Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 190-200

Received: 24-09-2017
Accepted: 27-10-2017

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A survey in Tamil Nadu of *Varroa jacobsoni* (Oudemans) ectoparasitic on Indian honey bees, *Apis cerana* F

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Abstract
Worldwide honey bee colonies are on the decline due to the colony collapse disorder (CCD). The ectoparasitic mite, *Varroa jacobsoni* (Oudemans) (Mesostigmata: Varroidae) and the diseases it transmits are considered one of the causal factors. We conducted a month-long roving survey for the mite syndrome in Indian honey bees, *Apis cerana indica* Fabricius (Hymenoptera: Apidae) in 12 districts of Tamil Nadu, South India in 2016, in addition to a year-long study in Coimbatore. The results indicated that colonies in all the surveyed districts had *V. jacobsoni* infestation and associated diseases (varroosis). Among the detection methods, examination of the debris on bottom board and of the pupae in brood comb cells was found superior to other methods. The need for monitoring and managing *V. jacobsoni* in Indian bee colonies is discussed.

Keywords: *Varroa jacobsoni*, *Apis cerana indica*, varroosis, Tamil Nadu, South India

1. Introduction
Honey bees play a key role in pollination of crops, besides providing honey, beeswax, beepollen, royal jelly, propolis, etc. However, of late their populations have been declining all over the world due to the colony collapse disorder (CCD), a syndrome caused by different types of stress, working in combination or synergistically [23, 25, 7, 37, 22]. Among the stressors, pests and diseases, especially the ectoparasitic mite, *Varroa* spp. (Mesostigmata: Varroidae) have caused irreparable damage to the beekeeping industry with destruction of numerous bee hives each year [16, 4, 29]. They feed on the haemolymph of honey bees at different developmental stages such as broods of drones and workers (Plate 1) as well as adults [6, 8, 11, 20, 21, 44, 46, 28, 36]. They reproduce in the brood cells, especially that of drones [38, 1, 43], transmitting viral diseases, especially the Deformed Winged Virus (DWV) [5, 34, 33, 10, 50]. If untreated, high infestation levels may cause the colonies to collapse, characterized by sudden disappearance of bees from the hive, leaving behind the queen and a small worker bee population [7, 37, 22]. The *Varroa*-associated diseased pupae show uncapped or perforated cappings, from which the adults emerge with heavily deformed wings [47, 48] and they may act as an activator of inapparent viral infections [2, 5,]. The association between *Varroa* and honey bee viruses is termed as 'bee parasitic mite syndrome', also referred to as *Varroosis* [12, 33, 29, 30]. Chemical pesticides against the mites often lead to resistance in mites coupled with contamination of the hive products [33, 26]. This may affect pollination as well [13]. Beekeepers in Tamil Nadu have recently been struggling to keep the Indian bee, *Apis cerana indica* Fabricius (Hymenoptera: Apidae) colonies, citing various reasons, especially under performance and unknown diseases. Though Thai sac brood virus (TSBV) disease has been a recurring a problem [41], in the absence of any authenticated report in India on CCD related syndrome, *Varroa jabobsoni* (Oudemans) was identified as the cause on a campus-level research during 2013-14 at Agricultural College & Research Institute, Killikulam [29]. However, no information was available on the occurrence of the mite and its syndrome from elsewhere in India. Therefore this study was undertaken with the objective to assess the extent of *Varroa* infestation and associated diseases in Tamil Nadu, South India.

2. Materials and Methods
The one-spell survey was conducted during 2016 in 12 districts of Tamil Nadu (Figure 1),

namely, Dharmapuri, Salem, Krishnagiri, Karur, Erode, Coimbatore, Trichy, Madurai, Tirunelveli, Tuticorin, Kanyakumari and Namakkal. With 3.96 per cent geographical area of India, Tamil Nadu lies between 8^0 05'N-13^0 35'N latitude and 76^0 15'-80^0 20' E longitude. The temperature in the state ranges from 2^0C in hills to 45^0C in other areas. The average rainfall ranges from 925 mm to 1170 mm. A simple random sampling was done while selecting honey bee colonies for inspection. Three beekeeper-maintained colonies were selected at random in each district and examined for the occurrence of *V. jacobsoni* and associated diseases. Beekeepers were interviewed on different aspects of beekeeping with the aid of a check list of questions related to seasons, hives per apiary, hive type, pests, diseases and predators. Colony strength was assessed based on the presence of brood and food store.

