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Effect of probiotic supplement feed on the foraging activity of Indian honeybee (*Apis cerana indica* F.)

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Abstract

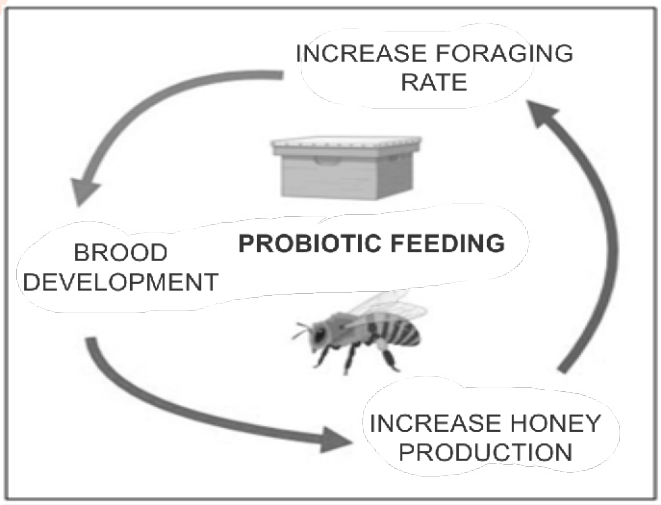
Aim: To investigate the effect of probiotic supplement feed on the foraging behaviour Indian honeybee colonies to resolve the excessive usage of antibiotics.

Methodology: The effect of sugar syrup feed (Untreated hives) and sugar syrup + probiotic supplement feed @ 10 ml 100 ml^{-1} syrup (Treated hives) on Indian honeybees was tested. The foraging activity and foraging rate were recorded and subjected to paired t- test analysis.

Results: The present observation showed a significant difference in the foraging rate between the colonies fed with sugar syrup + probiotic supplement (2.33 ± 0.11 min^{-1}) than the colonies that received sugar syrup alone (1.35 ± 0.06 min^{-1}). The highest foraging rate (2.80 ± 0.39 min^{-1}) was recorded in treated hives.

Interpretation: The experiment revealed that the brood development was directly proportional to brood pheromone levels, which positively affects the foragers, consequently foraging rate and honey production. Thus, the probiotic supplement feeding may influence the foraging behaviour of honeybee colonies.

Key words: Foraging rate, Honey, Indian honeybees, Probiotic supplement



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Introduction

The geographical position of India, and agro-climatic conditions supports the growth of a wide variety of cultivated and natural flora. Thus, an extended area of forest and cultivatable lands can sustain the bee keeping practices and honey production (Johnson *et al.,* 2010). Honeybees have many benefits other than income generation from honey production (Gupta *et al*., 2014). They are required for effective pollination of crops and are to improve the food production (Chantawannakul, 2018). Bee products are also considered as an important source of nutrition and have many medicinal applications (MusaOzcan and Juhaimi, 2015). Worldwide, 400 crop species are pollinated by honeybees (James and Pitts-Singer, 2008). Globally, 87 major food crops depend on animal pollination and they account for 35 per cent of the global food production (Van der Sluijs and Vaage, 2016). The crops pollinated by animals for reproduction are dependent on the managed honeybee for pollination (Breeze *et al.,* 2011). Hence, they play vital role for the economic, sustainable agriculture, food security, pollination of most wild flowers and maintaining the biodiversity.

These beneficial honeybees are often affected by various biotic and abiotic factors. Among the factors honey bee diseases caused by viruses, bacteria, fungi, protozoan and parasitic mites have been found to play a major role in increased honeybee mortality and collapse of the colony (Bailey, 1968; Kemp and Kross, 2000). Acaricides and antibiotics were used for the management of parasitic mites and diseases (Bogdanov, 2006). Among antibiotics oxytetracycline is commonly used to treat the bacteria *Paenibacillus larvae* and *Melissococcus plutonis*, the causal organisms of European foulbrood disease and American foulbrood disease, respectively (Johnson *et al.,* 2010). Intensive use of antibiotics, acaricides and other chemicals for management practices of diseases and parasites in bee keeping has consequently led to numerous side effects on the honeybees, residues in bee products, genotoxicity in consumers (Genersch, 2010).

