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Infestation of ectoparasitic mite *Varroa jacobsoni* Oudemans on pupal brood of *Apis cerana* F. in selected locations of Southern Karnataka

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Abstract
Among the mites associated with honey bees, ectoparasitic mite *Varroa jacobsoni* Oudemans is one of the important pest of bees, its infestation levels in *Apis cerana* colonies was recorded during September 2019 to March 2020 at three different locations of Southern Karnataka. The study revealed that, despite of different ecological conditions, infestation of *V. jacobsoni* on *Apis cerana* pupal brood was found at all the three locations with varying levels. Although, their peak infestation was observed during January 2020 at all the three locations. However, in the present study, *V. jacobsoni* infestation was seems to be at low level and not causing any detrimental effect to *A. cerana* colonies. This study also revealed the higher preference of *V. jacobsoni* towards drone pupal brood for their reproduction as the number of infected drone pupal brood was significantly higher than the number of infected worker pupal brood at all the three locations.

Keywords: Mites, *Varroa jacobsoni*, honey bees, *Apis cerana*, Karnataka

Introduction
Honey bees play a major role in pollination along with providing other commercial products like honey, pollen, beeswax, royal jelly, propolis and bee venom etc. However, like other plants and animals, they are also liable for different types of both abiotic and biotic stressors. Among the biotic stressors, mites which parasitizes bees are one of the major problem along with other pests and diseases. *Varroa jacobsoni* (Mesostigata: Varroidae) is one among those parasitic mites which is causing major problem in beekeeping industry all over the world [11, 9, 5, 20, 32, 21]. Oudemans (1904) was first to report the mite, *Varroa jacobsoni* (Varroidae: Mesostigmata) from Asian honey bee, *Apis cerana* F. from Java. *V. jacobsoni* is an ectoparasite of brood and adult honey bees. They feed on haemolymph of brood and adult bees. It has dorso-ventrally flattened, reddish brown colored body (Plate 2) and it can be easily seen by naked eyes [3, 28]. Their reproduction take place inside the capped brood. The females get copulated within capped brood. When the adult bee emerges, female mites also comes out along with bee and continue their life cycle. The feeding period is short and development usually takes only a week [18, 31, 8]. Varroa mites also transmits the viral diseases, especially the deformed winged virus (DWV) [8, 25]. The adult emerged from mite infested pupa will be with deformed appendages. If unchecked, the infestation of Varroa mites will lead to weakening and absconding of bee colonies. Bee keeping with *A. cerana* colonies are common in Southern part of Karnataka as its caring and management practices are easy [14]. Hence, the present study was undertaken with an objective to discover the extent of *Varroa jacobsoni* infestation in *A. cerana* colonies in selected locations of Southern Karnataka.

Materials and Methods
Study area
The study was conducted on *Apis cerana* Fabricius colonies of three different locations of Southern Karnataka namely, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK) campus, Bengaluru; University of Agricultural and Horticultural Sciences (UAHS), Navile campus, Shivamogga and College of Forestry (COF), Ponnampet campus, Kodagu.

Sampling method

To assess the infestation of *V. jacobsoni* in *A. cerana* colonies the below mentioned sampling method was conducted on ten randomly selected *A. cerana* colonies at each of the three apiary locations once in a month for a period of total seven months starting from September 2019 to March 2020.

Examination of pupal brood

At each of the three apiary locations, 10 colonies of *A. cerana* were randomly selected. From each colony, 40 pupal brood cells (20 Worker pupal brood and 20 Drone pupal brood) were individually de-capped with the help of forceps and pupa were examined for mite infestation. Number of pupal brood cells infested per each colony were recorded and then pupal brood which were infested with mites were collected in vials containing 70 percent ethyl alcohol, it was then labeled with hive number and were brought to the laboratory for counting number of mites per colony (Plate 1).

Its potential in terms of percent infestation was calculated by using the below mentioned formula:

$$\text{Percent infestation} = \frac{\text{Number of pupal brood cells infested}}{\text{Total no. of pupal brood cells examined}} \text{ X } 100$$

Varroa jacobsoni infestation on worker and drone brood was recorded separately. Preference of host selection ratio by mite between worker (W) and drone (D) pupal brood was calculated by using following formula.

$$\text{W: D ratio} = \frac{\text{Number of worker pupal brood infested}}{\text{Number of drone pupal brood infested}}$$

Plate 1: Sampling method followed for pupal brood examination

Data analysis

The data was subjected to suitable statistical analysis. The infestation of *V. jacobsoni* on worker and drone pupal brood during different months in different apiary locations were analysed by Students 't'- test. The numerical data on per cent infestation of mite were transformed arcsine transformation and suitable statistical analysis was performed to evaluate the results.