Adopting different methods, *V. jacobsoni* population density was assessed from the debris on the bottom board, from the brood inside the comb cells and from the body of adult bees at the time of hive examination. In debris examination, the number of mites that had fallen on to the bottom board (dead or alive) in each colony was counted in a 100 cm^2 area using a transparent grid (Plate 2). In brood examination, worker and drone pupae, preferably the latter, were collected from an area of 50 cm^2 from the brood combs by decapping the brood cells with a knife (Plate 3). The cell walls were examined under illumination and magnification to assess the number of mites in the cell or on the brood. To examine the adult bees, ca. 50 worker bees from the brood combs were transferred to a transparent polythene bag and examined carefully to count the number of mites clinging on to the bees (Plate 4). The captured bees were then released to the hive after examination. In yet another adult bee examination method, ca. 50 adult bees were captured from the brood combs, transferred to a 400 ml screen-capped PET jar containing 20 g powdered table sugar, and shaken vigorously (Plate 5). After a few minutes, the sugar powder was poured into water in an autoclavable Petri dish to isolate and count the dislodged mites as the sugar gets dissolved in water (Plate 6). The sugar powder-laden bees in jars were released into the hives later. The mite infestation rate on adult bees and broods was calculated as follows [14]:

$$\text{Adult bees mite infestation rate / 100 adult bees} = \frac{\text{Number of mites}}{\text{Number of bees}} \times 100$$

$$\text{Brood mite infestation rate /100 cells} = \frac{\text{Number of infested cells}}{\text{Total number of opened cells}} \times 100$$

The transparent 1.0 cm^2 grid was used to count the number of diseased pupae inside uncapped cells or of cells with perforated cappings (Plate 7, 8). In one of the districts, Coimbatore, the population dynamics of *V. jacobsoni* was assessed at monthly interval all through the year. The data collected were summarized and analysed with Microsoft Excel.

4. Results

The results indicated the presence of *V. jacobsoni* in all the 12 districts of Tamil Nadu where the survey was undertaken (Figure 1), irrespective of the hive type (Marthandam, BSI, Janata, Newton), agro-climatic conditions and vegetation, with 78.5 per cent colonies infested (Table 1 & 2). Most infested colonies were weak (62.1 %) to moderate (34.5 %) in strength (Figure 2). Only 16.2 per cent colonies were strong and only 21.6 per cent of the colonies were mite-free. None of the infested colony was strong. Diversified pests and predators were noticed in and around the hive (Table 3). Wax moth larvae (*Galleria mellonella*) and ants of different species were common in all districts. Lizards were the second most common predator, followed by bee eaters. The overall mite population density was highest in Madurai (23.3 / colony), followed by Erode (6.8 / colony) and Coimbatore (5.0 / colony). They were fewer in Krishnagiri (0.3 / colony), followed by Salem and Namakkal (0.8 / colony) (Figure 3). On average, there were 5.0 mites per colony. Examination of the debris on the bottom board indicated the presence of dead mites in 11 districts, the largest number in Madurai (22.0 / colony / 100 cm^2 bottom board area), followed by Erode and Coimbatore (6.7 - 5.0 / colony / 100 cm^2 bottom board area) (Table 4). They were fewer in Namakkal, Salem and Tirunelveli (0.3 / colony / 100 cm^2 bottom board area) and nil in Krishnagiri. Brood examination revealed the mite infestation at all places (0.3-2.0 / colony / 50 cm^2 brood comb area) (Table 4). The adult bee examination from inside the polythene bag indicated the presence of mites only in Madurai and Coimbatore districts (0.3 / colony / ca. 50 adult bees), while the powdered sugar method exposed the mites in Erode, Tuticorin, Kanyakumari, Dharmapuri and Coimbatore (0.3 – 1.3 / colony/ ca. 50 adult bees) (Table 4). Comparatively, the debris examination method was found more effective in detecting *V. jacobsoni* infestation (3.6 mites / colony/ / 100 cm^2 bottom board area), followed by brood (pupae) examination (0.9 mites / 50 cm^2 brood comb area). The mite infestation rate on adult bees was highest in Erode and Coimbatore districts (1.3 and 0.6 / 100 adult bees), followed by Dharmapuri, Madurai, Tuticorin and Kanyakumari districts (0.3 / 100 adult bees) (Table 4). The mite infestation rate on brood was highest in Madurai (1.5 / 100 pupal cells), followed by Kanyakumari (1.0 / 100 pupal cells). The infestation rate was lowest in Karur and Krishnagiri districts (0.2 / 100 pupal cells) (Table 4). Month-wise *V. jacobsoni* population assessment in Coimbatore indicated its occurrence all around the year (Figure 4), with peak population density during the swarming season in January - February (2.0 - 2.4 / colony) and lowest during the rainy season in October - December (0.3 - 0.5 / colony).

