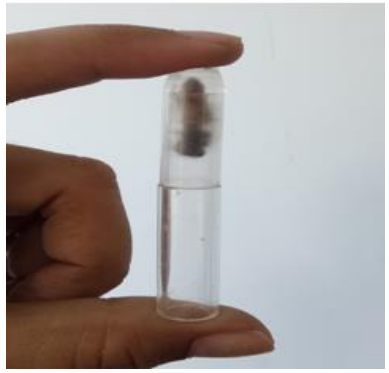


Fig. 5. Sting chamber of queen

A. Queen cages

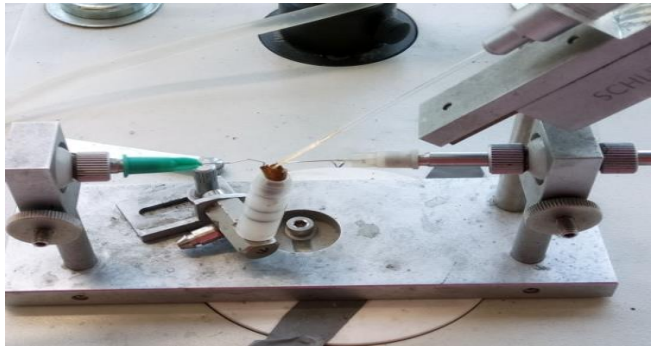
B. Passing queen to plastic tube



C. Passing queen to holder *via* plastic tube

D. Queen placed on queen holder

E. Opening of vaginal orifice



F. Injection of semen

G. *Apis mellifera*L. push-in cage

Fig. 6(A-G). Preparation of virgin queen for artificial insemination

syringe tip was dorsally positioned above the "V", defining the vaginal orifice (Fig. 5). 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. 6F). 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. 6G), 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 [4].

3. RESULTS AND DISCUSSION

Selection of viable colonies of *Apis mellifera* L.: The selection of viable colonies was worked out from September'2015-January'2016, and out of 99 *A. mellifera* L. colonies, ten viable colonies were selected. The results in Table 1 revealed the average data of the ten selected colonies which was later used for preparation of queens and drone selection. The strength of most of the colonies ranged between 7.7±0.3 to 7.9±0.3 number of frames. The brood area of the colonies were found to have ranged between 2643.6±209.4 to 2715.8±207.8 sq.cm. All the ten colonies showed significant differences with regard to area of pollen store which ranged between 657.01±87.1 to 712.1±95.07 sq.cm. The nectar area of the colonies were recorded between 704.7±96.4 to 740.2±95.08 sq.cm. The pollination efficiency of the *A. mellifera* L. colonies were found to have ranged between 5.8±0.9 to 6.4±0.9 milligram of pollen load per five minutes. The honey yield of the colonies ranged between 1.8±0.9 to 1.9±0.9 kilogram. In the selected colonies of *A. mellifera* L. no incidence of diseases and pests were observed during the study period. Swarming and absconding behaviour were also observed to be absent. Moreover, the colonies of were found as very calm in nature.

Comparative performance of *Apis mellifera* L. in artificially inseminated and naturally inseminated colonies: During the course of queen rearing in 2016, all total twenty queens of *Apis mellifera* L. were raised in the month of February'2016 for executing artificial insemination. And out of twenty queens, six queens survived after artificial insemination (AI). For comparison, similar number of colonies having naturally inseminated (NI) queens were maintained. When the AI queens started effective egg laying, the comparison between AI and NI colonies were made (Table 2). Guler et. al. [5] performed similar work and found significant difference between naturally mated queen (NMQC) and instrumentally inseminated queen colony (IIQC) groups in terms of hygiene behavior, there was no significant difference between the groups in terms of performance phenotypes. Delaney et al. [6] reported that when the queens were sufficiently inseminated (3.99 ± 1.504 million sperm) and mated with an appropriate number of drones (effective paternity frequency: 16.0 ± 9.48), very few of the queens were parasitized by tracheal mites and none were found with either Nosema species. Buescu et al. [3] also reported higher performance of artificially inseminated queens compared to naturally mated queen, however, the factors like rearing conditions, mating age, treatment of queens before and after insemination, semen dosage and handling, pheromone development, effects of CO2 treatments and environmental conditions can affect their performance.

