



The Influence of Brood Survival Rates on the Behavioral Patterns, Economic Aspects and Resistant Traits of Italian Honeybee *Apis mellifera* L. Reared on Indian Mustard in Bihar

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Authors' contributions

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

Article Information

DOI: 10.9734/JEAI/2024/v46i52425

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/115637>

Original Research Article

Received: 04/02/2024

Accepted: 07/04/2024

Published: 11/04/2024

ABSTRACT

To better understand *Apis mellifera* foraging behaviour for mustard, this study examined how brood survival rates affected the species behavioural, economic and resistant traits. Out of 10 colonies used for the study, the results showed that 1 colony had a low rate of brood survival, 3 colonies had a medium rate, and 6 colonies had a high rate. In the experiment on scattered brood patterns, poor, medium and excellent brood patterns were found in 1, 2, and 7 colonies, respectively.

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According to the data on bee activity collected, the majority of bees were observed leaving between the hours of 9:00 and 10:00 and arriving between the hours of 16:00 and 17:00. According to the economic attributes data, colonies with high and moderate brood survival rates had more honey and pollen reserves. According to the hygienic behaviour results, colonies with high and moderate brood survival rates had 100 per cent efficiency while colonies with low brood survival rates had the lowest efficiency at 76 per cent.

Keywords: Bee activity; brood; mustard; pollen.

1. INTRODUCTION

Honey bees are highly advanced and familiar groups of social insects. Bees are capable of increasing the yield of cross-pollinated and to some extent in the case of self-pollinated crops through their pollination activities. It has been found that presently there are equal or more than 25,000 species of honey bees in the world and this holds for about 65 per cent of pollination activities of different flowering plants. They are working as important pollinating agents for more than 80 per cent of all flowering plants including the majority of agricultural and horticultural crops [1]. Being the main pollination service provider, the honey bees add quite well in increasing the overall production and productivity of these kinds of cross-pollinated crops with their efficient pollination in an obscure and silent way [1-2]. Honey bees are the most common and important pollinators of several agricultural or field crops and mustard (*Brassica juncea*) is one of them. Mustard belongs to the Cruciferae family and is generally cultivated as a *rabi* annual herb crop [3]. In Bihar, it is grown throughout the winter season. It is one of the most popular and widely grown oil-seed crops next to sunflowers. Mustard is generally called rai. There are a total of six cultivated species under the genus *Brassica* and among them, more than 80 per cent of the total cultivated area is held by Indian mustard scientifically known as *Brassica juncea* [4]. Mustard seeds are rich in protein content, total oil content in them is around 37-49 per cent [5-6] while the amino acids present in mustard proteins are very finely balanced, which are much richer in essential amino acids required by human beings, it contains 20-28 per cent oleic acid, linoleic acid in 10-20 per cent and lastly 30-40 per cent erucic acid present in mustard protein [7]. Mustard is a highly cross-pollinated crop and heavily depends upon pollinators like honey bees for successful fruiting. Mustard is one of the entomophilous crops that require many insect pollinators specially and they provide a rich source of pollen and nectar flow throughout the whole flowering period. Among different insect

pollinators *A. mellifera* is one of the major and most dominant species, so their contribution should be studied thoroughly in respect to pollinating mustard to understand the gaps to be fulfilled in future. Keeping all these aspects in mind the experiment had been chalked out.

2. MATERIALS AND METHODS

2.1 Location

RPCAU, Pusa, Samastipur in Bihar state is located at a latitude of 25.980N and a longitude of 85.670E.

2.2 Behavioural Characters

Nest Cleaning: To determine the nest cleaning ability of each colony, observation of the debris content at the bottom board was taken and a total of 4 observations were recorded at 7-day intervals. Then the colonies were designated as a) Very clean b) Clean c) Poor cleaning ability.

Bee Activity: Observation on the activity of honeybee colonies was done by counting the number of bees leaving the hive (outgoing) and those landing at the entrance (incoming) per minute during different day hours (09:00 to 10:00, 13:00 to 14:00 and 16:00 to 17:00 hours of the day) were taken.

