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Mites associated with honey bee colonies with special reference to phoretic mite, *Neocypholaelaps indica* Evans in selected locations of Southern Karnataka

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Abstract

The mite fauna associated with *Apis cerana* colonies was recorded during September 2019 to March 2020 at three different locations of Southern Karnataka which are belongs to three different agro-climatic zones of Karnataka. The study revealed the association of total 12 species of mite fauna from three locations. Phoretic mite, *Neocypholaelaps indica* Evans was found most abundant than other mites followed by ectoparasitic mite, *Varroa Jacobsoni* Oudemans. The remaining ten species were scavengers. Despite of different ecological conditions, incidence of *N. indica* in *A. cerana* colonies was found in all the locations. Higher incidence of *N. indica* was observed during September to December 2019 at all the three locations. Further, our study also revealed the infestation of *N. indica* on the body surface of three honeybee species foraging on niger during post monsoon season, but infestation of *N. indica* on those honeybees were not found during summer season.

Keywords: Mites, phoretic mites, *Neocypholaelaps indica*, honey bees, *Apis cerana*, Karnataka

Introduction

Honey bees are eusocial insects which are well known to human kind since the pre-historic times. The presence of honey, bees wax, brood, pollen, nectar and favorable microhabitat conditions inside the hive is a boon for many insect and non-insect associates. Among the associates of honey bees, mites constitute a predominant fauna. Several mite species have been found in association with bee colonies throughout Asia, but not all of them are associated with the bees as parasites. Till now, around 100 mite species are found to be associated with honey bees [11, 14]. But only few species actually parasitize the bees [13]. The other mites are those which feed on pollen and nectar. Mites which are associated with honeybees are categorized into three types viz., parasitic, phoretic and scavenger mites. Parasitic mites are serious pests of honey bees, which are reported to destroy hives in many countries in the world [15]. Hence, many detailed studies were done on these mites [1, 9]. However, not much studies were done on phoretic mites by researchers, because they are not causing any direct damage to bees. But indirectly by attaching to the thorax, abdomen and legs of bees, they reduce their nectar, pollen carrying capacity thereby affecting honey production and pollination [3, 12, 2]. Hence, the present study was undertaken on the incidence of phoretic flower mite, *Neocypholaelaps indica* Evans in *Apis cerana* F. colonies.

Materials and Methods

Study area

The study was conducted on *A. cerana* colonies at three different locations of Southern Karnataka viz., 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 methods

The incidence of *N. indica* in *A. cerana* colonies were observed by examination hive debris from ten randomly selected colonies at each of the three apiary locations once in a month

during September 2019 to March 2020 and their incidence on adult foraging bees were detected by capturing and examination foraging bees which visits niger and sunflower during peak flowering period at ZARS, GKVK, Bengaluru.

Examination of hive debris

Hive debris which is present on the bottom board of ten randomly selected *A. cerana* colonies at each of the three different apiary locations was collected in plastic boxes, then it was labeled with hive number and were brought to the laboratory (Plate 1). Later the debris samples were keenly observed under stereo binocular microscope, number of different mites were recorded and mounted on glass slides for

identification. The mites were identified with the help of available literature and later got confirmed by Dr. C. Chinnamade Gowda, Professor of Entomology and Acarologist, AINP on Agril. Acarology, UAS, GKVK, Bengaluru. This method allows the sampling of mites in the colony those which die due to natural phenomenon *i.e.*, natural mite fall, those that fall off from the adult honey bee body due to grooming behavior, those which are removed from infested brood because of hygienic behavior of honey bees, those that fall down during their natural course of movements within the colony. This method also allows the sampling of scavenger mites which are feeding on the debris in the colony.



Plate 1: Sampling method followed for examination of hive debris

Examination of adult foraging bees

However, to assess the number of phoretic mites which are attached to the body of foraging bees, during the peak flowering period foraging bees that visits the niger and sunflower at Zonal Agricultural Research Station (ZARS), GKVK, Bengaluru were captured with the help of sweep net (Plate 2). Here three *Apis* spp. *viz.*, *A. cerana*, *A. dorasta* and

A. florea bees were captured from 8 AM to 2 PM at the rate of 10 *Apis* species each at every 2 hour intervals. This type of sampling was done for 7 days from 18th of November, 2019 to 24th of November, 2019 (Post monsoon season) on niger and during summer season *i.e.*, from 3rd February, 2020 to 9th February, 2020 and from 27th February, 2020 to 5th March, 2020 on sunflower and niger, respectively.