Symbionts are microorganisms establishing interactions with their animal host, including insects and honeybees. They are involved in many aspects of host physiology, including nutrition, reproduction, immune homeostasis and defence (Sansonno, 2014). Understanding the interactions between the indigenous larval gut flora, nutrition and disease progression are important because the larval gut act as the target for many pathogenic bacteria and fungi (Vojvodic *et al.,* 2013). Thus, the indigenous gut bacteria play a role in withstanding the colonization of the gut by non-indigenous species, including pathogens (Dillion and Dillion, 2004). The gut microbiota modulation is considered a successful approach in the entomological field for the management of pest and pathogens (Alberoni *et al.,* 2018) and Gram-positive bacteria, such as lactic acid bacteriawhich are the promising options for pest and pathogen management in Apiculture (Audisio, 2017). According to the Food and Agriculture Organization of the United Nations and World Health Organization, probiotics are live microorganisms that confers health benefit to the host when administered in adequate amounts (FAO, 2001). Administration of different *Lactobacillus* and *Bifidobacterium* strains to infected larvae of *P. larvae*, significantly reduced their mortality (Forsgren *et al.,* 2010). Similar results were observed in the case of infection with the bacterial pathogen *M. plutonius* (Vasquez *et al.,* 2012; Wu *et al.,* 2014). Thus, this paper investigates the effect of probiotic supplement feeding on the foraging behaviour of Indian honeybee.

Materials and Methods

Field experiments were conducted in the Bee garden, Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli district during 2018-19 to evaluate the effect of both sugar syrup feeding (Untreated hives) and sugar syrup + probiotic supplement feeding @ 10 ml $100ml^{-1}$ (Treated hives) on foraging activity of Indian honeybees . Each set of treatments had 10 bee hives kept at Bee garden with equidistant between each experimental bee hives. Foraging activity of bees were determined by counting the number of worker bees moving out and returning to the hive with and without the pollen loads by using hand tally counter and stop watch. The bees, carried pollen on their legs and empty foragers were noted separately. Bees returning without pollen loads were considered as non-pollen foragers/nectar gatherers. The foraging activity were recorded for three months during the period 2018-19 at fortnightly intervals and the observations were recorded three times a day *viz*., 8:00, 12:00 and 16:00 h for 5 minutes while the value obtained by sum of the three intervals were taken as the total foragers of the hive per 15 minutes for that particular day (Reddy *et al.,* 2015; Hemalatha *et al.,* 2018). The foraging rate was determined by counting the homing bees which were recorded three times a day *viz*., 8:00, 12:00 and 16:00 h for one minute. The mean of the values obtained at three intervals were taken as the foraging rate. The experimental data on the total foragers and foraging rate from the field experiments between treated and untreated were subjected to paired t- test analysis with Microsoft Excel using square root transformation ($\sqrt{x+0.5}$).

Observations on the number of honey cells and pollen cells stored in the brood frame were recorded for three months,during the period 2018-19 at weekly intervals using a transparent 1.0 cm grid in $100cm^2$ brood area (Delaplane*et al.,* 2013).The experimental data on the number of honey cells and pollen cells were also subjected to paired t- test analysis with Microsoft Excel using square root transformation ($\sqrt{x+0.5}$).

Results and Discussion

The foraging in honeybee is altruistic and socially regulated, but the individual nutritional physiology may also play a role in the foraging activity (Toth *et al.,* 2005). The foraging activity was highest in the colonies which received probiotic supplement feed. The foraging activity varied between both probiotic supplement-fed colonies and probiotic supplement non- fed colonies (29.60 ± 1.67 to 48.30 ± 2.45/15 min) (Table 1). The number of foraging bees that entered the hives were significantly highest ($P \leq 0.05$)

when probiotic supplement + sugar syrup was fed to the bees (48.30 ± 2.45/15 min) than in untreated hives (33.60 ± 1.63/15 min).

The foraging rate in terms of number of bees entering the hive per minute showed significant difference between those colonies fed with sugar syrup + probiotic supplement (2.33 ± 0.11/min) than in those colonies that received sugar syrup without probiotic supplement (1.35 ± 0.06/min) (Table 1). The highest foraging rate of 2.80 ± 0.39/min was recorded in treated hives. The present observations had a significant difference in the foraging behaviour between the treated and untreated hives.

Foraging efficiency of a colony was measured in terms of number of bees with pollen load entering into the hive. The foraging behaviours affected by both nectar and pollen, their quality and quantity (Pushpalatha, 2018). The pollen foraging bees that entered the hives ranged from 14.80 ± 0.73 to 20.70 ± 0.70/15 min in the colonies fed with probiotic supplement+ sugar syrup and sugar syrup without probiotic supplement (Table 2). The pollen foraging bees that entered the hives were highly significant (P ≤ 0.05) when probiotic supplement + sugar syrup were fed to the bees (20.20 ± 0.38 per 15 min) and without probiotic supplement (15.83 ± 0.35 per 15 min). The probiotic supplements are involved in the health status and brood development of the honeybee colonies (Hamdi *et al.,* 2011; Alberoni *et al.,* 2016). The brood increase generates an

