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Population dynamics of ectoparasitic bee-mite (*Tropilaelaps clareae*) in European honey bee (*Apis mellifera*) colonies in north-western India

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Abstract
Studies on year round population fluctuation of *Tropilaelaps clareae* Delfinado and Baker in *Apis mellifera* Linnaeus colonies through different seasons under with two strength colonies (8-10 and 18-20 bee frame strength) in north-western India revealed the maximum mite population during March-April that synchronized with the peak worker brood rearing in the bee colonies. The maximum mite population on adult bees and mite fall were also observed in March-April but the population of the mite on adult bees was very small. This showed that this mite has negligible phoretic behaviour. This is in contrast to *Varroa destructor* Delfinado and Baker which showed a strong phoretic behaviour. The mite population in worker brood was positively correlated with worker brood rearing in 8-10 and 18-20 bee frame strength *A. mellifera* colonies (r = 0.74, r = 0.79, respectively). Since the residual mite population starts multiplying quickly to increase in brood rearing with the spring onset, the strategy of reducing its carry over to spring brood rearing period by hitting it with some effective management measures before the spring onset when mite population is low, could be a promising strategy. Further, in double strength (18-20) honey bee colonies wherein brood rearing had been higher than in low strength (8-10) honey bee colonies, the mite population had been the lowest in the brood in the former colonies which could be attributed to high adult nurse worker bee population resulted in a more mite fall which resulted in lower incidence of the mite in the brood. Thus, it can be inferred that honey bee colonies must be maintained stronger to take these to at least one super.

Keywords: *Apis mellifera*, brood rearing, Ectoparasitic mite, population dynamics, *Tropilaelaps clareae*

Introduction

Beekeeping is an important agricultural subsidiary occupation adopted by thousands of farmers of this region of India. The majority of the beekeepers are professional beekeepers, having adopted commercial apiculture by hiving exotic European honey bee species (*Apis mellifera* Linnaeus) which is capable of yielding higher honey in comparison to Asiatic honey bee species (*Apis cerana* Fabricius). However, still, average honey production per colony is low which is attributed to several biotic and abiotic factors. Infestation by ecto-parasitic mites is one of the major biotic causes for the low apiary yields. *Varroa destructor* Anderson and Trueman and *Tropilaelaps clareae* Delfinado and Baker are the two ecto-parasitic mites causing severe losses to honey bee colonies was given by FAO in 2006 [6]. Though various aspects of the *V. destructor* Has already been studied in details, lesser is known about the interaction of *T. clareae* with *A. mellifera*. The natural host of this mite is *Apis dorsata* Fabricius, which later spread to other bee species including *A. mellifera*. Now this mite is presumed to be widely disseminated by globally distributed bee species, *A. mellifera* was found by Sammataro in 2004 [14]. In Indian Punjab, after the successful introduction of *A. mellifera* in 1962-64, a serious attack of the mite was noticed during 1966 by Stephen in 1968 [19]. In a report, the incidence of this mite was recorded to be as high as 39.83 per cent during 2006-07 which during the following years declined to 9.00 and 3.91 per cent during 2008-09 and 2009-10, respectively was found by Chhuneja *et al*. in 2011 [3]. Its incidence was comparatively higher (4.67 %) during brood rearing period of summer 2009 was reported by Singh *et al*. 2011a [17], Chhuneja and Singh 2012 [4]. The highest incidence was attributed to some unscientific practices being followed by the beekeepers was found by Singh *et al*. in 2011b [18]. The feeding on brood by this mite results in producing malformed bees and may result in the death of brood and bees under conditions of heavy infestation. Some workers have also reported transmission of deformed wing virus (DWV) by the mite during the course of the infestation was given by Burgett *et al*. in 1983 [1], Sihag and Singh 1991 [16], Nagaraja and Rajagopal 2001 [10]. Keeping in view the potential of causing economic damage to *A. mellifera* colonies by this mite and lack of scientific knowledge on various aspects regarding this mite in the region, the present study of population dynamics of *T. clareae* in *A. mellifera* colonies were undertaken during 2013-14 at *Apis mellifera* Apiary of the Punjab Agricultural University, Ludhiana. This would help in identifying the period of peak activity of this mite in relation to *A. mellifera* so as to undertake timely management practices for curbing the mite pest. *Tropilaelaps* belong to family Laelapidae (Mesostigmata). *Tropilaelaps* species feed on bee larvae and pupae and like *Varroa*, causes brood malformation and the death of bees in heavy infestation was reported by Sammataro in 2004 [14]. Rinderer *et al* in 1994 [13] reported that if the population of *T. clareae* was allowed to develop unchecked, the mite can rapidly cause death of the colony. Griffiths in1988 [8], Nagaraja and Reddy in 1999 [10] also reported that this mite is an opportunistic parasite which exploited honey bee colony during brood rich period. Although not yet recorded from Britain, *T. clareae* is notifiable menace and Wilkins and Brown in 2005 [22] have reported its potential risks in the UK.