Plate 2: Field view during flowering of niger (left) and sunflower (right)

The captured bees were killed by using ethyl acetate and brought to the laboratory for counting the number of phoretic mites on the body of bees under stereo binocular microscope. The mite specimens which were present on the body surface of three *Apis* species which were collected during different time intervals, were counted and recorded separately, then these mite specimens were collected in a vial containing ethyl alcohol and mounted on glass slide.

Results and Discussions

Mite fauna associated with *Apis cerana* colonies

In total, from three different apiary locations, the present study revealed the association of 12 species of mite fauna which belonging to 3 order and 10 families (Table 1; Plate 3) with *A. cerana* colonies during September 2019 to March 2020. Among these mite fauna, phoretic mite, *Neocyphoaelaps indica* Evans was found to be most

abundant followed by *Varroa jacobsoni* Oudemans. The phoretic mite, *N. indica*, although harmless to bees, reduce their nectar, pollen carrying capacity thereby affecting honey

production and pollination. Mites were found on the thorax and abdomen of foraging bees [7].

Table 1: List of mite fauna observed in *Apis cerana* colonies at three different locations

Order	Family	Common name / Scientific name	Role	L1	L2	L3
Mesostigmata	Ameroseiidae	Phoretic mite <i>Neocyphophylax indica</i> Evans	Phoretic on adult bees, feed on pollen and stored honey in hives	+	+	+
	Varroidae	Varroa mite <i>Varroa jacobsoni</i> Oudemans	Ectoparasitic on pupal broods and adults, suck the haemolymph	+	+	+
	Uropodidae	<i>Trichouropoda</i> sp.	Scavengers	+	+	+
	Parasitidae	Species 1	Scavengers	-	-	+
	Unidentified family	Species 1	Scavengers	+	-	-
Trombidiformes	Cheyletidae	<i>Cheletophyes</i> sp.	Scavengers	+	+	+
	Tarsonemidae	<i>Tarsonemus</i> sp.	Scavengers	+	-	-
	Acaridae	<i>Sancassania</i> sp.	Scavengers	+	-	-
Sarcoptiformes	Aeroglyphidae	<i>Glycycometus</i> sp.	Scavengers	+	+	+
	Unidentified family	3 Oribatid species mites	Scavengers	-	+	+

Note: L1, L2, L3 indicates apiary location viz., L1: UAS, GKVK, Bengaluru; L2: UAHS, Navile, Shivamogga, and L3: COF, Ponnampet, Kodagu, respectively; (+) indicates presence and (-) indicates absence