Varroa-associated disease symptoms (diseased pupae showing uncapped and perforated cappings) were also noticed in all the 12 districts. The disease intensity in terms of pupal cells with perforated cappings was highest in Madurai (3.8 / 50 cm^2 brood area), followed by Tirunelveli (3.8 / 50 cm^2 brood area) (Figure 5). Capped pupal cells showing perforations were at a moderate level in Coimbatore, Salem, Namakkal, Dharmapuri and Kanyakumari districts (2.4-2.8 / 50 cm^2 brood area). Only fewer cells were perforated in Krishnagiri district (1.3 / 50 cm^2 brood area). The perforated cells were fewer in Karur, Erode, Trichy and Tuticorin than in other districts but more than that in Krishnagiri (1.8- 2.0 / 50 cm^2 brood area). The diseased pupae in uncapped cells were most numerous in Salem and Namakkal districts (3.3 / 50 cm^2 brood area), followed by Kanyakumari, Tirunelveli Dharmapuri and Krishnagiri (2.2 - 2.5 / 50 cm^2 brood area) (Figure 6). Such pupae were fewer in Tuticorin district (0.8 / 50 cm^2 brood area) than in Trichy, Erode, Karur and Madurai (0.9 – 1.8 / 50 cm^2 brood area). The types of virus need to be identified and characterized in future.

Table 1: District-wise *A. cerana* colonies infested by *V. jabosoni*

Sampling date	District (village) surveyed	No. of colonies inspected / district	No. of infested colonies / district	Infestation %
19.09.2016	Dharmapuri (Theerthamazhai)	3.0	3.0	100.0
21.09.2016	Salem (Sandhiyur)	3.0	2.0	66.7
20.09.2016	Krishnagiri (Chaparam)	3.0	1.0	33.3
22.09.2016	Karur (Vellaikovil)	3.0	2.0	66.7
11.09.2016	Erode (Maanikampalayam)	3.0	3.0	100.0
23.09.2016	Coimbatore (TNAU)	3.0	3.0	100.0
07.09.2016	Trichy (Navalur Kuttapattu)	3.0	2.0	66.0
06.09.2016	Madurai (Agricultural college)	4.0	3.0	75.0
03.09.2026	Tirunelveli (Vasudevanallur)	3.0	2.0	66.0
04.09.2016	Tuticorin (Killikulam)	3.0	3.0	100.0
05.09.2016	Kanyakumari (Devicode)	3.0	3.0	100.0
18.09.2016	Namakkal (Senthamangalam)	3.0	2.0	66.7
Mean				78.5

Table 2: The geographical positions, climatic conditions and vegetation types in the surveyed districts

District (village) surveyed	Latitude	Longitude	Altitude (M)	Maximum Temperature/day (^{0}C)	Rainfall (mm / year)	Relative Humidity (%)	Vegetation type
Dharmapuri (Theerrthamazhai)	12°10'19"N	78°59'05"E	392.0	35.0	446.5	61.0	Acacia
Salem (Sandhiyur)	11°56'82"N	78°13'79"E	261.0	34.0	511.1	62.0	Coconut, Mango
Krishnagiri (Chaparam)	12°42'15"N	78°21'74"E	454.0	36.0	587.2	62.0	Mango
Karur (Vellaikovil)	8°60'85"N	78°05'46"E	231.0	32.0	455.9	74.0	Coconut, Moringa
Erode (Maanikampalayam)	11°14'10"N	77°05'86"E	240.0	33.0	307.7	82.0	Coconut, Moringa
Coimbatore (TNAU)	9°53'80"N	77°28'40"E	467.0	34.0	816.9	76.0	Coconut
Trichy (Navalur Kuttapattu)	10°65'74"N	78°74'64"E	147.0	36.0	675.9	62.0	Guava, Neem, Sapota, Mango
Madurai (Agri college)	9°92'94"N	78°11'58"E	121.0	35.0	650.1	76.0	Mango, Sapota, Guava, Neem, Antigonon, Coconut
Tirunelveli (Vasudevanallur)	9°23'96"N	77°41'14"E	182.0	37.0	385.7	57.0	Citrus, Coconut
Tuticorin (Killikulam)	8°70'34"N	77°85'96"E	40.0	36.0	227.8	72.0	Rubber, Neem, Guava, Mango, Tamarind, Antigonon, Coconut
Kanyakumari (Devicode)	8°21'41"N	77°21'94"E	37.0	29.0	902.2	87.0	Rubber
Namakkal (Senthamangalam)	11°74'44"N	79°38'10"E	107.0	33.0	381.2	64.0	Banana, Coconut, Arecanut

Table 3: Occurrence of other honey bee pests in the surveyed districts

District (village) surveyed	Other honey bee pests and predators noticed inside the hives								
	Wax moth larvae	Ants	Reduviid bugs	Dermestid beetles	Bee eaters	Yellow jacket wasps	Lizards	Pseudo scorpions	Hawk moths
Dharmapuri (Theerrthamazhai)	+	+	+	+	+	+	+	+	-
Salem (Sandhiyur)	+	+	+	+	+	+	+	-	-
Krishnagiri (Chaparam)	+	+	-	-	+	-	-	+	-
Karur	+	+	+	-	-	-	+	-	-

