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Effect of probiotic supplement on the disease management and brood development of the Indian honey bee, *Apis cerana indica* F. (Hymenoptera: Apidae)

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Abstract
Studies were carried out at the Bee garden, Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli district to investigate the effect of probiotic supplement for the management of diseases on honey bees. The European Foul Brood (EFB) affected cells per 100 cm^2 brood area showed a significant difference among the colonies that received treatment (0.07±0.03 / 100 cm^2 brood area) and in control (0.45±0.10 / 100 cm^2 brood area). The Thai Sac Brood (TSB) affected cells per 100 cm^2 brood area also showed significant difference among the colonies that received treatment (0.05±0.02 / 100 cm^2 brood area) and in control (0.59±0.18 / 100 cm^2 brood area). There were a decreased incidence of disease in the probiotic fed colonies (treated) than the probiotic non-fed colonies (control). The probiotic feed also resulted in a significant colony development as evidenced by increased brood comb area in probiotic fed colonies (treated) than probiotic non-fed colonies (control).

Keywords: Indian honey bees, probiotics, immunity, disease management and brood development

Introduction
Honey bees are required for the effective pollination of crops and are therefore critical to world agriculture for human food production [5]. Worldwide, bees pollinate more than 400 crop species [12]. Honey bees are affected by various diseases caused by viruses, bacteria, fungi, protozoan and mites. The ectoparasitic mites, *Varroa destructor* (Anderson and Trueman) that parasitise pupae and adult bees, and feed on the haemolymph of honey bee. These wound sites often harbour pathogen infections [17]. *Varroa* mites act as vectors for a number of honey bee pathogens and may weaken the immune systems of their hosts, leaving them vulnerable to infections. Honey bee pathogens include *Melissococcus plutonius* (Trüper and de' Clari), *Paenibacillus larvae* (White), *Ascosphaera apis* (Maasen ex Claussen) and *Nosema apis* (Zander) which causes European foulbrood disease (EFB), American foulbrood disease (AFB), Chalkbrood disease and *Nosema* disease [14]. The Thai Sac Brood Virus (TSBV) causing disease is the major threat to hives of *Apis cerana indica* F. in different parts of Southern India [3].

Oxytetracycline is commonly used to treat *P. larvae* and *M. plutonis* bacteria, causal organisms of European foulbrood disease (EFB) and American foulbrood diseases (AFB) respectively. There were reports of tetracycline resistance in these bacteria because of widespread usage of antibiotics against honey bee pathogens. Erythromycin, lincomycin, monensin, streptomycin and enrofloxacin were also reportedly used in bee products [13].

Antibiotics like sulfonamides (sulfathiazole, sulfamethazine, sulfamethaxazole and sulfanilamide), aminoglycosides (streptomycin), Tetracycline (oxytetracycline and chlortetracycline) and amphenicols (chloramphenicol) were also reported in honey which are capable for creating multi-drug resistant microorganisms because of widespread usage against pathogens [4]. In European countries, the use of antibiotics against *P. larvae* was banned due to several problems associated with their use such as due to the presence of antibiotic residues in honey bee products cause negative effects on honey bee longevity and vitality [10]. Since gut microbiota plays a significant role in bee health there is a risk of unbalancing the bee gut microbiota due to use of antibiotics [15].

These issues had created a demand for introduction of biocides that kill or inhibit bacterial, fungal pathogens and acarid infestation of honey bee.

Symbionts are microorganisms establishing interactions with their animal host, including insects and honey bees. They are involved in many aspects of the host physiology, including nutrition, reproduction, immune homeostasis and defence. The manipulation and exploitation of the insect microbiota could be effective for the development of strategies for the management of insect-related problems [18]. The indigenous gut bacteria plays an important role in the colonization of the gut against pathogens [8]. The gut microbiota modulation has been considered as a successful and practical approach in the entomological field for the management of pest and pathogen [2]. Therefore this investigation was carried out in *A. cerana* colonies to compare the effect of probiotic feeding and powdered sugar spreading on the brood development and honey bee disease incidence.