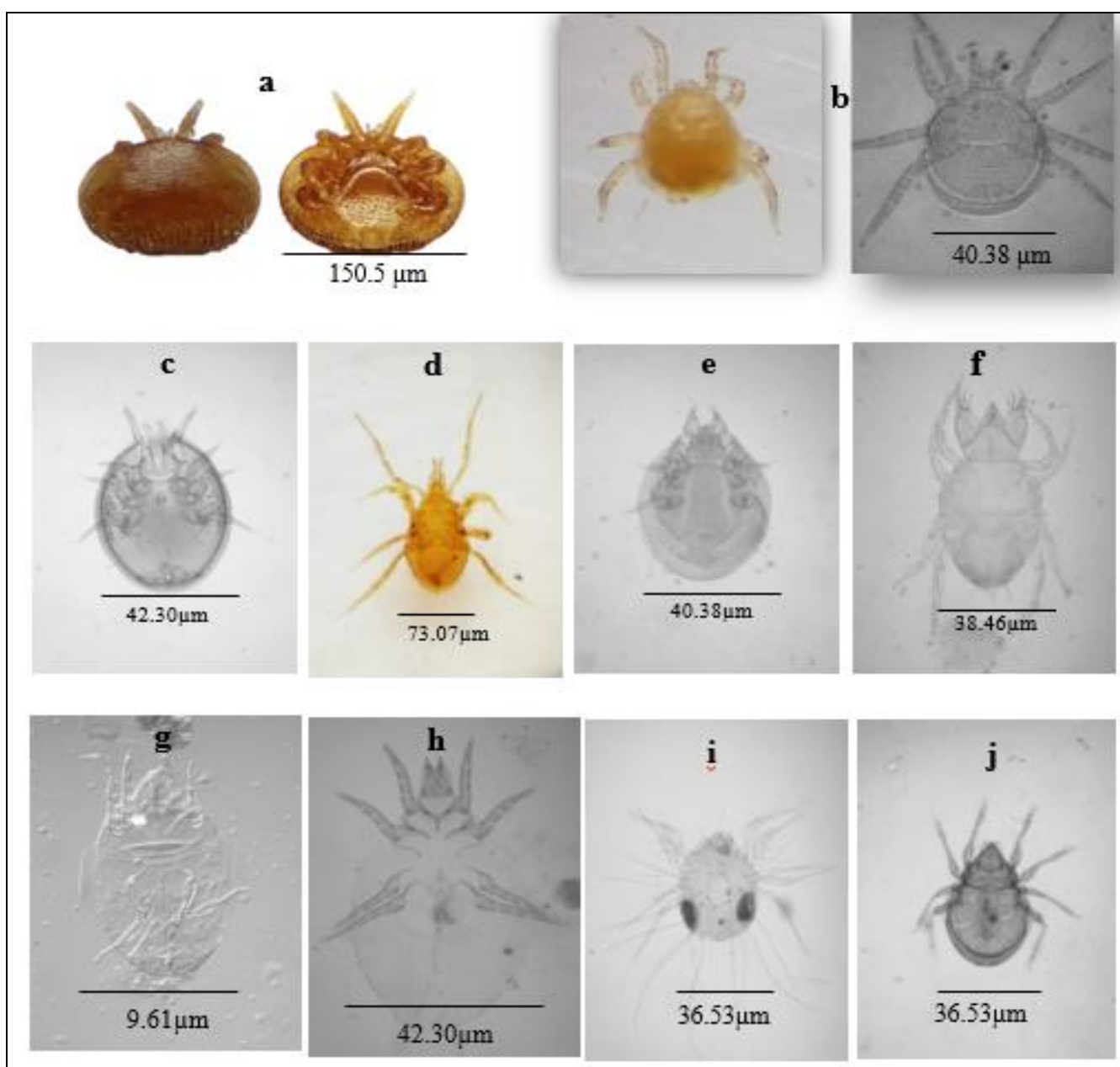


Plate 3: Mite fauna observed in *Apis cerana* colonies, (a) *Varroa jacobsoni*, (b) *Neocyphophylax indica*, (c) *Trichouropoda* sp., (d) Species 1 of Family: Parasitidae, (e) Species 1 of unidentified family, (f) *Cheletophyes* sp., (g) *Tarsonemus* sp., (h) *Sancassania* sp., (i) *Glycycometus* sp., (j) Oribatid mite

Incidence of phoretic mite, *Neocypholaelaps indica* in *Apis cerana* colonies

The data on the *N. indica* population recorded from hive debris examination at UAS, GKVK, Bengaluru showed the peak incidence of *N. indica* during September 2019 (493.80 ± 87.08 mites / colony) later on during further months it showed decreasing trend with minimum incidence during March 2020 (15.20 ± 6.71 mites / colony), whereas, the peak incidence of *N. indica* was found during December 2019 at both UAHS,

Navile, Shivamogga (336.90 ± 68.93 mites / colony) and COF, Ponnampet, Kodagu (343.70 ± 28.58 mites / colony). Later on during further months the incidence of *N. indica* in *A. cerana* colonies showed decreasing trend with minimum incidence during March 2020 both UAHS, Navile, Shivamogga (12.70 ± 0.83 mites / colony) and COF, Ponnampet, Kodagu (21.50 ± 7.80 mites / colony) (Table 2; Fig. 1).