Strength of colony: The data on the strength of colony revealed that there was significant difference between AI and NI colonies of *A. mellifera* L. during the month of November (8.2±0.2 numbers of frames in AI and 7.3±0.1 numbers of frames in NI) and December (8.9±0.1 numbers of frames in AI and 8.4±0.1 numbers of frames in NI).

Brood area: Perusal of data on brood area presented on Table 2 revealed significant difference between AI and NI colonies in all the months from April' 2016 to March' 2017. In both AI and NI colonies, brood area reached peak during May (4091.2±10.5sq.cm in AI and 3882.4±4.9 sq.cm in NI) and lowest was recorded in the month of October (2039.2±10.4 sq.cm in AI and 1813.8±11.3 sq.cm in NI). And in all the months the brood area was significantly higher in AI colonies than the NI colonies since the fecundity of AI queen is more as compared to the NI queens. The maximum temperature coupling with shortage of bee flora affected the brood rearing during July to October. Therefore, the duration from July to October was referred to as lean period for brood rearing. Artificial feeding to the colonies with sugar solution was practiced during this period. Further, it was observed that

Table 1. Selection of viable *Apis mellifera*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	7.9±0.3	2668.2±210.7	672.4±89.1	717.0±92.09	6.4±0.9	1.8±0.9
Sept'2015 - Jan'2016	colony 2	7.8±0.3	2680.0±205.3	668.0±89.6	727.2±57.3	5.8±0.9	1.8±0.9
Sept'2015 - Jan'2016	colony 3	7.7±0.3	2677.6±207.9	657.0±87.1	718.8±96.3	6.2±0.9	1.9±0.9
Sept'2015 - Jan'2016	colony 4	7.8±0.3	2643.6±209.4	688.8±97.8	735.0±97.8	6.0±0.8	1.8±0.9
Sept'2015 - Jan'2016	colony 5	7.9±0.3	2663.0±204.6	665.0±88.4	727.6±93.5	6.2±1.01	1.8±0.9
Sept'2015 - Jan'2016	colony 6	7.9±0.3	2672.2±198.9	680.2±90.3	729.0±90.1	6.2±0.9	1.8±0.9
Sept'2015 - Jan'2016	colony 7	7.8±0.3	2675.8±204.9	673.2±88.7	732.2±99.5	5.8±0.9	1.8±0.9
Sept'2015 - Jan'2016	colony 8	7.9±0.3	2715.8±207.8	712.1±95.07	723.6±87.6	6.0±0.8	1.8±0.9
Sept'2015 - Jan'2016	colony 9	7.7±0.3	2692.6±206.5	661.8±90.5	740.2±95.08	5.8±0.9	1.8±0.9
Sept'2015 - Jan'2016	colony 10	7.8±0.3	2664.4±205.04	664.1±88.07	704.7±96.4	6.4±0.9	1.8±0.9
Sept'2015 - Jan'2016	C.D. 5%	NS	33.1	17.8	NS	NS	NS

Data based on mean of 10 colonies

Table 2. Comparative performance between artificially and naturally inseminated colonies of *Apis mellifera*