Results and Discussions

Like other plants and animals, honey bees are also liable to different types pests and diseases. Among the pests, mites which parasites the bees are one of the major threat. *Varraoa jacobsoni* is one such parasitic mite which is an important pest of honey bees. Original host of *V. jacobsoni* is *A. cerana*. In the later days, co-existence of *A. cerana* and *A. mellifera* triggered the switching of Varroa mite from its original host, *A. cerana* to *A. mellifera*. Later *A. mellifera* proved to be its highly susceptible host [12]. The first observations of a mite spill over from *A. cerana* to *A. mellifera* were made in Japan in 1957 [29]. The results of the present study which was conducted on *A. cerana* colonies at three different locations of southern Karnataka are discussed below.

Percent infestation of *V. jacobsoni* on *A. cerana* pupal brood

The present study revealed that, percent infestation of *V. jacobsoni* during September 2019 to March 2020 are on par with each other at both UAHS, Navile, Shivamogga and COF, Ponnampet, Kodagu. However, at UAS, GKVK, Bengaluru, except for September 2019 (0.0 percent) infestation during remaining months were on par with each other (Table 1; Fig. 1). This may be attributed to continuous availability of pupal brood throughout the year and hygienic behavior of *A. cerana* bees which regulates the mite population level. Even though there is no significant difference in the infestation level during the period of seven months, their peak infestation was observed during January 2020 at both UAHS, Navile, Shivamogga (14.00 percent) and COF, Ponnampet, Kodagu (10.00 percent), whereas, during November 2019 and January 2020 at UAS, GKVK, Bengaluru, (4.75 percent) (Table 1; Fig. 1). Hence,

Table 1: Per cent infestation of *Varroa jacobsoni* on *Apis cerana* pupal brood during September 2019 to March 2020 at three different apiary locations

Locations / Month/Year	UAS, GKVK, Bengaluru	UAHS, Navile, Shivamogga	COF, Ponnampet, Kodagu
September 2019	0.00 (0.00 ± 0.00) [b]	3.50 (9.16 ± 2.51)	2.75 (7.77 ± 3.82)
October 2019	3.00 (8.87 ± 2.30) [a]	8.50 (11.43 ± 6.75)	4.25 (9.67 ± 3.55)
November 2019	4.75 (10.86 ± 3.24) [a]	8.75 (15.78 ± 3.63)	3.25 (8.30 ± 3.19)
December 2019	3.25 (9.05 ± 2.58) [a]	7.75 (13.50 ± 4.63)	5.25 (12.72 ± 1.90)
January 2020	4.75 (12.10 ± 1.92) [a]	14.00 (20.09 ± 4.95)	10.00 (16.51 ± 4.20)
February 2020	3.50 (9.31 ± 2.76) [a]	7.75 (14.11 ± 3.74)	8.25 (15.81 ± 2.80)
March 2020	3.25 (7.43 ± 3.70) [a]	7.25 (14.78 ± 2.62)	8.50 (15.16 ± 3.87)
F- test	*	NS	NS
SE (m)	2.46		
SE (d)	3.48		
CD (p =0.05)	7.22		
CV	66.89 %		

Note: Pupal brood cells observed in each month: 400 (n = 400)

Figures in parentheses (mean ± SE) are arcsin transformed values; NS – Non significant

Table 2: Comparative per cent infestation of *Varroa jacobsoni* on *Apis cerana* pupal brood during September 2019 to March 2020 between three different apiary locations