Materials and Methods

Three naturally infested colonies each of 8-10 bee-frame (single chamber) and 18-20 beeframe (double chamber) strengths were examined continually during all the five seasons for complete one year (2013-14). The mite population was recorded in worker brood and on adult bees, and mite fall was also recorded. As *T. clareae* is chiefly a brood mite, year round brood rearing was also recorded in all the colonies. Long term weekly average of maximum and minimum temperatures recorded at Ludhiana revealed mean maximum and minimum temperatures to be 39.7 and 5.40C, respectively. The average rainfall is 400-1300 mm with approximately 80 per cent of the rainfall received during July to September

Brood rearing

Brood rearing was recorded in terms of brood area that was measured with the help of a special measuring frame of the size of a normal Langstroth frame, fitted with wire grid and each square measured one square inch area. The brood comb was taken out of the colony and its bees were brushed off. This measuring frame was apportioned against the brood comb and the number of squares of capped brood were counted with the help of the measuring frame and the total area of such brood available in the colony was then calculated. The recorded brood area (in square inches) was multiplied by 6.45 to obtain it in sq cm. The observation was made at every fortnightly interval.

T. clareae population in worker brood

Older capped brood cells were opened from each of the experimental colonies with the help of Varroa fork at fortnightly interval and the number of mites present per 20 cells were counted and recorded. Three such observations were made at a time in each of the experimental colonies was according to Dietemann *et al*. in 2013 [5].

T. clareae population on adult bees

The mite population on the adult bees was counted by dislodging them from honey bees at fortnightly interval. More than 300 adult bees were caught from the combs by a single shake from each of the experimental colonies and were colony-wise separately transferred to glass bottles. After pouring powdered sugar into the bottle, the bottle was shaken to dislodge the mites. The number of mites dislodged for every 300 adult bees was calculated for the every colony was according to Macedo *et al*. in 2002 [9].

T. clareae fall

Mite fall was recorded on sticky paper spread on Varroa board during this period. For recording the mite fall, bottom boards of Langstroth hives were replaced with Varroa bottom boards which is a modified bottom board with a sliding board moving through grooves on the inner side of long bars of the bottom board and a mesh above. Sticky paper of the size corresponding to inner dimensions of the bottom board of Langstorth hive was spread on the sliding board of Varroa bottom board in every experimental colony. The number of mites per sq inch were counted at five different places on the sticky paper and added up as the mite fall per five square inches.

The apiary observations were recorded in the morning (0800-1000 h) during summer months (April to July), in the noon (1200-1400 h) during winter months (November to February) and during the late forenoon (1100-1300 h) during the rest of the months.

Ambient weather parameters

Ambient weather parameters (maximum temperature, minimum temperature and relative humidity) were recorded on every day of the apiary observation from the meteorological observatory of the university which is situated just 150 m from the apiary.

Statistical Analysis

Data recorded on various aspects were analyzed using CPCS 1 software for CRD. Besides, correlation coefficients were worked out between the brood rearing and the incidence of the mite (mite population in worker brood, on adult bees, and mite fall) and the coefficients were statistically tested for significance at the 5 per cent level of significance. Correlation coefficients were also worked out between the incidence of the mite and ambient weather parameters (maximum temperature, minimum temperature and relative humidity). These correlation coefficients were also tested at 5 per cent level of significance.