Materials and Methods

The honey bee, *A. cerana* colonies available in the Bee garden at the Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli district were collectively utilized to conduct this experiment over a period of ten weeks. Ten bee hives per treatment were maintained. Each bee hives had equal frames frame strength (four frames) and the queens were of uniform age. Feeding of sugar syrup for control colonies and sugar syrup + probiotic for treated colonies were given at weekly interval at the rate of 250 ml per colony using the plastic cup feeder. Honey and pollen (bee bread) stored in the cells, number of eggs laid by the queen, number of larvae and pupal cells (capped), the number of diseased cells showing symptoms and uncapped or perforated cappings were recorded at weekly interval using a transparent 1.0 cm-grid [6]. In addition, the number of *Varroa* mites that had fallen on the bottom board were assessed every week in a 1250 cm^2 area using the same transparent grid. Mite assessment was also made by keeping 50 workers inside a 400 ml PET jar and by shaking them vigorously after adding a heaped table spoon of powdered sugar. The dislodged mites were counted after dissolving the powdered sugar in water contained in a whitish container and the dust-laden bees in jars were released. The powdered sugar was dusted every week after all the above observations were recorded [7]. The data from experiments were subjected to student t – test with square root ($\sqrt{x+0.5}$) transformation.

Results and Discussion

The experimental results showed that there were a significant difference in the queen bees fecundity due to the increased number of egg cells among the colonies fed with sugar syrup + probiotic supplement (97.00±3.62 / 100 cm^2 brood area) while not in the colonies fed without probiotic supplement (58.54±3.63 / 100 cm^2 brood area). A significant difference in the number of larva after feeding the bee hives with sugar syrup (80.33±6.70 / 100 cm^2 brood area) and sugar syrup + probiotic feeding (133.16±10.62 / 100 cm^2 brood area). A significant difference in the number of pupae was observed in brood cells after feeding with sugar syrup (165.09±11.92 / 100 cm^2 brood area) and probiotic feeding (265.04±12.80 / 100 cm^2 brood area). The honey filled cells recorded at treatment hives ranged from 62.50±5.46 to 154.00±20.86 /100 cm^2 brood area and the number of honey cells recorded at control bee hives ranged from 40.20±5.33 to 95.70±13.16/100 cm^2 brood area. The pollen / bee bread filled cells of *A. cerana* combs differed significantly between probiotic supplement feeding colonies (24.28±2.54 / 100 cm^2 brood area) and probiotic non-fed colonies (14.07±1.54 / 100 cm^2 brood area). The EFB affected cells per 100 cm^2 brood area showed a significant difference among the colonies that received treatment (0.07±0.03 / 100 cm^2 brood area) and in control (0.45±0.10 / 100 cm^2 brood area). The TSB affected cells per 100 cm^2 brood area also showed significant difference among the colonies that received treatment (0.05±0.02 / 100 cm^2 brood area) and in control (0.59±0.18 / 100 cm^2 brood area) (Table 1). The results of the experiments suggested that there was significant difference on the number of eggs, larva and pupa between the treated colonies and untreated colonies. The increase in eggs, larvae, pupa, pollen cells and honey cells in treated colonies were 39.65, 39.67, 37.71, 42.05 and 42.79 per cent increase in the number of egg cells, larval cells, pupal cells, pollen cells and honey cells respectively than the untreated ones.