Month	Strength (No. of frames with bees)			Brood area (sq.cm)			Pollen area (sq.cm)			Nectar area (sq.cm)			Pollination efficiency (mg)			Honey yield (kg)		
	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'16	10±0	10±0	NS	3829.8±7.5	3678±9.1	28.92	730.2±10.7	573.2±5.68	35.28	3619.8±6.9	3443.2±9	26.20	14±0.31	10.8±0.5	1.11	3.10±0.006(8.62)	2.96±0.01(7.76)	0.03
May'16	10±0.12	10±0.12	NS	4091.2±10.5	3882.4±4.9	23.82	629.8±9.26	519.4±9.28	50.12	3201.8±17.9	2903.4±14.1	68.14	10.6±0.4	9±0.3	NS	2.94±0.01(7.66)	2.76±0.01(6.62)	0.05
Jun'16	9.2±0.12	9±0.12	NS	3517.8±11.5	3381.6±6.9	42.98	532.2±9.25	479.6±7.55	34.19	2333.8±6.5	2030.2±8.7	32.76	8±0.31	7±0.3	0.87	2.51±0.017(5.34)	2.22±0.02(3.93)	0.08
July'16	7.4±0.12	7.3±0.12	NS	2680.8±5.2	2524.6±8.7	25.36	497.4±8.17	362.2±8.01	29.04	1524.8±8.3	1323.8±6.5	33.79	7.2±0.37	5±0.3	1.03	1±0.015(0)	1±0.01(0)	NS
Aug'16	6.8±0.2	6.6±0.18	NS	2427.2±8.4	2229±8.6	38.24	420.2±7.39	321.8±7.69	28.60	1233.6±8.1	1038.2±6.5	35.25	6.6±0.24	2.8±0.2	1.03	1±0.006(0)	1±0.01(0)	NS
Sept'16	6.6±0.24	6.4±0.24	NS	2102.6±11.5	1946.2±13.1	48.68	436.8±10.43	342.6±12.6	47.21	992.2±4.4	847.8±13	34.22	4.4±0.24	1.8±0.2	1.11	1±0.0102(0)	1±0.01(0)	NS
Oct'16	6.4±0.24	6.2±0.2	NS	2039.2±10.4	1813.8±11.3	41.80	440.8±8.73	349±10.97	35.84	872±7.6	695.2±11.8	24.44	5.6±0.24	2.4±0.2	1.03	1±0.0102(0)	1±0.01(0)	NS
Nov'16	8.2±0.2	7.3±0.12	0.80	2926.8±5.5	2507.8±14	52.27	527.8±8.62	486.4±5.97	38.13	845±10.5	654.4±12.4	22.87	10±0.31	7±0.3	1.52	1±0.0102(0)	1±0.01(0)	NS
Dec'16	8.9±0.1	8.4±0.18	0.43	3449.8±10.1	3342.8±10.8	29.19	1287.4±6.27	1108±15.23	41.61	783.4±5.3	612.2±12.4	49.10	11.6±0.4	10.6±0.2	NS	2.47±0.015(5.14)	2.34±0.02(4.48)	0.10
Jan'17	9.3±0.12	9±0.22	NS	3494.2±5.1	3368.4±9	28.57	1349.2±8.63	1295.2±14.96	40.72	671.8±7.4	556±12.5	44.72	14±0.54	11.6±0.4	NS	2.52±0.015(5.37)	2.36±0.01(4.60)	0.05
Febr'17	9.6±0.1	9.3±0.12	NS	3581.8±6.9	3423.6±10.3	35.96	1052.4±8.38	922.8±9.52	25.02	832.8±10.4	625.2±11.1	39.77	8±0.31	7.4±0.2	NS	2.69±0.0108(6.27)	2.55±0.03(5.52)	0.08
Mar'17	10±0.12	9.8±0.12	NS	3672.6±9.5	3503.6±10.6	54.71	882.4±4.65	714.4±13.63	43.28	2712.6±10.9	2411±13.3	52.38	11.4±0.24	9.2±0.3	1.03	2.76±0.009(6.66)	2.71±0.01(6.39)	NS
Mean	6.40±.17	6.20±.18	0.12	3151.15±86.3	2966.81±89.3	7.71	798.46±75.24	622.88±40.40	132.91	1626.96±131.8	1421.71±126.9	8.11	9.28±0.40	7.13±0.44	0.27	3.75±.11	3.27±.10	0.01

Data based on mean of 6 colonies

Table 3. Comparative performance between artificially and naturally inseminated colonies of *Apis mellifera*

