Evaluating the influence of brood survival rates on the behavioral patterns, economic aspects, and resistance traits of the Apis mellifera L. honeybee species when reared on mustard crops in the Bihar region

ABSTRACT
In order to better understand *Apis mellifera* forageing behaviour for mustard, this study examined how brood survival rates affected the species behavioural, economic and resistant traits. Out of the 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 pattern, poor, medium and excellent brood patterns were found in 1, 2, and 7 colonies, respectively. 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% efficiency while colonies with low brood survival rates had the lowest efficiency at 76%.
KEYWORDS: Mustard, Bee activity, Pollen, Brood

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INTRODUCTION

Honey bees are highly advanced and familiar groups of social insects. Bees are capable of increasing to the yield of cross pollinated and to some extent in 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 exist in the world and this holds for about 65 per cent pollination activities of different flowering plants. They are working as important pollinating agent for more than 80% of all flowering plants including the majority of agricultural and horticultural crops. Being the main pollination service provider, the honey bees adds quite well in increasing the overall production and productivity of these kind of cross pollinated crops with their efficient pollination in an obscure and silent way **[1] [2]**. Honey bees are most common and important pollinators of several agricultural or field crops and mustard (*Brassica juncea*) is one of them. Mustard belongs to Cruciferae family and generally cultivated as *rabi* crops in India. In Bihar it is cultivated in winter season. It is one of the popular and mostly cultivated oilseed crops next to sunflower. Mustard is generally called as rai. There are total six cultivated species under the genus Brassica and among them more than 80% of total cultivated area holds by Indian mustard or scientifically known as *Brassica juncea* **[3]**. Mustard seed are rich in energy content, total oil content in them is around 28-32 % with relatively good amount of protein (28-36%), while the amino acid presents in mustard proteins are very finely balanced, it is much richer in essential amino acid required by human beings, it contains 20-28% oleic acid, linoleic acid in 10-20% and lastly 30-40% erucic acid present in mustard protein **[4]**. Mustard is highly cross-pollinated crops 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 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 there 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

MATERIAL AND METHOD

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 total 4 observations were recorded at 7 days interval. Then the colonies were designated as a) Very clean b) Clean c) Poor cleaning ability.

Bee activity: Observation on the activity of honeybee colonies were 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.

Economic traits:

Honey stores: Firstly, estimation the honey stores with the help of grid frame was done then converting the number of squares covering honey area into grams by multiplying with 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.

Figure.1: Field view of experimental plots

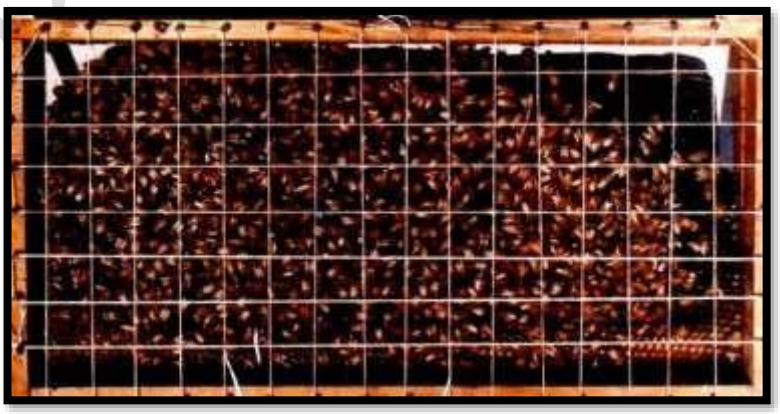

Figure. 2: Standard grid frame made of iron wire

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Pollen stores: Measurements of the pollen stores were done in cm^2 by counting the number of cells in the wire grid covered by pollens.

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 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:

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

RESULTS AND DISCUSSION

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 days interval. The collected debris was weighted in weighing balance and the obtained results are listed down in table 2. The experiment on 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 categorized under very clean colony while 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 week was 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 were died and total debris content was also much more compared to other colonies and categorized under colony with poor cleaning ability and remaining 8 colonies were remarked as clean colony. It was observed that the weight of the debris content kept increasing as the month proceeds and for almost all colonies the maximum value of debris content was during the 4th week. Now from the experiment it was noticed that the colony no. 5 was very clean colony and this colony witnessed the highest brood survival rate with per cent brood survival rate of 96.87 while on the other side colony no. 9 with the lowest per cent brood survival rate of 75.75 was the dirtiest colony and same trend was found with the remaining colonies.

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	

Bee activity:

Observation on 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

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hours (09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs.) was taken for the

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whole month (01.12.2020 to 31.12.2020) was done. 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 OPSTAT programme. The obtained data regarding bee activity are listed down in table 2. So, from the data of outgoing bees, it was concluded that almost all the colonies having 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 were only 9.00. On the other hand, maximum number of outgoing bees was noticed in colony no. 3 with 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 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 and it showed least activity during late hour of the day or during 16:00 to 17:00 hours with only 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 in relation to brood survival rate and bee activity it was observed that colony no.9 with the lowest brood survival rate witnessed 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 colony having high and medium brood survival rate having a greater number of active outgoing bees and with low brood survival rate 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 were only 7.33, 9.00 and 9.33 during 09:00 to 10:00 hrs., 13:00 to 14:00 hrs. and 16:00 to 17:00 hrs. respectively.