Table 1 : Effect of probiotic supplement feeding on the total number of foraging bees and foraging rate of Indian honeybee colonies

Month 2019	Fortnightly	Foraging bees per 15 min. (no.)			Foraging rate per min (no.)		
	count	Untreated hives	Treated hives	*t* – value	Untreated hives	Treated hives	*t* - value
March	I	31.20±1.64 (5.63)	46.60±1.62 (6.86)	6.41 (P=4.89E-6)**	1.33±0.17 (1.34)	2.10±0.23 (1.61)	2.32 (P=0.033)**
	II	33.60±1.04 (5.84)	47.60±2.42 (6.94)	5.18 (0.0002)**	1.60±0.27 (1.45)	2.80±0.39 (1.82)	2.14 (P=0.047)**
April	I	32.70±1.63 (5.76)	48.30±2.45 (6.99)	5.04 (P=0.0001)**	1.20±0.20 (1.30)	2.50±0.37 (1.73)	2.74 (P=0.016)**
	II	33.10±0.96 (5.80)	42.60±0.81 (6.57)	6.98 (P=2.24E-6)**	1.30±0.15 (1.34)	2.30±0.37 (1.67)	2.10 (P=0.056)**
May	I	30.40±1.35 (5.56)	47.90±1.94 (6.96)	7.16 (P=1.14E-6)**	1.40±0.22 (1.38)	2.20±0.20 (1.64)	2.30 (P=0.034)**
	II	29.60±1.67 (5.49)	46.50±1.47 (6.86)	7.19 (P=2.14E-6)**	1.30±0.15 (1.34)	2.10±0.28 (1.61)	1.99 (P=0.064)**
Mean		**31.77±0.65 (5.68)**	**46.58±0.85 (6.86)**	**13.84 (P=7.51E-8)****	**1.35±0.06 (1.36)**	**2.33±0.11 (1.68)**	**8.38 (P=3.11E-5)****

Mean ± SE; Mean of observations of 10 hives; Figures in parentheses are square root (√x+0.5) transformed values;* significant 0.01% level; **significant 0.05% level.

Table 2 : Effect of probiotic supplement feeding on pollen foraging bees and non-pollen foraging bees or nectar gatherers of Indian honeybee colonies

Month 2019	Fortnightly	Foraging bees per 15 min. (no.)			Foraging rate per min (no.)		
	Count	Untreated hives	Treated hives	*t* – value	Untreated hives	Treated hives	*t* - value
March	I	16.00±0.77 (4.06)	20.40±0.84 (4.57)	3.38 (P=0.003)**	23.20±0.61 (4.87)	28.50±0.50 (5.39)	5.97 (P=1.53E-5)**
	II	14.80±0.73 (3.91)	20.00±1.14 (4.53)	3.50 (P=0.003)**	22.90±0.57 (4.84)	27.30±0.61 (5.27)	4.66 (P=0.0002)**
April	I	17.20±0.88 (4.21)	20.70±0.70 (4.60)	2.70 (P=0.015)**	23.60±0.40 (4.91)	29.90±0.94 (5.51)	5.82 (P=5.93E-5)**
	II	16.40±0.65 (4.11)	20.00±0.77 (4.53)	3.06 (P=0.007)**	22.80±0.71 (4.83)	29.20±0.68 (5.45)	5.92 (P=1.34E-5)**
May	I	15.20±0.57 (3.99)	20.00±0.93 (4.54)	4.05 (P=0.001)**	22.10±0.69 (4.81)	29.80±0.92 (5.48)	6.39 (P=2.32E-5)**
	II						
Mean		**15.83±0.35 (4.04)**	**20.20±0.38 (4.55)**	**11.05 (3.27E-5)****	**22.87±0.21 (4.83)**	**29.03±0.40 (5.43)**	**5.97 (P=1.53E-5)****

Mean ± SE; Mean of observations of 10 hives; Figures in parentheses are square root (√x+0.5) transformed values; * significant 0.01% level; **significant 0.05% level.

Table 3 : Effect of probiotic supplement feeding on honey cells and pollen cells of Indian honeybee colonies