MONTH	Strength (No. of frames with bees)			Brood area (sq.cm)			Pollen area (sq.cm)			Nectar area (sq.cm)			Pollination efficiency (mg)			Honey yield (kg)		
	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	10±0	10±0	NS	3888.6±9	3774.2±8.8	36.32	823.4±12.97	616.6±15.25	63.49	3620.8±10.7	3321.2±8	18.78	15.2±0.32	12±0.316	1.03	3.11±0.009(8.7)	3.01±0.011(8.11)	NS
May'17	10±0.12	10±0.122	NS	4203.8±21.6	3836.8±9.4	66.10	758±11.67	595.2±12.94	67.72	3145±11.1	2989.8±10.1	44.24	10.2±0.37	9±0.316	1.03	2.95±0.008(7.7)	2.77±0.011(6.69)|	0.02
Jun'17	9.3±0.12	8.7±0.122	NS	3674.8±8.2	3421.4±8.3	22.01	581.8±8.44	386.8±8.36	9.25	2391.2±10.8	2071.6±10.6	23.65	7±0.31	5.6±0.245	1.11	2.47±0.011(5.11)	2.26±0.016(4.12)	0.02
July'17	7.6±0.24	7.2±0.2	NS	2842.6±9.2	2564±8.6	28.45	478.2±7.28	384.6±7.57	40.85	1438.6±7.7	1286.4±8.6	40.09	6.6±0.24	3.8±0.2	1.03	1±0.012(0)	1±0.0134(0)	NS
Aug'17	7±0	6.8±0.2	NS	2376±7	2123.4±15.2	42.10	427.2±13.25	342.6±11.28	37.68	1310.2±13.6	1172.2±9.3	40.35	6±0.31	3±0.316	0.87	1±0.009(0)	1±0.0119(0)	NS
Sept'17	6.8±0.12	6.6±0.244	NS	2113.6±15.5	1974.6±8.5	46.50	419±11.42	340.4±8.38	44.01	1006±6.9	865.8±10	34.08	4.6±0.24	1.6±0.245	0.87	1±0.008(0)	1±0.0118(0)	NS
Oct'17	6.2±0.2	6.0±0.244	NS	2018.8±12.5	1944.2±9.7	45.66	395.4±5.92	337.2±11.58	31.23	867.8±13.9	742.4±10.7	14.06	2±0.31	`1.8±0.374	NS	1±0.008(0)	1±0.0118(0)	NS
Nov'17	8±0.12	8±0.122	NS	2953±8.6	2729.8±5.5	15.56	569.8±6.7	428.8±9.35	14.77	818±12.6	640.8±9.1	48.20	12.2±0.37	6.6±0.245	1.41	1±0.008(0)	1±0.0118(0)	NS
Dec'17	8.8±0.2	8.4±0.244	NS	3426.4±12.6	3239.4±9.2	45.61	1305.6±12.7	1115±14.64	52.28	796±11.8	605±15.4	73.67	12±0.31	9.2±0.374	1.36	2.57±0.011(5.65)	2.36±0.012(5.6)	0.05
Jan'18	9±0.12	8.6±0.244	NS	3483±4.7	3317±12.1	35.85	1382.2±6.69	1226.8±6.04	33.30	664.4±7	510±10.8	47.05	15.2±0.37	13±0.316	0.55	2.74±0.012(6.53)	2.47±0.0134(5.12)	0.03
Febr'18	9.2±0.12	8.7±0.122	0.51	3556.4±9.8	3423.2±10.7	4.59	1021±11.73	961.6±10.57	42.85	848.6±10.8	615±9.5	39.52	9.2±0.37	8.4±0.245	0.55	2.75±0.01(6.6)	2.56±0.011(5.57)	0.02
Mar'18	9.2±0.12	8.8±0.122	NS	3583.6±9.7	3463±7.9	44.31	971.6±7.17	952.4±10.55	28.08	2835.8±12.2	2436±7.4	41.17	11.2±0.2	11±0.316	NS	2.72±0.012(6.48)	2.73±0.01(6.42)	NS
Mean	8.42±.16	8.26±.16	0.09	3155.46±92.1	3015.08±86.9	8.59	742.30±42.03	651.41±40.44	8.15	1589.38±130.0	1471.33±130.6	8.21	9.11±0.51	7.50±0.45	0.25	3.89±.11	3.38±.10	0.006

Data based on mean of 8 colonies

bee flora was abundant from November to June and were responsible for better performance in brood rearing during this period. Hence, the period beginning from November to June was referred to as honey flow period.

Pollen area: The observations of pollen area revealed significant difference between AI and NI colonies in all the months from April' 2016 to March' 2017. The lowest pollen area was recorded in the month of August (420.2±7.3sq.cm) and peak was recorded in January (1349.2±8.6sq.cm) in AI colonies. Similarly, pollen area in the NI colonies was lowest in the month of August (321.8±7.6 sq.cm) and highest in the month of January (1295.2±14.9 sq.cm). The pollen was gathered throughout the year in variable quantity depending on the availability of bee flora. However, pollen gathering activity was slowed down from July to October due to dearth of flora. The results were in agreement with the findings of Bisht and Pant [7]. Although there was variations in pollen area in different months, but in all the months the pollen area was higher in AI colonies than NI colonies due to high vigour of the workers of AI colonies as compared to the NI colonies.