2.3 Economic Traits

Honey Stores: First, an estimation of the honey stores with the help of a grid frame was done then converting the number of squares covering the honey area into grams by multiplying by 8.06 (considering that an average frame full of honey, contained 1000 g of honey) was done. Total honey yielding from all 10 hives was recorded.

Pollen Stores: Measurements of the pollen stores were done in cm² by counting the number of cells in the wire grid covered by pollens.



Fig. 1. Field view of experimental plots



Fig. 2. Standard grid frame made of iron wire

2.4 Resistance Traits

Hygienic behaviour: To determine the hygienic behaviour pin killing of 25 capped pupae was done. Killing the pupae in the sealed stage by piercing the caps with a sharp needle was done. Observation in the pierced area after 24 hours for removal of pin-killed broods was taken. Per cent removal of killed brood was calculated by using the below formula [8].

$$\text{Per cent removal} = (\text{Number of pin killed pupae removed} / 25) * 100$$

3. RESULTS AND DISCUSSION

3.1 Behavioural Characters

Nest Cleaning: To determine the nest-cleaning ability of each colony, observation of the debris

content at the bottom board was recorded. Four observations were taken at 7-day intervals. The collected debris was weighed in weighing balance and the obtained results are listed in Table 2.

The debris includes excreta, dead insects, and parasitised or predator-eaten insect carcasses. The experiment on the nest cleaning ability of *A. mellifera* showed that among all 10 colonies colony no. 5 was the cleanest colony and its debris content for the 1st, 2nd, 3rd and 4th week was only 1.24, 1.31, 1.29 and 1.87 g respectively and this colony was categorised under very clean colony. In contrast, the average debris content for the whole month of that colony was only 1.43 g. On the other hand colony no. 9 was found to be the dirtiest colony with their debris content for the 1st, 2nd, 3rd and 4th weeks being only 2.10, 3.14, 3.23 and 3.76 g

respectively and the average weight of debris for the whole month was 3.05 g. Colony no. 9 faced high levels of mite attack and for that reason, some bees died total debris content was also much higher compared to other colonies and categorised under colony with poor cleaning ability and the remaining 8 colonies were remarked as clean colonies. It was observed that the weight of the debris content kept increasing as the month proceeded and for almost all colonies the maximum value of debris content was during the 4th week. Now from the experiment, it was noticed that colony no. 5 was a very clean colony and this colony witnessed the highest brood survival rate with a per cent brood survival rate of 96.87. In contrast, on the other side colony no. 9 with the lowest per cent brood survival rate of 75.75 was the dirtiest colony and the same trend was found with the remaining colonies.

3.2 Bee Activity

Observation of the activity of honeybee colonies by counting the number of bees leaving the hive (outgoing) and those landing at the entrance (incoming) per minute during different day hours (09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs.) was taken for the whole month (01.12.2020 to 31.12.2020) was done. A stopwatch was used and for each colony 3 observations were taken during different day hours. The data was analysed in factorial RBD with 2 factors in the OPSTAT programme. The obtained data regarding bee activity are listed in Table 2. So, from the data of outgoing bees, it was concluded that almost all the colonies had good activity except colony no 9. In colony no. 9 the average number of outgoing bees were

11.33, 9.33 and 6.33 during 09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs. respectively. The average number of outgoing bees for the whole month of colony no. 9 was only 9.00. On the other hand, a maximum number of outgoing bees was noticed in colony no. 3 with an average number of outgoing bees were 15.67, 12.00 and 8.67 during 09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs. respectively and the average number of active outgoing bees was 12.11 for the whole month in colony no. 3. While in respect to daytime, it was observed that activity or number of outgoing bees were maximum in the early morning that is during 09:00 to 10:00 hrs. with 14.11 average outgoing followed by during 13:00 to 1400 hrs. With 11.79 average outgoing bees. It showed the least activity during the late hour of the day or 16:00 to 17:00 hours with only an average 7.63 mean number of bees found to leave their colony. It was observed that bee activity almost completely stopped by 17:30 hrs. Now, about brood survival rate and bee activity, it was observed that colony no.9 with the lowest brood survival rate witnessed the least number of active outgoing bees and on the other side colony no. 3 having the third highest brood survival rate, and in other colonies the same trend was observed that colonies having high and medium brood survival rates had a greater number of active outgoing bees and with low brood survival rates a smaller number of active outgoing bees were noticed. From the data of incoming bees, it was noticed that the lowest active incoming bees were recorded in colony no. 9, in colony no. 9 the average number of incoming bees was only 7.33, 9.00 and 9.33 from 09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs. respectively.