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

Period of observation	Colony Number	Outgoing bees during different day hours				Incoming bees during different day hours			
		09:00-10:00 hrs.	13:00-14:00 hrs.	16:00-17:00 hrs.	Average no. of outgoing 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, maximum number of incoming bees were recorded in colony no. 5 with average number of incoming bees were 10.33, 10.67 and 14.33 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 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 late hour of the day or during 16:00 to 17:00 hours with average 12.33 number of incoming bees

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were noticed in all 10 colonies followed by during 13:00 to 1400 hrs. with 10.43 average incoming bees and it showed least activity with only average 8.70 mean number of bees found to came back to their colony during early hours of the day with the time range of 09:00 to 10:00 hrs. More or less the present findings are agreement with the findings of **[5]** and **[6]** where they observed that the activity of foraging of Indian bee (*Apis cerna indica*) and European bee (*Apis mellifera*) increased at 10:00 hr of the day. Now in relation to brood survival rate and bee activity it was observed that colony no.9 having the lowest brood survival rate witnessed least number of active incoming bees and on the other side colony no. 5 having the highest brood survival rate of 96.87% and in 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.

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 the honey stores with the help of grid frame was done then converting the number of squares covering 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 was done in cm^2 by counting the number of cells in the wire grid covered by pollens were taken. One square of standard grid frame contains 10 cells in to it and area of Single Square of grid frame is 1 square inch (6.45 cm^2). So based on this, 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. Quantity of honey was maximum in colony no. 5 with 394.94 g as total 49 squares were filled with honey and by multiplying with the factor 8.06 the total quantity of honey was calculated. Some others colonies with higher brood survival rate like colony 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 colony 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. While the colony no. 9 that 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 in to colony no. 9. While the average honey content for the all 10 colonies was 334.49 g. The data regarding pollen stores showed the same trend like honey stores as the pollen store was maximum in colony no. 5 with 36.13 cm^2 pollen area as total 52 cells were filled with pollen and by multiplying with 6.45 the total area of pollen in cm^2 was calculated. Some others colonies with higher brood survival rate like colony no. 3, 6, 7, 8 and 10 having also good amount of pollen area with 31.61 cm^2, 33.55 cm^2, 27.74 cm^2, 32.90 cm^2 and 29.03 cm^2 pollen are respectively. On the other side colony no. 1, 2 and 4 were categorised under medium brood survival rate colony and their yield of pollen was 26.45 cm^2, 29.03 cm^2 and 28.39 cm^2 respectively. While the colony no. 9 that having the lowest brood survival rate among all the colonies able to produce only 25.16 cm^2 pollen area as only 39 cells were filled with honey of that particular frame inserted in to colony no. 9. Average pollen area of all 10 colonies was 26.45 cm^2. This data is relevant to **[7]** where more quantity of bees in the colony then that colony shows higher activity related foraging and pollen collection.

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Table no. 3: Quantity of honey (g) and pollen stores (cm^2) in different colonies						
Colony Number	Brood survival rate (%)	No. of square filled with honey	Quantity of honey (g) (No of square filled with honey × 8.06)	No. of cells filled with pollen	No. of squares in grid frame covered with pollen	Pollen stores(cm^2) (No. of squares in grid frame covered with pollen × 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

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 sharp steel needle was done. Recording the pierced area after 24 hours to count the number of dead pupae removed by worker bees and then per cent removal of killed brood was calculated by using the below formula:

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

The obtained data of hygienic behaviour showed that there is significant impact of brood survival rate with resistant traits of *A. mellifera*. As the result showed that colonies having higher rate of survival rates were much efficient to remove the dead pupae and thereby per cent removal was more in those colonies and on the other side colony with low rate of survival were less efficient in removing the dead pupae. The highest per cent removal of dead pupae was recorded in colony no. 3 as the worker bees of that particular colony were able to remove all 25 dead pupae and thereby per cent removal was 100.

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

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

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90.80%. Our research is in accordance with the study of **[8]** where they had quantified removal of freeze-killed and chalk brood-infected larvae. This was done in open cells and total 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 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 understand why hygienic behaviour is less common in *A. mellifera*, which is puzzling as it decreased many diseases without adverse effects.

Conclusion

1. Colonies having high and medium brood survival rates were much cleaner, more active, showed higher economic and resistant traits and good hygienic behaviour.
2. Colony with low brood survival rates was less clean, less active and poor in resistant and hygienic behaviour.
3. 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.
4. Colonies with high and moderate brood survival rates had 100% efficiency while colonies with low brood survival rates had the lowest efficiency at 76%.
5. 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.

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