Results and Discussion

To assess the year rounds population fluctuation of *T. clareae* in *A. mellifera* colonies through different seasons, the mite population in brood and on adult bees and the mite fall were recorded at two bee strengths (8-10 and 18-20 bee-frames) of the honey bee colonies during 2012-13. In 8-10 bee-frame colonies, the mean mite population per 20 brood cells at the start of the experiment (on 15th November) was 0.44±0.36 (Table 1) which increased continuously to peak population (7.44±3.78) on 1 st March. Worker brood too was continuously increasing with its peak (2120.6±417.1 cm^2) observed on 15th April. However, after the first mite population peak, the mite population decreased to 2.88±0.92 on 15th April. Two smaller peaks were observed on 1st May (4.22±2.03) and 1st June (4.33±1.08). For the rest of the observation dates, the mite population was very low, ranging from 0.11±0.13 to 2.33±1.64. Although a smaller peak of brood area was recorded on 15th October, but this increase in brood rearing did not result in corresponding mite population build up.

In 18-20 bee-frame colonies, the mite population per 20 cells on 15th November was 0.33±0.23 which increased over the time and peak (4.33±1.77) was observed on 1st March (Table 1). At the same time, the worker brood to continuously increase with a peak on 1st April (3074.7±405.19 cm 2). However, after this first peak, the mite population decreased to 1.55±0.27 on 1st April and smaller peaks were observed on 15th April (4.11±1.18) and on 1st June (3.00±1.24). Brood rearing during this period had been decreasing too. During the rest of the year, the mite population was low, ranging from 0.11±0.13 to 1.66±0.62. Although a smaller peak in brood rearing was recorded on 1st September but it could not translate the corresponding mite population build up. The peak mite population observed on 1st March was also at par with that recorded in 15th February (2.88±1.42) and 15th March (3.22±1.44).

Gatoria *et al*. in 1995[7] and Padhi and Rath in 2012 [12] recorded peak mite population during October and November months under the Punjab and Odisha conditions, respectively. Sharma *et al*. in 2011[15] observed peak mite population in *A. mellifera* colonies during September-October in Himachal Pradesh. The results found in present findings are not supported by these studies in respect of the period during which peak mite population was observed. But all the workers found peak mite population coinciding with the peak in brood rearing which supports the present findings.

Like the results of the current study, Chahal *et al*. in 1986 [2] also observed two peaks in brood infestation by mite, one during February-May and the other during September-November, both coinciding with the peak of brood rearing activity in Ludhiana, which support the present findings partially because the second peak was observed much earlier i.e. during May-June. Thus, over the spell, the population dynamics of the mite has changed, becoming adaptable and commensurating with the changing brood rearing pattern of the bee, providing an abundant supply of the food for the mite for its multiplication.

Thakur *et al*. in 2009 [20] from Himachal Pradesh also observed two peaks in mite population (May-June and September-October) the difference from the present findings could be due to the difference in the climatic conditions of the two localities which affect the brood rearing in *A. mellifera* colonies, and thus the peaks of the mite.

The mite population on adult bees ranged from 0.00±0.00 to 2.66±1.08 per 300 bees in 8-10 beeframe strength colonies (Table 1). There were non-significant differences among all the fortnightly observations. The mite population on adult bees being very low indicated negligible phoresis. This was in accordance with the results of Waghchoure-Camphor and Martin in 2009 [21] who had reported very low infestation level on the adult bees implying a very short phoretic period.

The mite population ranged from 0.00±0.00 to 3.00±2.12 per 300 adult bees in 18-20 bee-frame strength colonies (Table 1). The maximum number of mites per 300 adult bees was recorded on 15th March (3.00±2.12) which was statistically at par with that recorded on 1st March (2.00±1.41), the latter being further at par with that on 15th February (1.33±0.40). On the other dates, it was either nil or negligible.

In 8-10 bee-frame colonies, the mite fall per 5 inch2 on 15th November was 1.33±0.81 (Table 1) which increased over the time and reached to peak (12.16±7.8) on 15th March and it was at par with the mite fall recorded in 1st April (8.66±1.59) and 15th April (7.66±0.73).