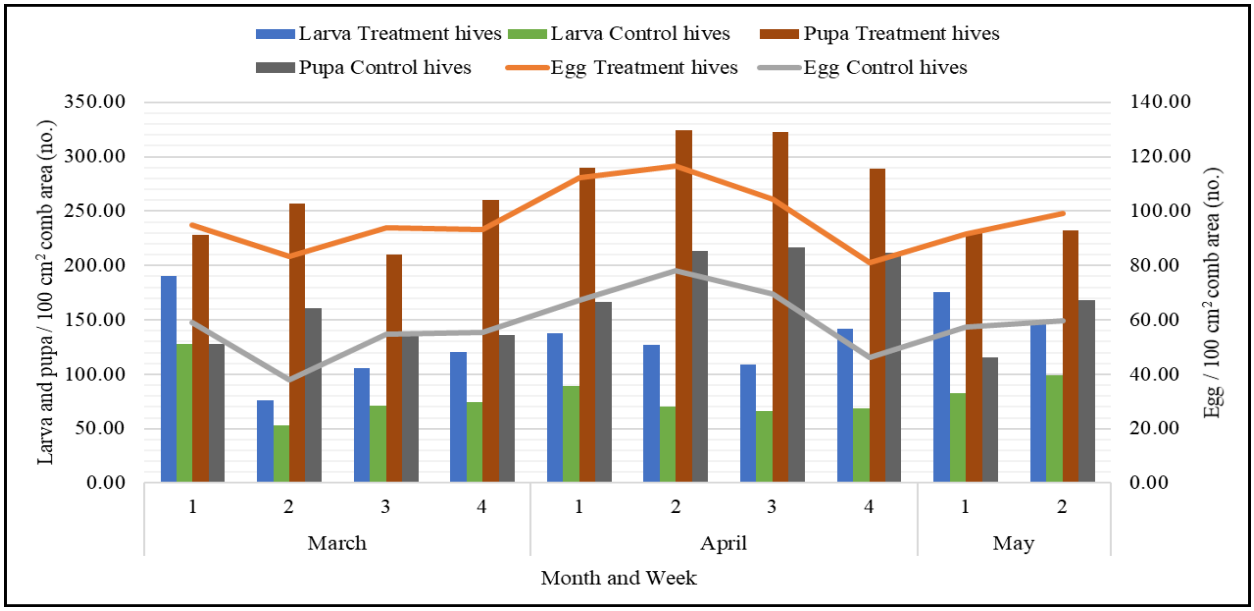
Alberoni *et al.* (2018), reported that the bacterial supplementation led to a significant increase of brood population (46.2%), pollen (53.4%) and harvestable honey in honey supers (59.21%) (Fig. 1 & 2). Patruica *et al.* (2011) also reported that prebiotic and probiotic supplementation of colony with sugar syrup showed better in colony development with increased populated brood comb area this was in accordance with the findings. Evans and Amstrong (2006) suggested that supplementation of colonies with naturally occurring bacteria or their antagonistic products can provide a protection against foulbrood disease and thus the probiotic treated colonies have significant effect on the diseased larval cells. There was a decreased incidence of disease in the probiotic fed colonies (treated) than the probiotic non fed colonies (control) in experimental hives (Fig. 3). In accordance Pătruică *et al.* (2011) the experimental groups which were fed with sugar syrup incorporated with lactic or acetic acid showed statistical differences in terms of the number of brood cells and showed 10.67 to 20.34 per cent more brood growth than the control group. Alberoni *et al.* (2016) also reported that the honey bee gut microbiota enhanced the nutritional status of honey bees. The significant bee brood development in the probiotic fed colonies clearly indicated that the probiotic not only enhanced the immune status of the honey bees but also contributed indirectly to the nutritional and energetic status of honey bees. Alberoni *et al.* (2016) also suggested that other than enhancing the nutritional status of honey bees, they have direct stimulation of the bee's immune system and other host protection strategies like antimicrobial, biofilm formation, biosynthesis of cell wall exopolysaccharides and genes encoding.

This can be well documented by earlier findings that LAB had microbial mechanisms that mediate the protection of gut epithelium; production of antimicrobial compounds to inhibit pathogens; stimulation of the honey bee immune system mediated by the microbial symbionts against the pathogens; modulation of host intestinal pH by the microbial symbionts; microbial prebiotic effect on the insect gut consortium; competitive exclusion of microbial symbionts counteracting the pathogens and they can be exploited in managing honey bee stresses, especially in nutrition, parasites and diseases [11].

Table 1: Effect of probiotic feeding (treated) and sugar syrup feeding (untreated) on the brood development and mite population in *A. cerana* colonies.