Locations / Month/Year	UAS, GKVK, Bengaluru	UAHS, Navile, Shivamogga	COF, Ponnampet, Kodagu
September 2019	0.00 (0.00 ± 0.00)	3.50 (9.16 ± 2.51)	2.75 (7.77 ± 3.82)
October 2019	3.00 (8.87 ± 2.30)	8.50 (11.43 ± 6.75)	4.25 (9.67 ± 3.55)
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January 2020	4.75 (12.10 ± 1.92)	14.00 (20.09 ± 4.95)	10.00 (16.51 ± 4.20)
February 2020	3.50 (9.31 ± 2.76)	7.75 (14.11 ± 3.74)	8.25 (15.81 ± 2.80)
March 2020	3.25 (7.43 ± 3.70)	7.25 (14.78 ± 2.62)	8.50 (15.16 ± 3.87)
Mean ± SE	3.21 (8.22 ± 1.48) [b]	8.18 (14.12 ± 1.31) [a]	6.03 (12.29 ± 1.40) [a]
F-test	*		
SE (m)	1.33		
SE (d)	1.87		
CD (p = 0.05)	3.74		
CV %	67.96 %		

Note: Figures in parentheses (mean ± SE) are arcsin transformed values; * Significant at 5%

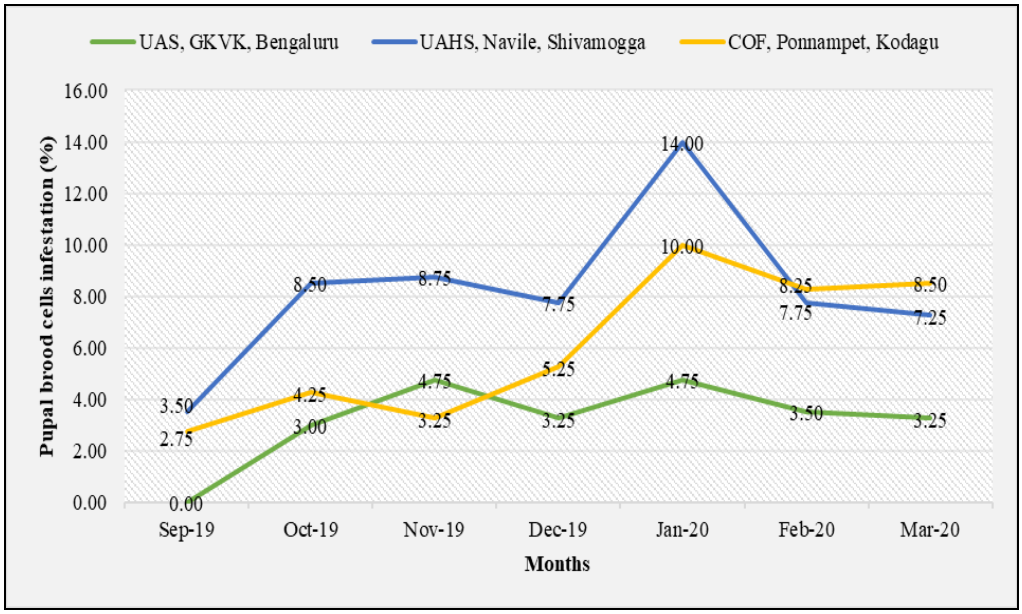

Fig 1: Per cent infestation of *Varroa jacobsoni* on *Apis cerana* pupal brood during September 2019 to March 2020 at three different locations