Table 2: Populations dynamics of *Neocypholaelaps indica* in *Apis cerana* colonies under hive debris examination method at three different apiary locations

Month / Year	UAS, GKVK, Bengaluru	UAHS, Navile, Shivamogga	COF, Ponnampet, Kodagu
September 2019	493.80 ± 87.08^a	264.60 ± 63.42^a	184.40 ± 48.78^{bd}
October 2019	310.90 ± 31.87^b	269.40 ± 29.23^a	243.40 ± 14.80^{cd}
November 2019	323.00 ± 14.66^b	314.10 ± 64.21^a	254.00 ± 50.59^{ad}
December 2019	282.20 ± 24.98^b	336.90 ± 68.93^a	343.70 ± 28.58^a
January 2020	107.80 ± 27.86^c	121.60 ± 18.39^b	294.30 ± 47.92^{ac}
February 2020	116.60 ± 53.46^c	28.80 ± 3.13^b	124.70 ± 29.11^b
March 2020	15.20 ± 6.71^c	12.70 ± 0.83^b	21.50 ± 7.80^e
F-test	*	*	*
SE (d)	42.43	47.41	32.46
SE (m)	60.00	67.05	45.91
CD (p = 0.05)	124.58	139.22	95.32
CV	40.26%	55.05%	34.56%

Note: Values under each month are the mean of 10 colonies observed (n=10); * Significant at 5%

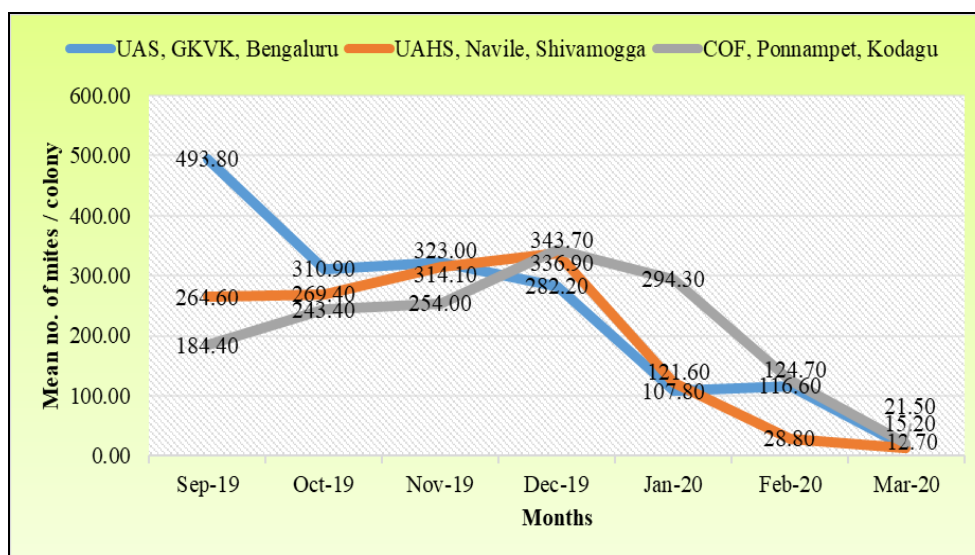


Fig. 1: Population dynamics of *Neocypholaelaps indica* in hive debris examination method in *Apis cerana* colonies during September 2019 to March 2020 at three different locations

In the comparison of mean number of *N. indica* /colony /month between three different locations maximum incidence was observed at UAS, GKVK, Bengaluru (235.64 mites/colony/month), followed by COF, Ponnampet, Kodagu (209.43 mites/colony/month) and UAHS, Navile, Shivamogga (192.57 mites/colony/month) during September 2019 to March 2020 and there is no significant difference in the incidence of *N. indica* between three different apiary locations

(Fig. 2). However, our study revealed the presence of significant difference in the incidence of *N. indica* in *A. cerana* colonies across the seven months at all the three apiary location, this may be attributed by the fact that reproduction of *N. indica* is takes place on flowers. Hence, based on availability of suitable hosts (flowers) for their reproduction significant difference across the months was observed in the present studies.