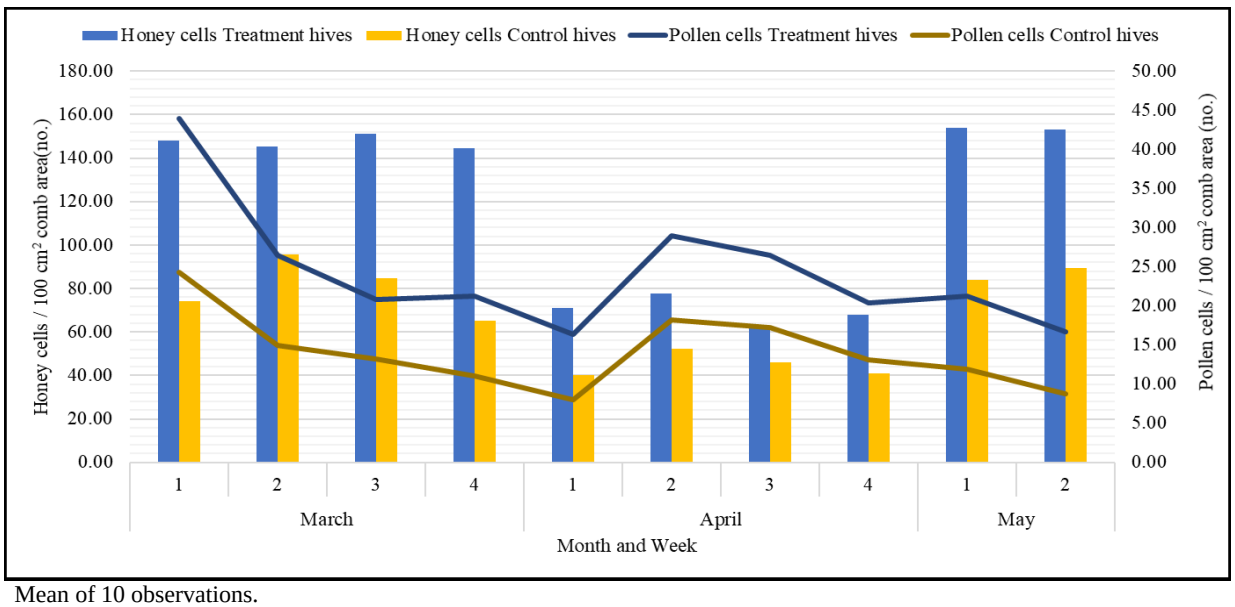
Categories		Sugar syrup + dusting of powdered sugar + screened bottom board (Untreated)	Probiotic supplement + powdered sugar spreading+ screened bottom board (Treated)	*t* - value
Brood examination / 100 cm^2 (no.)	Eggs	58.54±3.63 (7.68)	97.00±3.62 (9.87)	7.32 (P=1.19E-6)**
	Larval cells	80.33±6.70 (8.99)	133.16±10.62 (11.56)	4.35 (P=0.0004)**
	Capped cells	165.09±11.92 (12.87)	265.04±12.80 (16.30)	5.67 (P=2.24E-5)**
	Honey cells	67.24±6.70 (8.23)	117.54±13.08 (10.86)	3.37 (P=0.004)**
	Pollen cells	14.07±1.54 (3.82)	24.28±2.54 (4.98)	3.76 (P=0.002)**
	EFB affected cells	0.45±0.10 (0.97)	0.07±0.03 (0.75)	3.78 (P=0.003)**
	TSB affected cells	0.59±0.18 (1.04)	0.05±0.02 (0.79)	3.10 (P=0.011)**
Debris examination (no.)	Fallen mites/1250 cm^2	2.00±0.76 (1.58)	0.16±0.03 (0.81)	2.67 (P=0.025)**
Mite population (no.)	Phoretic mites/50 workers	1.38±0.53 (0.91)	0.13±0.04 (1.11)	2.36 (P=0.042)**

Figures in parentheses are $\sqrt{x+0.5}$ transformed values
Mean ± SE of 10 observations
**significant 0.05% level