(Vellaikovil)									
Erode (Maanikampalayam)	+	+	-	+	-	+	+	+	-
Coimbatore (TNAU)	+	+	+	+	+	+	+	+	-
Trichy (Navalur Kuttapattu)	+	+	+	+	+	+	+	+	+
Madurai (Agri college)	+	+	+	+	-	+	+	+	+
Tirunelveli (Vasudevanallur)	+	+	-	+	+	-	+	+	-
Tuticorin (Killikulam)	+	+	+	+	+	+	+	+	-
Kanyakumari (Devicode)	+	+	+	+	+	+	+	+	-
Namakkal (Senthamangalm)	+	+	-	-	+	+	-	-	+
Percentage (%)	100.0	100.0	66.7	75.0	75.0	66.7	83.3	75.0	25.0

Table 4: District-wise mite population density and infestation rate.

District (village) surveyed	Method-wise mite population density / colony				Mite infestation	
	No. / 100 cm^2 bottom board area	No. / ca. 50 adult bees in polythene cover	No. / ca. 50 adult bees in sugar powder dissolved water	No. in pupal cells / 50 cm^2 brood comb area	Rate / 100 pupal cells	Rate / 100 adult bees
Dharmapuri (Theerrthamazhai)	0.3	0.0	0.3	0.7	0.5	0.3
Salem (Sandhiyur)	0.3	0.0	0.0	0.7	0.5	0.0
Krishnagiri (Chaparam)	0.0	0.0	0.0	0.3	0.2	0.0
Karur (Vellaikovil)	1.3	0.0	0.0	0.3	0.2	0.0
Erode (Maanikampalayam)	6.7	0.0	1.3	1.0	0.8	1.3
Coimbatore (TNAU)	5.0	0.3	0.3	1.0	0.8	0.6
Trichy (Navalur Kuttapattu)	2.0	0.0	0.0	0.7	0.5	0.0
Madurai (Agri college)	22.0	0.3	0.0	2.0	1.5	0.3
Tirunelveli (Vasudevanallur)	0.3	0.0	0.0	1.0	0.8	0.0
Tuticorin (Killikulam)	1.7	0.0	0.3	0.7	0.5	0.3
Kanyakumari (Devicode)	2.7	0.0	0.3	1.3	1.0	0.3
Namakkal (Senthamangalam)	0.3	0.0	0.0	0.7	0.5	0.0
Mean	3.6	0.05	0.2	0.9	0.7	0.3

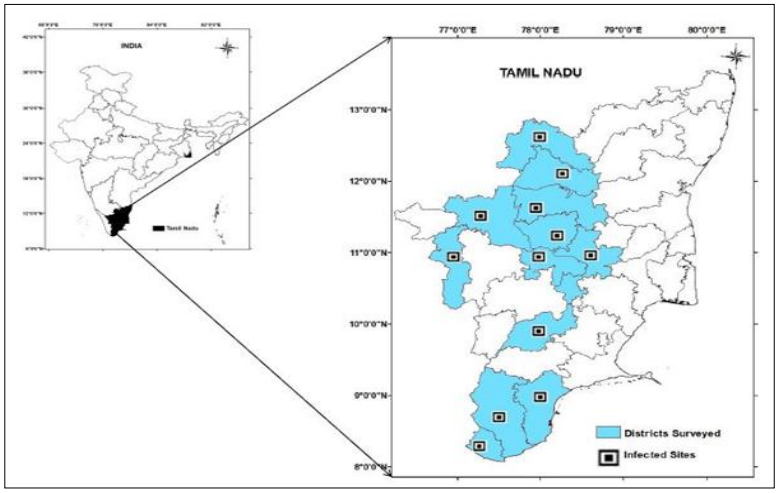

Fig 1: Map showing the districts surveyed for *V. jacobsoni* in Tamil Nadu. The mite infestation and associated diseases were found in all the surveyed districts.