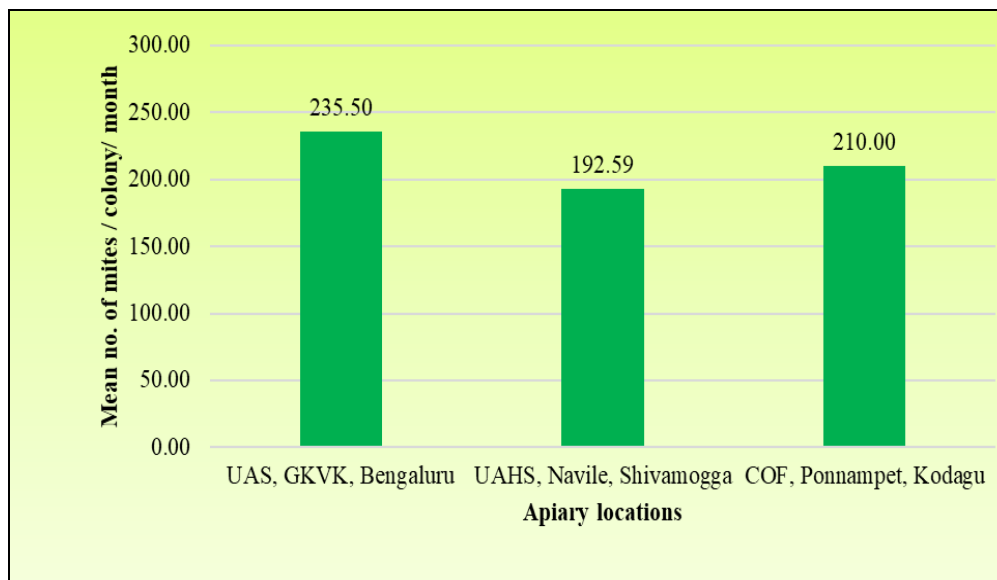


Fig 2: Mean number of *Neocypholaelaps indica* per *Apis cerana* colony during September 2019 to March 2020 in hive debris examination method at three different apiary locations

Phoretic mite, *N. indica* were not observed throughout the year. During 2006-07 they were found in colonies as well as on the bodies of *A. mellifera* from January to July and their number ranged from a minimum of 0.00 to a maximum of 3.32 per cent. Whereas, they were found in range of 0.00 to a maximum of 2.50 per cent with peak infestation in the month of February-April during 2007-08 [6]. However, their maximum incidence was observed during July-August [7]. Since the present study was conducted only for seven months from September 2019 to March 2020 and the results were not comparable with others studies.

Other scavenger mites associated with *Apis cerana* colonies

The mites other than *N. indica* and *V. jacobsoni* which were found associated with *A. cerana* colonies were scavenger mites, as they found to feed on hive debris. A total of ten species of scavenger mite fauna belonging to three orders and ten families was found to be associated with *A. cerana* colonies from three different apiary locations during September 2019 to March 2020. Among those twelve scavenger mite fauna, not all the mites were observed at all

the study locations and throughout the study period. Some of the mites were observed only during certain months. Their numbers were very less. However, *Glycycometus* sp. was the most abundant among scavenger mites followed by *Trichuropoda* sp. during September 2019 to March 2020 at three different apiary locations in our study. In southern Tagalog region, Philippines, 23 species of mites belonging to 15 different families were reported to be associated with *A. mellifera* and *A. cerana*. The abundant bee associated mites were the scavengers, *Trichouropoda stercoraria* (116 specimens) and *Forcellinia faini* (77 specimens); *Tarsonemus* sp. with uncertain feeding habits (94 specimens); the predator, *Blattisocius* sp. (103 specimens) and the pollen feeder, *Neocypholaelaps* sp. (58 specimens), and *Glycycometus* spp. (21 specimens) [8].

Infestation of phoretic mite, *Neocypholelaps indica* on foraging honey bees

During the present study, phoretic mite *Neocypholaelaps indica*, was found to be attached on thorax, legs, abdomen, and the space between thorax and abdomen on foraging bees of three *Apis* species (Plate 4).



Plate 4: Infestation of phoretic mite, *N. indica* on the body surface of foraging *Apis* bees

Table 3: Mean number of phoretic mite, *Neocypholaelaps indica* on three *Apis* species foraging on niger during peak flowering period at ZARS, GKVK, Bengaluru