Table 1. Weight of debris content (g) of different colonies

| Colony Number | 24.11.2020 | 01.12.2020 | 08.12.2020 | 15.12.2020 | Mean | Status |
|---------------|------------|------------|------------|------------|-------|---------------|
| 1 | 1.89 | 1.87 | 1.92 | 2.94 | 2.15 | Clean |
| 2 | 1.91 | 1.83 | 1.89 | 2.95 | 2.14 | Clean |
| 3 | 1.63 | 1.77 | 1.71 | 2.72 | 1.96 | Clean |
| 4 | 1.76 | 1.79 | 2.82 | 2.87 | 2.31 | Clean |
| 5 | 1.24 | 1.31 | 1.29 | 1.87 | 1.43 | Very clean |
| 6 | 1.52 | 1.55 | 2.59 | 2.69 | 2.08 | Clean |
| 7 | 1.71 | 2.72 | 2.71 | 1.74 | 2.22 | Clean |
| 8 | 1.46 | 2.45 | 1.53 | 3.61 | 2.26 | Clean |
| 9 | 2.10 | 3.14 | 3.23 | 3.76 | 3.05 | Poor cleaning |
| 10 | 2.65 | 1.68 | 1.75 | 1.76 | 1.96 | Clean |
| | | | | CD (<5%) | 0.73 | |
| | | | | CV (%) | 12.92 | |

Table 2. Average number of outgoing and incoming bees during different day hours

| Period of observation | Colony Number | Outgoing bees during different dayhours | | | | Incoming bees during different day hours | | | |
|--------------------------|---------------|---|------------------|------------------|-------------------------------|--|------------------|------------------|------------------------------|
| | | 09:00-10:00 hrs. | 13:00-14:00 hrs. | 16:00-17:00 hrs. | Avera geno. of out going bees | 09:00-10:00 hrs. | 13:00-14:00 hrs. | 16:00-17:00 hrs. | Average no. of incoming bees |
| 01.12.2020 to 31.12.2020 | 1 | 14.77 | 11.33 | 6.33 | 10.78 | 7.67 | 9.67 | 11.33 | 9.55 |
| | 2 | 13.33 | 11.00 | 7.67 | 10.67 | 9.67 | 9.00 | 11.67 | 10.11 |
| | 3 | 15.67 | 12.00 | 8.67 | 12.11 | 9.33 | 11.33 | 12.33 | 11.00 |
| | 4 | 16.33 | 10.00 | 9.33 | 11.89 | 6.67 | 10.00 | 12.33 | 9.67 |
| | 5 | 15.00 | 14.33 | 6.00 | 11.78 | 10.33 | 10.67 | 14.33 | 11.78 |
| | 6 | 14.67 | 12.67 | 6.00 | 11.11 | 10.67 | 12.00 | 12.33 | 11.67 |
| | 7 | 13.00 | 11.33 | 9.33 | 11.22 | 8.33 | 10.33 | 12.00 | 10.22 |
| | 8 | 14.67 | 12.00 | 7.67 | 11.44 | 9.67 | 10.33 | 14.33 | 11.44 |
| | 9 | 11.33 | 9.33 | 6.33 | 9.00 | 7.33 | 9.00 | 9.33 | 8.56 |
| | 10 | 12.33 | 14.00 | 9.00 | 11.78 | 7.33 | 12.00 | 12.33 | 10.56 |
| | Average | 14.11 | 11.79 | 7.63 | 11.17 | 8.70 | 10.43 | 12.23 | 10.45 |