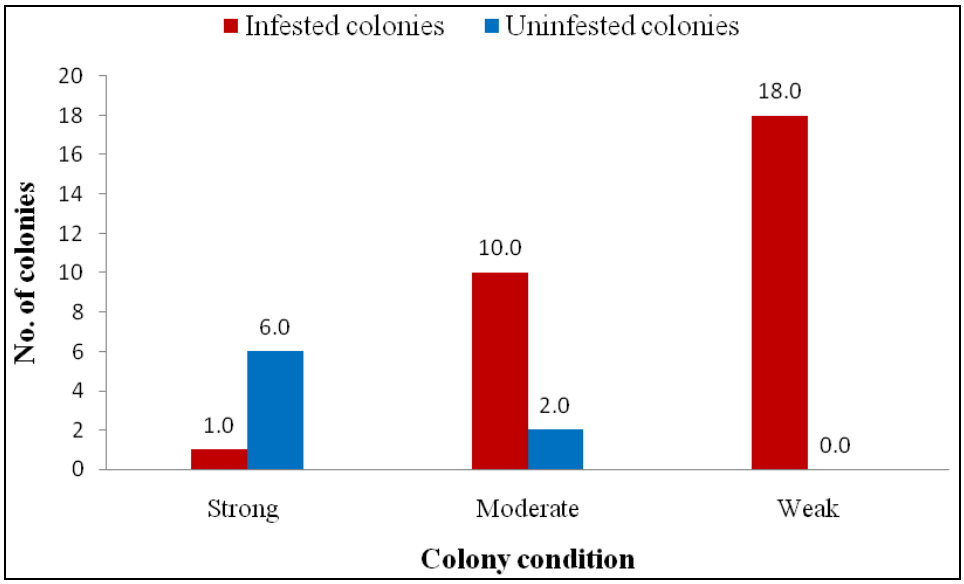

Fig 2: Status of *A. cerana* colonies in the surveyed sites.

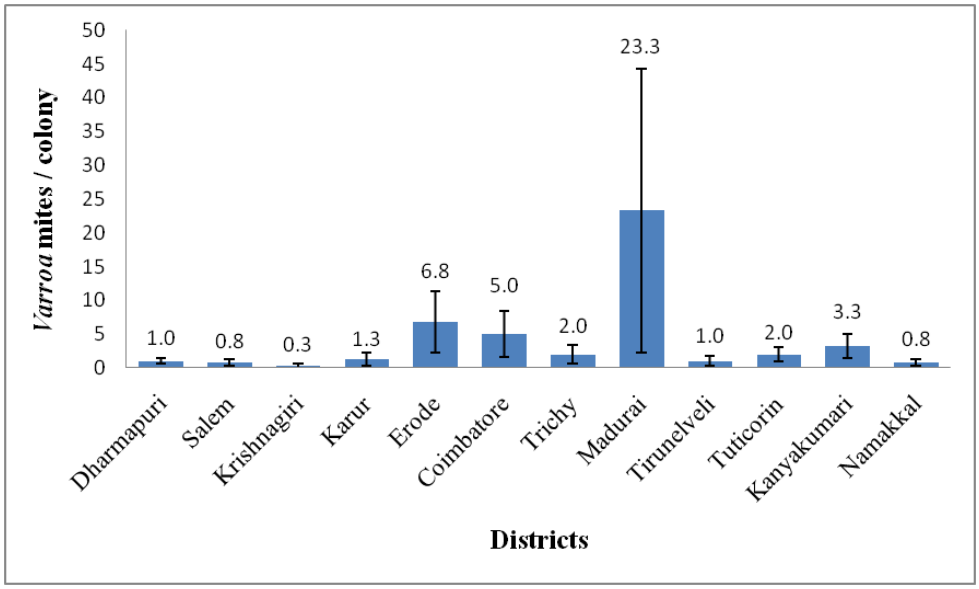

Fig 3: Population density of *V. jacobsoni* in the surveyed districts. Mean number of mites recovered from the bottom board, brood / brood cells and adults per colony. Vertical bars indicate the SE.