Month 2019	Fortnightly	Foraging bees of 15 min. (no.)			Foraging rate per min. (no.)		
	Count	Untreated hives	Treated hives	*t* – value	Untreated hives	Treated hives	*t* - value
	I	74.30±7.24 (8.65)	148.00±19.33 (12.19)	3.81 (P=0.002)**	24.30±2.92 (4.98)	43.90±4.62 (6.66)	3.70 (P=0.002)**
	II	95.70±13.16 (9.81)	145.30±16.75 (12.07)	2.36 (P=0.030)**	15.00±1.76 (3.94)	26.50±2.86 (5.20)	3.51 (P=0.003)**
March	III	84.80±21.70 (9.24)	151.20±22.80 (12.32)	2.38 (P=0.029)**	13.20±1.35 (3.70)	20.80±2.17 (4.62)	2.63 (P=0.018)**
	IV	65.20±7.58 (8.11)	144.60±31.99 (12.05)	2.46 (P=0.032)**	11.00±1.55 (3.39)	21.30±2.83 (4.67)	3.27 (P=0.004)**
	I	40.20±5.33 (6.38)	71.20±12.29 (8.47)	2.44 (P=0.027)**	8.00±0.77 (2.92)	16.40±1.66 (4.11)	4.73 (P=0.0002)**
	II	52.20±5.29 (7.26)	77.50±9.27 (8.83)	2.62 (P=0.019)**	18.20±2.08 (4.32)	29.00±0.94 (5.43)	4.33 (P=0.001)**
April	III	46.00±3.87 (6.82)	62.50±5.46 (7.94)	2.35 (P=0.031)**	17.20±2.59 (4.21)	26.50±2.68 (5.20)	2.72 (P=0.014)**
	IV	40.80±3.81 (6.43)	67.90±11.40 (8.27)	2.40 (P=0.032)**	13.10±1.31 (3.69)	20.40±2.17 (4.57)	2.64 (P=0.017)**
May	I	83.80±8.55 (9.18)	154.00±20.86 (12.43)	3.14 (P=0.007)**	11.90±1.27 (3.52)	21.30±2.83 (4.67)	3.21 (P=0.006)**
	II	89.40±11.69 (9.48)	153.20±25.74 (12.40)	2.36 (P=0.035)**	8.80±0.84 (3.05)	16.70±1.71 (4.15)	4.18 (P=0.001)**
Mean		**67.24±6.70 (8.23)**	**117.54±13.08 (10.86)**	**3.37 (P=0.004)****	**14.07±1.54 (3.82)**	**24.28±2.54 (4.98)**	**3.76 (P=0.002)****

Mean ± SE; Mean of observations at 10 hives; Figures in parentheses are square root (√x+0.5) transformed values; *significant 0.01% level; **significant 0.05% level.

expansion of bee colonies because of increase in the foragers. Moreover, a higher amount of pollen in this study is due to high foraging activity in honeybees which support both brood and bee health status (Di Pasquale *et al*., 2013).The number of non-pollen foraging bees per nectar gatherers that entered hives ranged from 22.10 ± 0.69 to 29.90 ± 0.94 per 15 min in the colonies fed with probiotic supplement + sugar syrup and sugar syrup without probiotic supplement (Table 2). The non-pollen foragers that entered the hives were significantly highest (P ≤0.05) when probiotic supplement + sugar syrup were fed to the bees (29.03 ± 0.40/15 min) than the probiotic supplement non-fed colonies (22.87 ± 0.21 per 15 min).

The number of honey filled cells recorded in treated hives ranged from 62.50±5.46 to 154.00±20.86 100cm^{-2} brood area and the number of honey cells recorded in untreated hives ranged from 40.20±5.33 to 95.70±13.16 100cm^{-2} brood area. The number of honey filled cells were highly significant in the colonies fed with probiotic supplement (117.54±13.08 100cm^{-2} brood area) (Table 3). The number of pollen/bee bread filled cells of *A. cerana* combs differed significantly between probiotic supplement fed hives (24.28±2.54 100cm^{-2} brood area) and probiotic non-fed hives (14.07±1.54 100cm^{-2} brood area) (Table 3). Comparatively the highest number of pollen cells (43.90±4.62 100cm^{-2} brood area) were recorded in the treated hives than the untreated hives.

The findings of this study showed that the pollen foragers (20.20 ± 0.38 per 15 min), non-pollen foragers (29.03 ± 0.40 per 15 min) and foraging rate (2.33 ± 0.11 per min) were high in treated hives which were fed with probiotic supplement feed than the unfed hives. Since the brood pheromone levels, was directly proportional to foraging behaviour which positively affects the number of foraging honeybees and their foraging rate, ultimately the honey production (Pankiw *et al*., 1998; Pankiw, 2004; Alberoni *et al*., 2018). Thus, they had influenced the foraging behaviour of the honeybee colonies and may influence the honey production since the number of foragers (46.58 ± 0.85 per 15 min) and honey cells (117.54±13.08 100cm brood area) were found to be more in treated hives.

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Add-on Information

Authors' contribution: I. Padmashree: Carried out research and drafted the manuscript; **S. S. J. Roseleen:** Major advisor of the research work; **C. G. L. Justin:** Supervised the research work and checked the manuscript.

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