The average number of incoming bees for the whole month of colony no. 9 was only 8.56. On the other hand, the maximum number of incoming bees was recorded in colony no. 5 with the average number of incoming bees were 10.33, 10.67 and 14.33 from 09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs. respectively and the average number of active outgoing bees was 11.78 for the whole month in colony no. 5. While in respect to day hour point of view, it was found that maximum number of incoming bees was recorded during the late hour of the day or 16:00 to 17:00 hours with average 12.33 number of incoming bees were noticed in all 10 colonies followed by 13:00 to 1400 hrs. with 10.43 average incoming bees and it showed the least activity with only an average 8.70 mean number of bees found to come back to their colony during the early hours of the day with the time range of 09:00 to 10:00 hrs. More or less the present findings are in agreement with the findings of [9,2] where they observed that the activity of foraging of Indian bees (*Apis cerna indica*) and European bees (*Apis mellifera*) increased at 10:00 hr of the day. Now about brood survival rate and bee activity, it was observed that colony no.9 having the lowest brood survival rate witnessed the least number of active incoming bees and on the other side colony no. 5 had the highest brood survival rate of 96.87 per cent and other colonies, the same trend was observed that colony having high and medium brood survival rate having a greater number of active incoming bees and with low brood survival rate a smaller number of active incoming bees were noticed. It is observed that there are no predators or parasites involved.

3.3 Economic Traits

Honey Stores: Firstly, to measure the economic traits quantitatively of all the colonies a standard grid frame made of iron was made, estimation of the honey stores with the help of the grid frame was done then converting the number of squares covering the honey area into grams by multiplying with 8.06 (considering that an average frame full of honey, contained 1000 g of honey). Total honey yielding from those selected 10 hives was recorded.

Pollen Stores: Measurements of the pollen stores were done in cm² by counting the number of cells in the wire grid covered by pollens. One square of the standard grid frame contains 10 cells and the area of the Single Square of the grid frame is 1 square inch (6.45 cm²). So based on this, the area of pollen was calculated. The obtained results of economic traits showed that colonies having high and medium brood survival rates contained more amount of honey and pollen stores. As shown in Table 3 the quantity of honey was maximum in colony no. 5 with 394.94 g as a total of 49 squares were filled with honey and by multiplying with the factor 8.06 the total quantity of honey was calculated. Some other colonies with higher brood survival rates like colonies no. 3, 6, 7, 8 and 10 witnessed 378.82, 346.58, 370.6, 330.46 and 338.49 g of honey respectively. On the other side colonies no. 1, 2 and 4 were categorised under medium brood survival rate colony and their yield of honey was 314.34, 298.22 and 322.40 g respectively. Colony no. 9 having the lowest

brood survival rate among all the colonies able to produce only 249.86 g of honey as only 31 squares were filled with honey of that particular frame inserted into colony no. 9. While the average honey content for all 10 colonies was 334.49 g. The data regarding pollen stores showed the same trend as honey stores as the pollen store was maximum in colony no. 5 with 36.13 cm² pollen area as a total 52 cells were filled with pollen and by multiplying with 6.45 the total area of pollen in cm² was calculated. Some other colonies with higher brood survival rates like colonies no. 3, 6, 7, 8 and 10 have also good amount of pollen area with 31.61 cm², 33.55 cm², 27.74 cm², 32.90 cm² and 29.03 cm² pollen respectively. On the other side colonies no. 1, 2 and 4 were categorised under medium brood survival rate colony and their yield of pollen was 26.45 cm², 29.03 cm² and 28.39 cm² respectively. Colony no. 9 having the lowest brood survival rate among all the colonies able to produce only 25.16 cm² pollen area as only 39 cells were filled with honey of that particular frame inserted into colony no. 9. Average pollen area of all 10 colonies was 26.45 cm². This data

is relevant to [10] where more quantity of bees in the colony than that colony shows higher activity related to foraging and pollen collection.