Time Date	<i>A. cerana</i>			<i>A. dorsata</i>			<i>A. florea</i>		
	08.00-10.00h	10.00-12.00h	12.00-14.00h	08.00-10.00h	10.00-12.00h	12.00-14.00h	08.00-10.00h	10.00-12.00h	12.00-14.00h
18.11.2019	2.60 ± 0.52	2.40 ± 0.85	2.20 ± 0.44	1.40 ± 0.37	1.60 ± 0.43	1.30 ± 0.40	1.00 ± 0.21	1.10 ± 0.23	1.50 ± 0.22
19.11.2019	2.60 ± 0.50	2.30 ± 0.58	2.70 ± 0.47	1.50 ± 0.45	1.70 ± 0.70	1.50 ± 0.40	1.00 ± 0.26	0.90 ± 0.18	0.90 ± 0.28
20.11.2019	2.20 ± 0.47	1.90 ± 0.46	2.10 ± 0.75	1.60 ± 0.34	1.10 ± 0.28	1.40 ± 0.31	1.10 ± 0.31	1.20 ± 0.33	1.40 ± 0.27
21.11.2019	2.20 ± 0.42	2.60 ± 0.54	2.10 ± 0.35	1.60 ± 0.37	1.40 ± 0.37	1.70 ± 0.37	1.30 ± 0.42	1.30 ± 0.37	1.50 ± 0.31
22.11.2019	1.80 ± 0.39	2.20 ± 0.55	2.00 ± 0.60	1.40 ± 0.31	1.60 ± 0.27	1.50 ± 0.40	1.10 ± 0.31	1.40 ± 0.34	1.20 ± 0.39
23.11.2019	2.50 ± 0.52	2.60 ± 0.81	2.40 ± 0.45	1.30 ± 0.26	1.30 ± 0.30	1.40 ± 0.40	1.00 ± 0.30	1.10 ± 0.35	0.90 ± 0.23
24.11.2019	2.80 ± 0.76	3.00 ± 0.71	2.90 ± 0.71	1.40 ± 0.37	1.90 ± 0.59	1.90 ± 0.62	1.70 ± 0.42	1.80 ± 0.36	1.20 ± 0.25
Overall mean	2.39 ± 0.13	2.43 ± 0.13	2.34 ± 0.13	1.46 ± 0.04	1.51 ± 0.10	1.53 ± 0.08	1.17 ± 0.10	1.26 ± 0.11	1.23 ± 0.10

Note: Values are the mean number of mites on 10 bees (n=10)

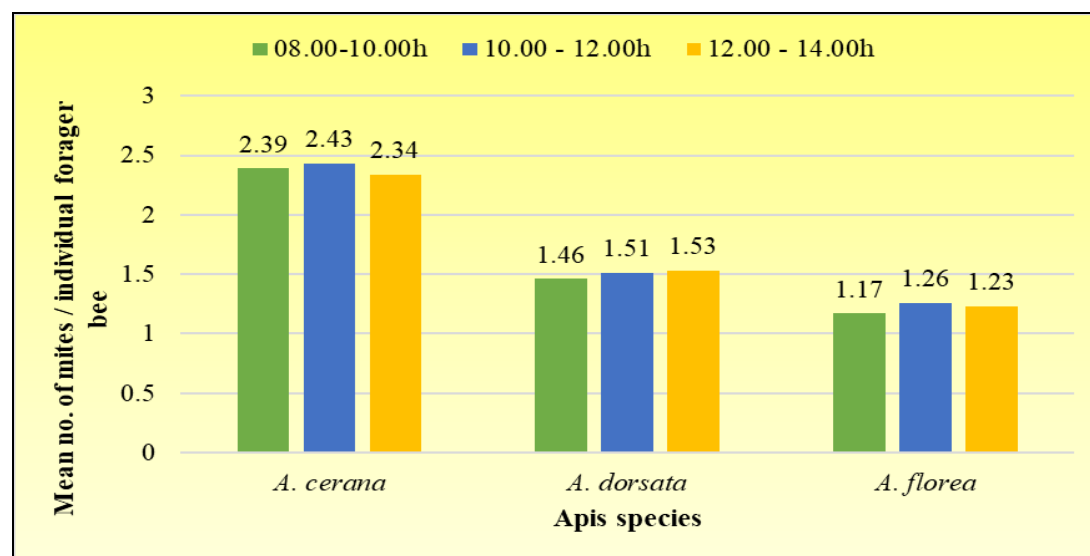
Table 4: Comparative infestation of *Neocypholaelaps indica* on three *Apis* species foraging on niger during different hours of the day at ZARS, GKVK, Bengaluru