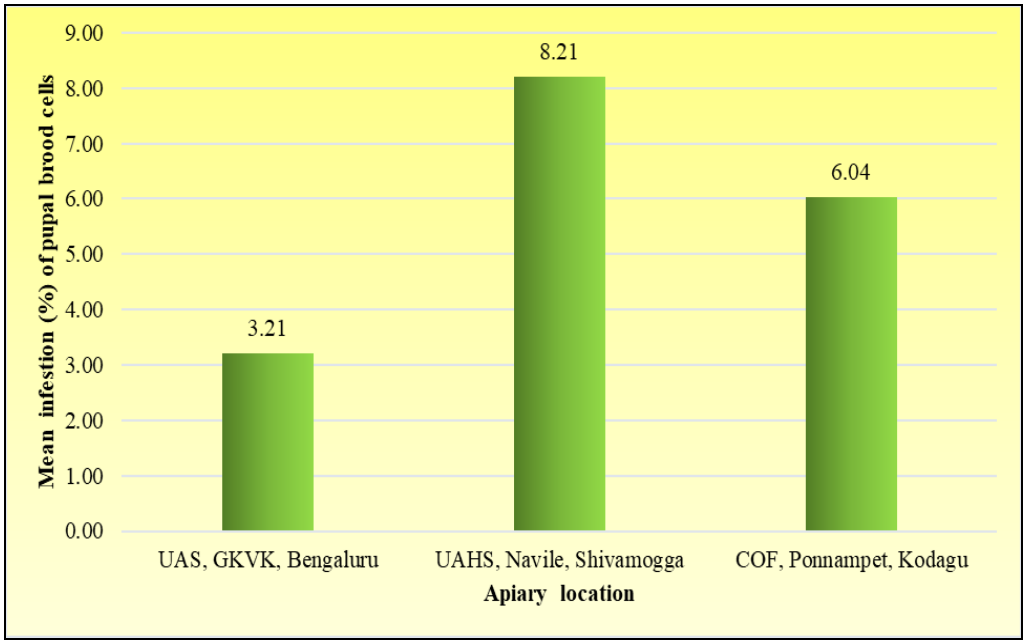

Fig 2: Mean infestation (%) of *Varroa jacobsoni* on *Apis cerana* pupal brood during September 2019 to March 2020 at three different locations

January month was found common at all the three locations as the peak infestation period because it coincides with the swarming seasons when more brood were reared, therefore higher infestation can be expected during that period. Similarly, a study from Coimbatore, Tamil Nadu also reported the occurrence of *V. jacobsoni* in *Apis cerana* colonies all around the year, with peak population density during January-February [21]. However, in case of *A. mellifera*, invasion of *V. jacobsoni* was low in spring and it increased considerably during summer [30] and also, the pest potential of another ectoparasitic mite, *Tropilaelaps clareae* on *A. mellifera* colonies in Hisar was reported maximum during April- May (7.5 percent) [15].

In the present study, infestation of *V. jacobsoni* was observed at all the three locations. However, not all the colonies were infested with *V. jacobsoni* at a single time at single locations. Out of ten randomly selected *A. cerana* colonies in a single location, most of the time only five to six colonies were infested with *V. jacobsoni*. Anyhow, the infected colonies are also working normal because the infestation percent was seemed to be lower as compared with other studies. For example, infestation of *Varroa* was recorded throughout the year with maximum abundance during the month of March, 40 percent and 31.84 percent during 2006-07 and 2007-08 respectively on *A. mellifera* [16]. Moreover, *A. mellifera* are more susceptible to these mites than *A. cerana* [27]. Varroa infested capped brood cells of *A. cerana* are not opened by the worker bees, when a pupa dies thus the mites also die. This presumably keeps the Varroa infestation of *A. cerana* at a lower level [16]. Also, the parasitism by *V. jacobsoni* induce workers of *A. cerana* to perform a series of cleaning behavior that effectively remove *V. jacobsoni* from the bodies of the adult bees as well as from brood. *A. mellifera* showed cleaning and hygienic behavior at low frequency and generally failed to remove *V. jacobsoni* from both the adult bees and the brood [4, 27].

Comparison of per cent infestation of *V. jacobsoni* on *A. cerana* pupal brood at three different apiary locations

Comparison between per cent infestation of *V. jacobsoni* on *A. cerana* pupal brood during September 2019 to March 2020 at three different apiary locations are made (Table 2). The results revealed that there is a significant difference in the per cent infestation of *V. jacobsoni* between apiary locations. Highest mean per cent infestation was observed at UAHS, Navile, Shivamogga (8.18 percent) followed by COF, Ponnampet, Kodagu (6.03 per cent) and UAS, GKVK, Bengaluru (3.21 per cent) (Fig. 2). Per cent infestation was relatively higher at UAHS, Navile, Shivamogga apiary throughout the observation period (3.50 to 14.00 per cent) (Fig. 1).

The results revealed the infestation of *V. jacobsoni* on *A. cerana* pupal broods is in varying levels across three different locations. Similar to the present study, a study from Tamil Nadu showed that, overall mite population density was highest in Madurai, followed by Erode out of total 12 districts surveyed for *V. jacobsoni* infestation [21]. Similarly, in Brazil, *V. jacobsoni* infestation rates on adult honey bees were measured at three different climatic regions for about a two-years period. The mean infestation rates were seen highest at Sao Joaquim, followed by Rio do Sul and Ribeirao Preto [22]. In Kenya also, *Varroa* mites were reported in all the eight study sites at varying levels on domesticated honeybee (*A. mellifera* L.) colonies [2]. In Tanzania also the presence of *Varroa* mites in honey bee colonies (*A. mellifera*) were reported from 23 out of 25 studied districts [23]. This shows that, ecological condition of an area is not a limiting factor for the distribution of *Varroa* mite. Hence, the present studies showed the infestation of *V. jacobsoni* at all the three different apiary locations with varying level. The probable reason for varying level of infestation between apiary location may be due to the ecological factors of locations and moreover, due to grooming behaviour and hygienic behaviour of bees which may vary between locations and also between colonies based on colony strength.