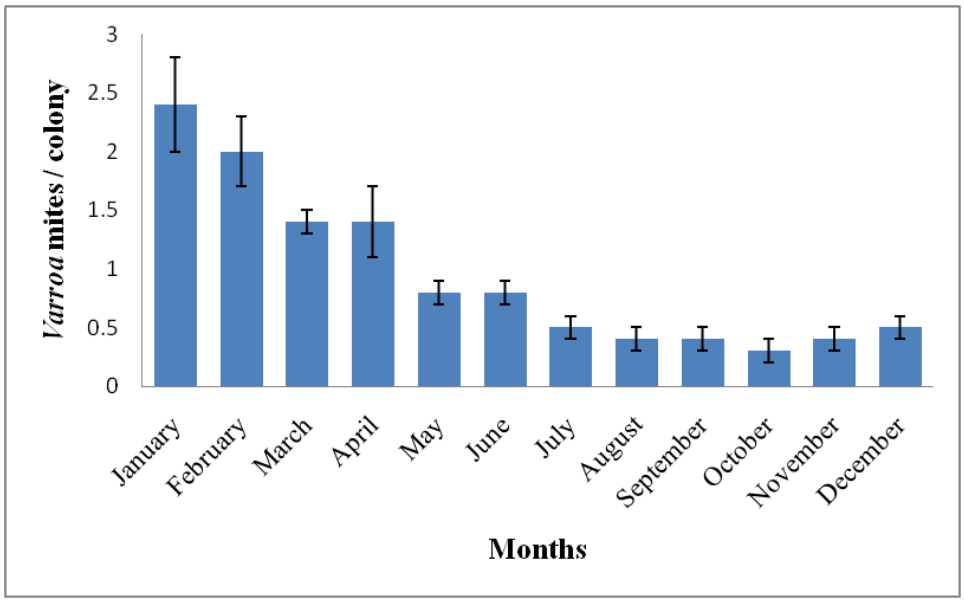

Fig 4: Population dynamics of *V. jacobsoni* in Coimbatore district in 2016. Mean number of mites recovered from the bottom board per colony. Vertical bars indicate the SE.

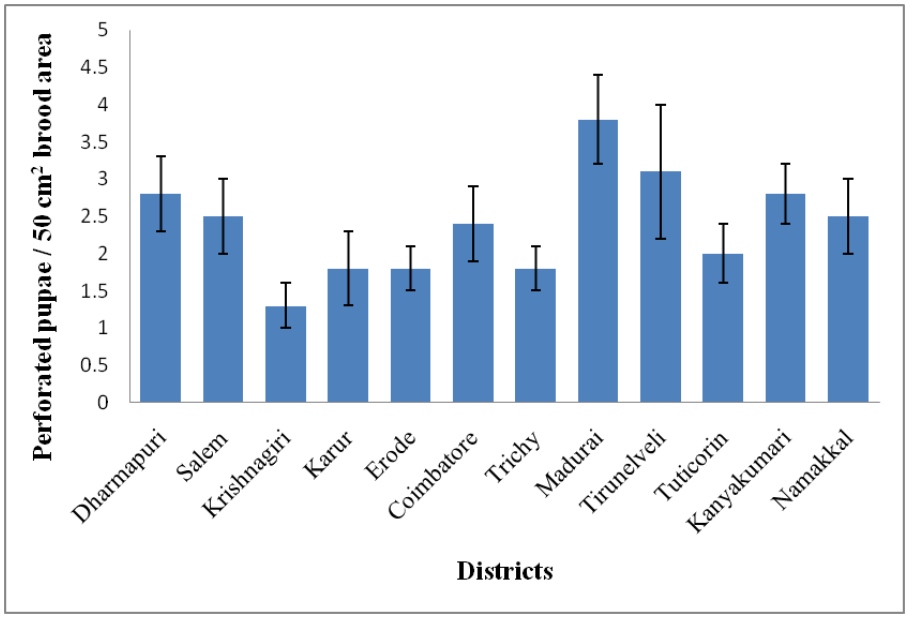

Fig 5: Number of diseased pupae showing perforated cappings in the brood comb of *A. cerana* colonies. Mean of 6 observations. Vertical bars indicate the SE.

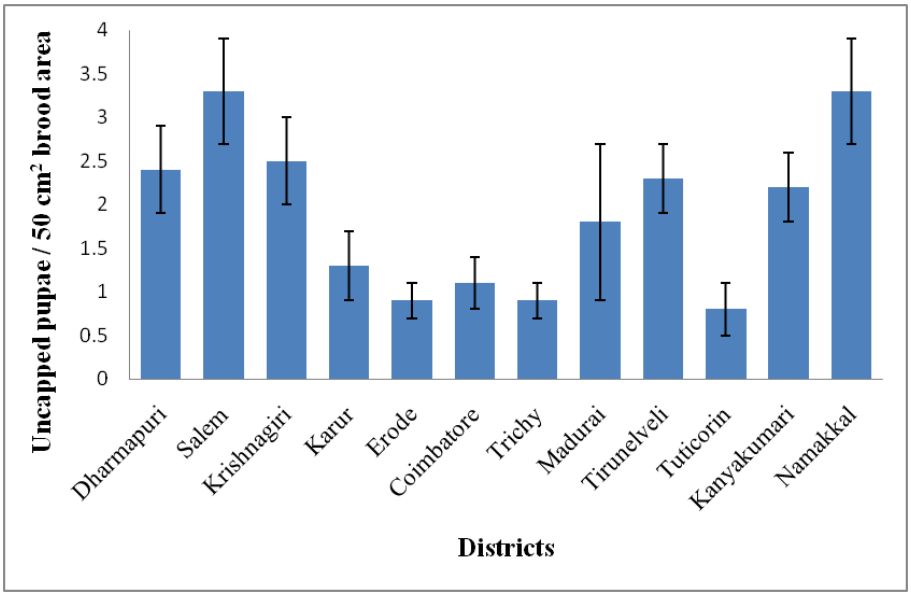

Fig 6: Number of diseased pupae in the uncapped cells in brood combs of *A. cerana* colonies. Mean of 6 observations. Vertical bars indicate the SE.