Nectar area: The observations on nectar area showed significant difference between AI and NI colonies in all the months from April'2016 to March'2017.The highest nectar area was recorded in the month of April (3619.8±6.9 sq.cm) and the lowest during January (671.8±10.4 sq.cm) in AI colonies. Similarly, the peak nectar area in the NI colonies was found to be 3443.2±9.0 sq.cm in the month of April and lowest nectar area was recorded in the month of January (556.0±12.5sq.cm). Thus, from the data we can draw the inference that nectar area in AI colonies was significantly higher than the NI colonies which was due to high vigour of the AI colonies as compared to the NI colonies.

Pollination efficiency: The observations on pollination efficiency revealed significant difference between AI and NI colonies in the month of April, June, July, August, September, October, November and March. The pollination efficiency during this month's were 14.0±0.3, 8.0±0.3, 7.2±0.3, 6.6±0.2, 4.4±0.2, 5.6±0.2, 10.0±0.3 and 11.4±0.2, milligram of pollen load in AI colonies and 10.8±0.5, 7±0.3, 5±0.3, 2.8±0.2, 1.8±0.2, 2.4±0.2, 7±0.3 and 9.2±0.3 milligram of pollen load in NI colonies. Thus, pollination efficiency was found to be higher in all the months in AI colonies than the NI colonies and this significantly higher collection of pollen loads by workers of AI colonies could be attributed to high vigour of the workers. Ratnikov [8] also made similar observation of greater forging efficiency in hybrid workers bees of various other races of *A. mellifera* as compared to their parents.

Honey yield: The observations on honey yield revealed significant difference between AI and NI colonies in the month of April, May, June, December, January and February, respectively. The respective values during this month's were 3.1±0.006, 2.94±0.01, 2.5±0.01, 2.4±0.01, 2.5±0.01 and 2.6±0.01kilogram honey yield in AI colonies and 2.9±0.01, 2.7±0.01, 2.2±0.02, 2.3±0.02, 2.3±0.01 and 2.5±0.03 kilogram honey yield in NI colonies, respectively.

The comparative performance of *Apis mellifera* L. was again observed between April' 2017 to March' 2018. A total of twenty numbers of queens were raised in the month of February'2017 and eight queens survived after artificial insemination. Similar number of naturally inseminated colonies were maintained for comparison. The regular month wise data of eight AI and NI colonies were recorded from April' 2017 to March' 2018 (Table 3). The average values of AI and NI colonies in terms of bee strength, brood area, pollen area, nectar area, pollination efficiency and honey yield have been discussed below:

Strength of colony: The data on the strength of colony revealed that there was significant difference between AI and NI colonies of *Apis mellifera* L. only in the month of February'2018 (9.2±0.1 numbers of frames in AI and 8.7±0.1 numbers of frames in NI).

Brood area: The observations on brood area was found to have highly significant difference between AI and NI colonies in all the months from April' 2017 to March' 2018. In both AI and NI colonies, brood area reached peak during May (4203.8±21.6 sq.cm in AI and 3836.8±9.4 sq.cm in NI) and lowest was recorded in the month of October (2018.8±12.5sq.cm in AI and 1944.2±9.7 sq.cm in NI). Hence, although in different months there was variation in brood area but in all the months the brood area was higher in AI colonies than NI colonies due to high egg laying capacity of AI queens than NI queens. This was in conformity with the observations of Oldroyd [9] who reported that hybrid queens had

significantly larger brood area than colonies headed by inbred queens.

Pollen area: The pollen area was found to be significantly different between AI and NI colonies in all the months from April' 2017 to March' 2018.Pollen area was at peak during January (1382.2±6.6 sq.cm) and lowest during October (395.4±5.9 sq.cm).Similarly, pollen area in the NI colonies was highest in January (1226.8±6.04 sq.cm) and lowest in October (337.2±11.5 sq.cm). Thus, in all the months the pollen area was significantly higher in AI colonies than the NI colonies, which was due to high vigour of the AI colonies as compared to NI colonies. This was in conformity with the observations of Roberts [10].

Nectar area: The data on nectar area also revealed highly significant difference between AI and NI colonies in all the months where peak nectar storage was recorded to be 3620.8±10.7 sq.cm in AI colonies and 3321.2±8.0 sq.cm in NI colonies in the month of April and lowest was recorded in the month of January in both AI and NI colonies (664.4±7.0 sq.cm and 510.0±10.8 sq.cm, respectively). However, in all the months of observation significant increase of nectar areas in AI colonies were observed than the NI colonies due to superior workers in AI colonies as compared to workers of NI colonies.