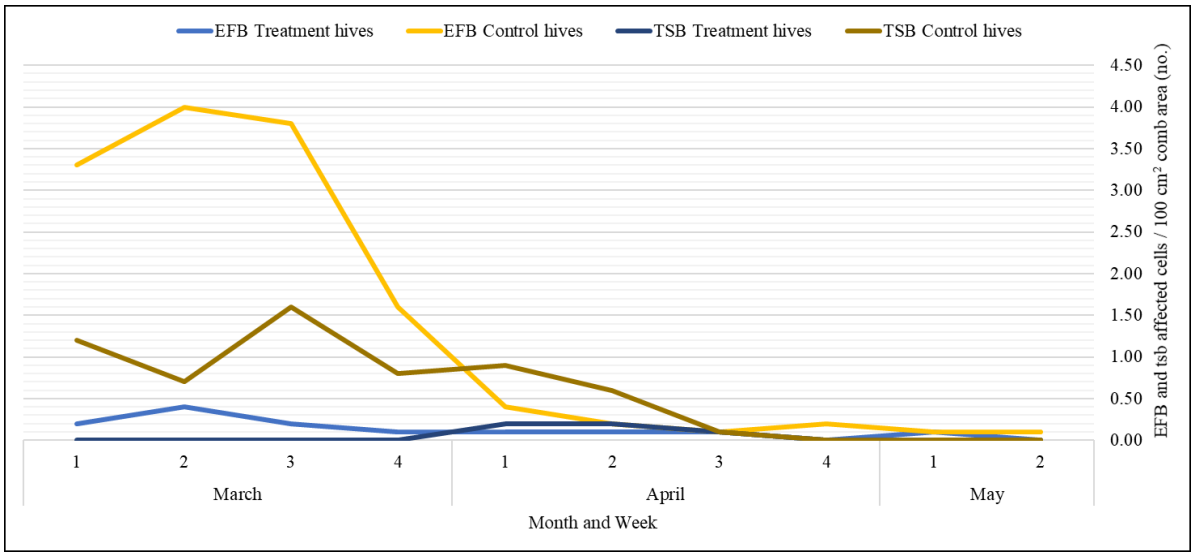
Mean of 10 observations.

Fig 1: Effect of probiotic feeding and powdered sugar spreading on the number of eggs, larva and pupa of *A. cerana* colonies



Mean of 10 observations.

Fig 2: Effect of probiotic feeding and powdered sugar spreading on the pollen and honey cells of *A. cerana* colonies



Mean of 10 observations.

Fig 3: Effect of probiotic feeding and powdered sugar spreading on the European Foulbrood (EFB) and Thai Sacbrood (TSB) affected larval cells of *A. cerana* colonies

Fig a: Feeding of sugar + probiotic supplement for treatment colonies

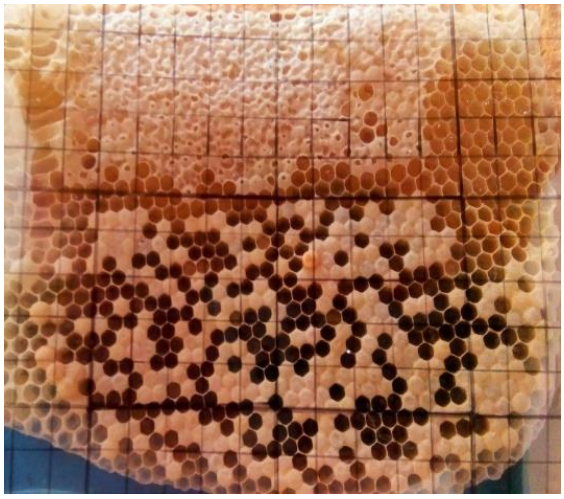


Fig b: Enumeration of brood strength using transparent grid

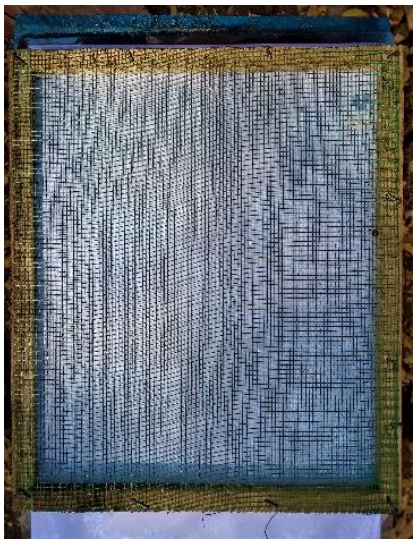


Fig c: Bottom board set up for mite assessment in bee hives

Fig d: Worker honey bees collected for *Varroa* mite assessment

Conclusion

The probiotic supplementation and sugar powder spreading enhanced the vital activities of Indian honey bees (*A. cerana*) such as significant difference in brood development and consequently led to increase the pollen storage, honey storage and resulted in increased harvestable honey production. They also play a major role in disease management by modulating the gut microbiota, altering the pH, modulating the immune system and production of non-specific and specific metabolites like lactic acid, bacteriocins, *etc*. this may decrease the usage of antibiotics used for the management of honey bee diseases and make way for residue free, cost effective and sustained production of honey.

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