Plate 1: An adult *V. jacobsoni*.

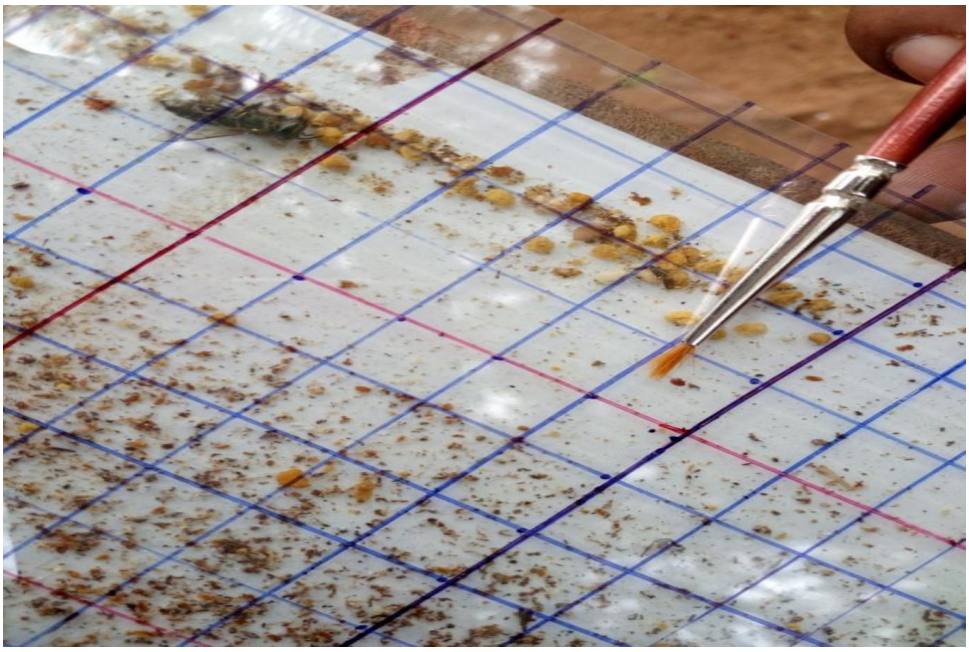

Plate 2: The transparent 1.0 cm grid sheet used to count the mites on the bottom board.

Plate 3: A *Varroa*-infested pupa.

Plate 4: ca. 50 *A. cerana* bees captured inside a polythene bag to record the mite numbers.

Plate 5: Ca. 50 *A. cerana* bees captured inside a screen-capped PET jar containing powdered table sugar and shaken vigourously to dislodge the mites.

Plate 6: Isolating the dislodged mites after the powdered sugar has dissolved in water.

Plate 7: Uncapped brood cells / cells with perforated cappings in an *A. cerana* colony.

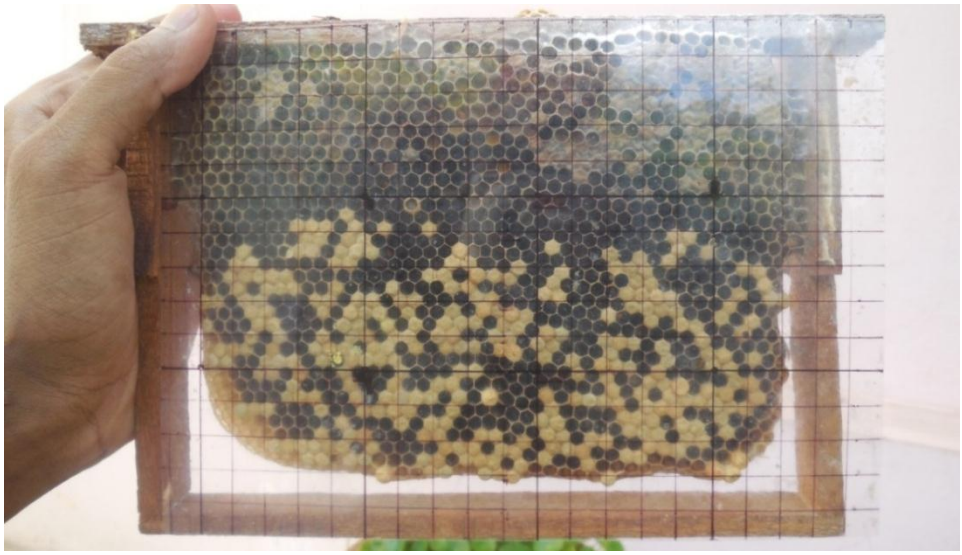

Plate 8: The transparent grid (1.0 cm^2) sheet used in sampling.