Time intervals	<i>Apis</i> sp.	<i>A. cerana</i>	<i>A. dorsata</i>	<i>A. florea</i>	Number of <i>N. indica</i> recorded during different time intervals of the day (Mean ± SE)
08.00 – 10.00h		2.39 ± 0.13	1.46 ± 0.04	1.17 ± 0.10	1.67 ± 0.37
10.00 – 12.00h		2.43 ± 0.13	1.51 ± 0.10	1.26 ± 0.11	1.73 ± 0.36
12.00 – 14.00h		2.34 ± 0.13	1.53 ± 0.08	1.23 ± 0.10	1.70 ± 0.33
Number of <i>N. indica</i> on <i>Apis</i> species (Mean ± SE)		2.39 ± 0.03 ^a	1.50 ± 0.02 ^b	1.22 ± 0.03 ^c	

Factors	F - Test	SE(m)	SE (d)	CD (p=0.05)	CV
<i>Apis</i> species (A)	*	0.05	0.07	0.14	13.50 %
Time (B)	NS	-	-	-	-
A X B	NS	-	-	-	-

Note: Values are the mean ±SE of seven days observations from 18/11/2019 to 24/11/2019

* Significant at 5%; NS - Non-significant

**Fig 3:** Mean number of *Neocypholaelaps indica* on body surface of three *Apis* species foraging on niger at different time intervals of the day during 18/11/2019 to 24/11/2019

The data regarding the infestation of *N. indica* on the body of three *Apis* species foraging on niger during peak flowering period at ZARS, GKVK, Bengaluru during post monsoon season (November 2019) revealed that, the mean number of *N. indica* on individual *A. cerana* ranged from 1.80 ± 0.39 to 3.00 ± 0.71 . On *A. dorsata* it ranged from 1.10 ± 0.28 to 2.90 ± 0.71 , whereas, on *A. florea* it ranged from 0.90 ± 0.18 to 1.8 ± 0.36 (Table 3). However, comparison between the infestation level of *N. indica* on the three *Apis* species during three different time intervals of the day was made (Table 4; Fig. 3). The results revealed that there was no significant

difference between the infestation of *N. indica* on bees during different time intervals of the day, whereas, significant difference was found between the infestation of *N. indica* on the body of three *Apis* species. It showed the highest levels of infestation on *A. cerana* (2.39 ± 0.39 mites/ bee), followed by *A. dorsata* (1.50 ± 0.02 mites / bee) and *A. florea* (1.22 ± 0.10 mites/bee). Overall, in the present study, the lower incidence of *N. indica* was observed on *A. florea*, this may be attributed to smaller body size of that bee species.

This mite is usually found on eucalyptus flowers and forages on pollen grains. Its number on individual forager bee ranges

from 35 to 60 and additional load on forager was equivalent to 131.3 per cent by body weight ^[10]. This phoretic mite is usually found on the dorsal side of the thoracic region of foraging honey bees which are transported frequently from flowers to hive and the mean number of mites per foraging bee varied between 20.6±16.12 and 53.00±12.02 ^[5]. *N. indica* are found to infest the flowers of *Pachysandra axillaris* and also *A. cerana* bees in Yunnan, China. The average number of mites on each *A. cerana* individual varied from 16.2 to 44.4 in 2006 and from 13.6 to 38.2 in 2008 ^[4].

The mean number of *N. indica* infesting foraging bees was found to be lesser in the present study when compared to the earlier studies. The possible reason for such differences may be overall scenario of mite incidence level during their study and the present study. Besides, this could be attributed to the study area and availability of suitable host (flowers) for mite reproduction. However, the infestation of *N. indica* on the body surface of foraging worker bees of three *Apis* species on niger and sunflower was not found during summer season (February and March 2020). Therefore, our study revealed the seasonal variation in the incidence of phoretic mite, *N. indica*

Conclusion

Our study revealed the association of parasitic, phoretic and scavenger mites with honey bee colonies. Phoretic mites do not cause any direct damage to bees. However, by attaching to body of bees, indirectly they reduce their nectar, pollen carrying capacity thereby affecting honey production and pollination. Since our study was carried for a period of seven months, there is a need to conduct this work for one complete year to obtain the proper data regarding population dynamics of mites and their effects of on honey production. So that, in the near future that data will be helpful for the better management of mites associated with honeybee colonies.

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