While some other colonies like in colony no. 6 the per cent removal were 96 while in colony no. 1, 5 and 8 the value of per cent removal was 92. While in colony no. 9 or the colony with the lowest brood survival rate was able to remove only 19 dead pupae out of 25 and thus per cent removal was only 76. The average per cent removal of dead pupae of all 10 colonies was 90.80 per cent. Our research follows the study of [11-12] where they quantified the removal of freeze-killed and chalk brood-infected larvae. This was done in open cells and a total of 20 colonies they used. They also quantified the removal of freeze-killed larvae from sealed cells. Categorization of colonies was done and ranged from non-hygienic to fully hygienic (52– 100% removal within 2 days). They observed that all larvae killed in open cells were completely removed. That behaviour showed that all colonies, including those with low hygienic

Table 3. Quantity of honey (g) and pollen stores (cm²) in different colonies

| Colony Number | Brood survival rate (%) | No. of square filled with honey | Quantity of honey (g) (No of a square filled with honey x8.06) | No. of cells filled with pollen | No. of squares in a grid frame covered with pollen | Pollen stores (cm ²) (No. of squares in grid frame covered with pollen x 6.45) |
|---------------|-------------------------|---------------------------------|--|---------------------------------|--|--|
| 1 | 84.78 | 39 | 314.34 | 41 | 4.1 | 26.45 |
| 2 | 89.18 | 37 | 298.22 | 45 | 4.5 | 29.03 |
| 3 | 93.93 | 47 | 378.82 | 49 | 4.9 | 31.61 |
| 4 | 89.74 | 40 | 322.40 | 44 | 4.4 | 28.39 |
| 5 | 96.87 | 49 | 394.94 | 56 | 5.6 | 36.13 |
| 6 | 95.45 | 43 | 346.58 | 52 | 5.2 | 33.55 |
| 7 | 92.10 | 46 | 370.76 | 43 | 4.3 | 27.74 |
| 8 | 95.55 | 41 | 330.46 | 51 | 5.1 | 32.90 |
| 9 | 75.75 | 31 | 249.86 | 39 | 3.9 | 25.16 |
| 10 | 92.68 | 42 | 338.52 | 45 | 4.5 | 29.03 |
| Average | 90.72 | 41.5 | 334.49 | 41 | 4.1 | 26.45 |

Table 4. Per cent removal of diseased or dead honey bees in different colonies

| Colony no. | Per cent brood survival | Pupae killed | Removed pupae (dead) | Removal (%) |
|------------|-------------------------|--------------|----------------------|-------------|
| 1 | 84.78 | 25 | 23 | 92.00 |
| 2 | 89.18 | 25 | 22 | 88.00 |
| 3 | 93.93 | 25 | 25 | 100.00 |
| 4 | 89.74 | 25 | 21 | 84.00 |
| 5 | 96.87 | 25 | 23 | 92.00 |
| 6 | 95.45 | 25 | 24 | 96.00 |
| 7 | 92.10 | 25 | 22 | 88.00 |
| 8 | 95.55 | 25 | 23 | 92.00 |
| 9 | 75.75 | 25 | 19 | 76.00 |
| 10 | 92.68 | 25 | 22 | 88.00 |
| Average | 90.72 | | | 90.80 |

behaviour against dead brood in sealed cells, are highly hygienic against dead brood in open cells and suggests that low hygienic behaviour against dead brood in sealed cells is a trait in its own right. The study also contributes to understanding why hygienic behaviour is less common in *A. mellifera*, which is puzzling as it decreases many diseases without adverse effects.

4. CONCLUSION

Colonies having high and medium brood survival rates were much cleaner, more active, and showed higher economic and resistant traits and good hygienic behaviour. Colonies with low brood survival rates were less clean, less active and poor in resistant and hygienic behaviour. Most of the outgoing bees were recorded during 09:00 to 10:00 hours while most of the incoming bees were recorded during 16:00 to 17:00 hours. Colonies with high and moderate brood survival rates had 100 per cent efficiency while colonies with low brood survival rates had the lowest efficiency at 76 per cent. There is a significant impact of brood survival rate with both physiological and economical traits of *A. mellifera* therefore to get good profit in terms of economic aspects of apiculture one must go with colonies having high brood survival rates.

FUTURE LINE OF WORK

Future studies should concentrate on improving brood survival rates through better hive management and disease control. Understanding bee behavioural patterns, particularly activity levels and sanitary habits is critical for improving colony health. Investigating temporal dynamics, such as the time of bee activity, might yield useful information for colony management. Strategies for increasing efficiency, especially in colonies with low brood survival rates, should be investigated. Economic evaluations should evaluate the cost-effectiveness of treatments designed to increase apiculture profitability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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