Plate 9: A *Varroa*-infested pupa showing deformities wings and legs.

5. Discussion

All over the world honey bees undergo different types of stress from both abiotic and biotic factors. Climate change, habitat loss and pesticides, especially neonicotinoids, are among the major abiotic factors [22, 24, 31]. Parasites and pathogens, especially *Varroa, Nosema* and viral diseases, are considered as major biotic stressors [22]. Scarcity of flora during the dearth period and the supplementary feeding with sugar syrup may also put the bees under nutritional stress [35]. Among the *Varroa* species, *Varroa destructor* Anderson and Trueman infests the Italian bees, *Apis mellifera*, whereas *V. jacobsoni* is common on Indian bees, *Apis cerana* [1, 34]. It was reported for the first time in 2015 from South India [29, 30]. Although this roving survey was conducted during the non-swarming season, it has confirmed that *V. jacobsoni* is widespread in all districts of Tamil Nadu but not in all colonies, most of them being weak, probably due to varroosis which the beekeepers were not aware of. However, though observed all round the year, their population density was highest in January - February, the swarming season when more drones are produced. Usually, *Varroa* spp. prefer the drone pupae for their reproduction [38, 1, 43]. *V. jacobsoni* also prefers the drone brood to worker brood [19], a key factor limiting the mite population growth [18]. Among the assessment techniques, examining the debris (wax fragments, pollen grains, broken body parts, etc.) on the bottom board of the hive was found easier with better results than other methods, probably because of the grooming behaviour of honey bees which dislodges mites [27], suppressing the mite numbers within tolerable limits. Grooming is of two types. In self or *auto-grooming*, adult bees detect and remove the mites from themselves by performing a grooming dance which increases especially at night [39]. In *allogrooming*, they may try to remove the mites from nest mates. Between the species, *A. cerana* bees groom more efficiently than *A. mellifera* [17]. The hygienic colonies uncap and remove the developing mite offspring by interrupting the reproductive cycle [34].

Most colonies in Tamil Nadu were weak with fewer bees, combs and food reserve not only due to the mite infestation but also due to the presence of diseases associated with *V. jacohsoni*, probably DWV (Plate 9). Its impact on colony strength may vary with climatic conditions and bee's races [32], which needs to be studied further. Often *Varroa*-associated disease symptoms are similar to that of American Foul Brood (AFB) but without any typical odour [1], including spotty brood pattern with punctured cappings, uncapped

pupae, disfigured and stunted adults with deformed legs and wings crawling on the comb / ground. Peak populations of *V. jacobsoni* coupled with disease intensity as punctured cappings were characteristic in Madurai district than in other places. Bees may discard the infested pupae and the queen may be superseded. The virgin queen may fail. Adult bees and pupae may have mites on their body as observed in this survey. The mites may shorten honey bee worker life span and cause precocious foraging [51]. All these might have weakened the colonies in the absence of proper management. At present, miticides and antibiotics are extensively used in apiculture to manage the mite syndrome, not only resulting in poor performance against and resistance in mites and pathogens [45, 3] but also ending in honey as residues [26]. Antibiotics also kill the beneficial honey bee microflora that is part of the natural honey bee defence against pathogens, including probiotics [52]. Having suffered huge colony losses due to CCD elsewhere, stress management strategies need to focus on non-chemical and non-antibiotic measures such as probiotics [33]. Providing a properly designed screened bottom board may prevent the dislodged mites from climbing up again by clinging to the incoming bees [42]. As a non-chemical measure, dusting the bees with powdered table sugar helps them dislodge the mites by enhanced grooming [15, 9]. As a reservoir of probiotic lactic acid bacteria (LAB), powdered sugar may also suppress the viral diseases in bees by augmenting LAB populations [29, 30], which produce antimicrobials [40, 49, 22].

Conclusion

This survey highlights the occurrence of *V. jacobsoni* and the disease infection that follows the mite infestation in *A. cerana* colonies in Tamil Nadu. Though not all colonies are affected, the syndrome is evident in all the districts where sampling was done. This may be true of other districts in Tamil Nadu and other States in India which needs to be investigated in future. Among the sampling and detection methods, examination of the debris that fall on to the bottom board is easier and more effective in detecting the mites than other methods. However, examining the pupae in brood comb cells helps determine the presence and intensity of the vector-transmitted diseases. The types of virus transmitted by the mite are to be characterized using latest technologies, especially molecular, for a holistic approach to the mite and disease management. Periodical monitoring and ecologically sound management strategies are required to manage varroosis in Indian bee colonies.

Acknowledgment

We are grateful to the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore for permission to inspect the bee colonies. This work was financially supported by the University Grants Commission, Rajiv Gandhi National Fellowship, New Delhi.

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