Pollination efficiency: The data on pollination efficiency revealed significant difference between AI and NI colonies in the month of April, May, June, July, August, September, November, December, January and February. The respective values during this month were 15.2±0.0.3, 10.2±0.3, 7.0±0.3, 6.6±0.2, 6.0±0.3, 4.6±0.2, 12.2±0.3, 12.0±0.3, 15.2±0.3 and 9.2±0.3 milligram of pollen load, respectively in AI colonies and 12.0±0.31, 9±0.31, 5.6±0.24, 3.8±0.2, 3±0.31, 1.6±0.24, 6.6±0.24, 9.2±0.37, 13±0.3 and 8.4±0.2, milligram of pollen load, respectively in NI colonies. 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 the NI colonies.

Honey yield: The data on honey yield showed significant difference between AI and NI colonies in the month of May, June, December, January and February. The respective values during this month's were 2.9±0.008, 2.4±0.01, 2.5±0.01, 2.7±0.01, 2.7±0.01 kilogram honey yield, respectively in AI colonies and 2.7±0.01, 2.2±0.01, 2.3±0.01, 2.4±0.01 and 2.5±0.01 kilogram honey yield, respectively in NI colonies. Thus, honey yield in AI colonies of *Apis mellifera* L. was higher than NI colonies. Ruttner [11,12] also reported such observations in *Apis mellifera* in West Germany [13-15].

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. mellifera* L. with respect to brood area, pollen area, nectar area and pollination efficiency which directly influences honey production. In the present studies, it has been found that brood area increased by five to ten per cent, pollen area increased by twelve to seventeen per cent and likewise nectar area was increased by ten to nineteen per cent in AI colonies as compared to NI colonies. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENT

The authors are thankful to Lilacharan Dutta, Owner at C.K. Udyogand 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. Mattu VK, Verma LR. Studies on the annual foraging cycle of *Apis cerana indica* F. in Shimla Hills of the North-West Himalayas. Apidologie. 1985;16:1-18.
2. Woyke J. Instrumental Insemination of *A. c. indica* queens. J. Apic. Res. 1973;12(3):151-58.
3. Buescu E, Gurau MR, Birțoiu AI. Artificial insemination on *Apis mellifera* – Aspects of artificial inseminated queen performances and factors that may affect their performance. Proc. Romanian Acadamy, Series B, Supplement 1. 2015;21-24.
4. Cobey SW, Tarpy DR, Wokey J. Standard methods for instrumental insemination of *Apis mellifera* queens. J. Apic. Res. 2013;52(4).
5. Guler A, Onder H, Kavak G, Toktay G, Ugurlutepe E, Biyik S, Aydin A. The effects of instrumental insemination on selected and unselected breeding characteristics in honeybee (*Apis mellifera* L.) Apidologie. 2022;53:35.
6. Delaney DA, Keller JJ, Caren JR, Tarpy DR. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). Apidologie. 2011;42:1–13.
7. Bisht DS, Pant NC. Studies on brood rearing activity of Indian Honeybee, Apis indica F. under Delhi condition. Indian J. Ent. 1968;30(3):109-111.
8. Ratnikov A. Ten years work with hybrids. Pchelovodstvo. 1960;37:20-22.
9. Oldroyd BP. Are queen characters and worker characters more important to honey production. In Africanized honey bees and bee mites.Chahester UK, Ellis. Horwood. 1986;91-96.
10. Roberts WC. Hybrid vigour in honeybees. Proc. XVI Int. Beekeep. Congr. Prelim. Sci. Meet. Vienna, Austria. 1956;235.
11. Ruttner F. Hybrid breeding. Proc. XXI Int. BeekeepCongr. Summ. Maryland, U.S.A. 1967;83.
12. Ruttner F. Methods of breeding honeybees: intraracial selection or inter-racial hybrids. Bee Wld. 1968;49:66-72.
13. Cobey SW. Comparision studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie. 2007;38:390-410.
14. Moritz RFA, Kohnert M. Seasonal effects on Artificial insemination of honey bee queens (*Apis mellifera* L.). Apidologie. 1984;15(2):223-231.
15. Taber S, Wandel J. Concerning the number of times queen bees mate. J. Econ. Entomol. 1958;51:786-789.

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The peer review history for this paper can be accessed here:
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