Table 3: Comparative preference of *Varroa jacobsoni* between worker and drone pupal brood of *Apis cerana* colonies at three different locations

	UAS, GKVK, Bengaluru			UAHS, Navile, Shivamogga			COF, Ponnampet, Kodagu		
Month/Year	**Worker (W) n=200**	**Drone (D) n=200**	**Worker to drone ratio (W: D)**	**Worker (W) n=200**	**Drone (D) n=200**	**Worker to drone ratio (W: D)**	**Worker (W) n=200**	**Drone (D) n=200**	**Worker to drone ratio (W: D)**
September 2019	0	0	0:0	0	14	0:1	0	14	0:1
October 2019	0	12	0:1	11	23	0.48:1	0	17	0:1
November 2019	4	15	0.26:1	2	33	0.06:1	0	13	0:1
December 2019	2	11	0.18:1	10	21	0.48:1	3	18	0.16:1
January 2020	5	14	0.35:1	22	34	0.65:1	14	26	0.54:1
February 2020	6	8	0.75:1	9	21	0.43:1	13	20	0.65:1
March 2020	3	10	0.30:1	7	22	0.32:1	15	19	0.78:1
Mean	2.86 ± 0.88	10.00 ± 1.89		8.71 ± 2.71	24.00 ± 2.69		6.43 ± 2.72	18.14 ± 1.62	
t test (worker and drone pupal brood)	$t_{cal.}$ -3.728 $t_{tab.}$ -2.179 and +2.179 (two tailed) Test is significant at 0.05 level			$t_{cal.}$ -4.65 $t_{tab.}$ -2.179 and +2.179 (two tailed) Test is significant at 0.05 level			$t_{cal.}$ -4.04 $t_{tab.}$ -2.179 and +2.179 (two tailed) Test is significant at 0.05 level		

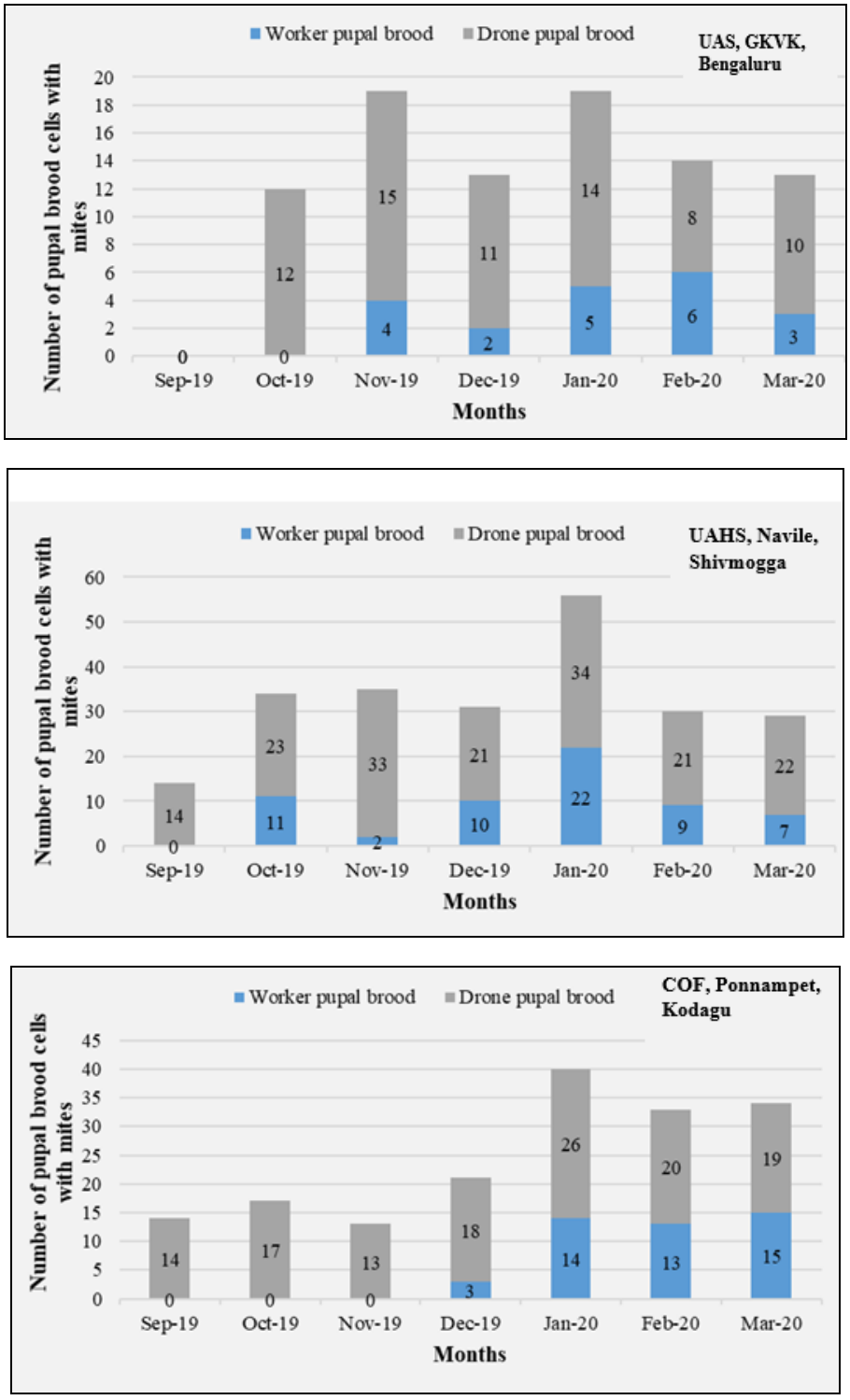

Fig 3: Infestation of *Varroa jacobsoni* between the worker pupal brood (n=200/month) and drone pupal brood (n=200/month) of *Apis cerana* colonies during September 2019 to March 2020 at three different locations

Preference of host by *V. jacobsoni* between worker and drone pupal brood of *A. cerana* colonies

During the present study, we observed the infestation of *V. jacobsoni* on both worker and drone pupal brood of *A. cerana* colonies in all the three locations. But the preference of *V. jacobsoni* for drone pupal brood was found to be significantly higher than worker pupal brood in all the three apiary locations (Table 3; Fig. 3). The mean number of infested worker and drone pupal brood during September 2019 to March 2020 was 2.86 ± 0.88 and 10.00 ± 1.89 respectively at UAS, GKVK, Bengaluru, 8.71 ± 2.71 and 24.00 ± 2.69 respectively at UAHS, Navile, Shivamogga and 6.43 ± 2.72 and 18.14 ± 1.62 respectively at COF, Ponnampet, Kodagu.

In all the three locations, the number of infected drone pupal brood was significantly higher because usually Varroa mites prefer drone brood for their reproduction [26, 1, 28]. In Sri Lanka, examination of capped brood cells showed that the *V. jacobsoni* preferred drone cells and that the mite reproduction occurs only in drone cells of *A. cerana* [16]. Another ectoparasitic mite i.e., *Tropilaelaps clareae* also prefers drone brood as compared to worker brood in *A. cerana* [24]. In case of *A. mellifera* also, on an average, infestation of *V. jacobsoni* was higher in drone pupal brood [13, 6, 7]. The methyl and ethyl esters of straight chain fatty acids, in particular methyl

palmitate is responsible for the attraction of *V. jacobsoni* towards drone brood [19]. However, the probable reason for *V. jacobsoni* to prefer drone pupal brood rather than worker pupal brood is that drone pupal broods usually take one or two days extra time for emergence as adults when compare to worker pupal brood. So this will benefit the mites for their proper feeding and development.

Plate 2: An adult *Varroa jacobsoni* mite

Plate 3: Infestation of ectoparasitic mite, *Varroa jacobsoni* on *Apis cerana* pupal brood

Conclusion

Varroa jacobsoni is one of the serious mite pest of the honey bees. Severe infection will lead to weakening and absconding of colonies. Our study showed the presence of *V. Jacobsoni* infestation at all the three different study locations, so there may be a chances infestation of *V. jacobsoni* at other locations of Karnataka also. However, in the present study, peak infestation was observed during January month at all the three locations, hence proper management practices should be followed during that time. Since our study was carried out only for seven months, there is a need to conduct thorough research on the population dynamics of *V. jacobsoni* during a span of one complete year and their effect of on honey production.

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