In 18-20 bee-frame colonies, the mite fall per 5 inch2 on 15th November was 1.00±0.70 which increased over the time and the peak (16.33±3.2) was observed on 15th March (Table 1). The mite fall during the 1st March (7.83±1.24), 1stApril (8.00±3.67), 15th April (8.83±4.21) and 1st May (5.33±3.39) were at par with one another. During the rest of the period, the mite fall had been very low. Waghchoure-Camphor and Martin in 2009 [21] also reported a peak in the mite fall during the months of peak in brood rearing. Data (Table 2) revealed that the mite population in worker brood was positive and significantly correlated with worker brood in both 8-10 and 18-20 bee-frame strength colonies (r = 0.74 and 0.79, respectively). This implies that the mite population in worker brood is significantly influenced by the worker brood rearing. Thakur *et al*. in 2009 [20] and Padhi and Rath in 2012 [12] too had observed the similar correlations.

Thus, the present study clearly indicated that there was a maximum mite multiplication during active brood rearing period of the honey bee which was also manifested as a little mite population on adult bees during the spring brood rearing. The increased mite population during this period also resulted in depicting higher mite fall. The mite being obligate brood parasite had its higher population around peak brood rearing period. Further, in double strength honey bee colonies wherein brood rearing had been higher than in low strength honey bee colonies, the mite population had been the lowest in the brood in the former colonies which could be attributed to high adult bee population (more nurse bees) resulted in a more mite fall which resulted in lower incidence of the mite in the brood. Thus, it can be inferred that honey bee colonies must be maintained stronger to take these to at least one super. Further, as the residual mite population starts multiplying quickly with increase in brood rearing with the spring onset, the strategy of reducing its carry over to spring brood rearing period by hitting it with some effective management measures before the spring onset when mite population is low, could be promising strategy.

Since *T. clareae* is a brood mite i.e. it multiplies within the capped brood, it is less influenced by either outside or within apiary ambient temperature and humidity conditions, except by the quantum of brood rearing itself, and that is why the correlations between the mite population and ambient temperature and humidity have been non-significant (Table 2).

Table 1: Annual dynamics of *Tropilaelaps clareae* (Delfinado and Baker) population and worker brood rearing *vis-à-vis* is the different bee strength of *Apis mellifera* Linnaeus colonies

Date of observation	Worker brood rearing (cm^2)*		Mite population in worker brood (per 20 cells)*		Mite population (per 300 adult worker bees)*		Mite fall (No. of mites/ 5 $inch^2$)*	
	8-10 bee-frame strength	18-20 bee-frame strength	8-10 bee-frame strength	18-20 bee-frame strength	8-10 bee-frame strength	18-20 bee-frame strength	8-10 bee-frame strength	18-20 bee-frame strength
15-Nov	693.73±9.65	959.61±131.59	0.44±0.36	0.33±0.23	0.00±0.00	0.00±0.00	1.33±0.81	1.00±0.70
1-Dec	717.38±51.00	885.08±138.81	0.33±0.00	0.33±0.23	0.00±0.00	0.00±0.00	1.16±0.54	0.83±0.20
15-Dec	835.63±67.50	866.44±168.86	0.33±0.23	0.33±0.00	0.00±0.00	0.00±0.00	0.83±0.54	0.50±0.00
1-Jan	701.61±190.20	866.44±177.87	0.44±0.36	0.66±0.23	0.00±0.00	0.00±0.00	0.83±0.54	0.50±0.00
15-Jan	882.93±168.30	1183.24±168.08	1.11±0.59	1.44±0.36	0.00±0.00	0.00±0.00	2.00±0.70	1.00±0.35
1-Feb	1229.80±153.29	2208.05±123.42	2.66±1.17	1.88±0.82	1.00±1.22	0.00±0.00	1.83±0.73	2.60±1.42
15-Feb	1395.35±186.24	2599.35±123.42	4.88±2.47	2.88±1.42	0.66±0.81	1.33±0.40	3.33±0.73	3.33±0.54
1-Mar	1568.78±251.06	2703.51±177.14	7.44±3.78	4.33±1.77	1.00±1.22	2.00±1.41	5.33±2.55	7.83±1.24
15-Mar	1655.50±352.43	2571.40±278.80	6.00±1.92	3.22±1.44	2.66±1.08	3.00±2.12	12.16±7.86	16.33±3.20
1-Apr	1986.60±420.02	3074.78±405.19	4.55±0.36	1.55±0.27	0.00±0.00	0.00±0.00	8.66±1.59	8.00±3.67
15-Apr	2120.61±417.18	2867.67±259.20	2.88±0.92	4.11±1.18	1.66±1.08	0.00±0.00	7.66±0.73	8.83±4.21
1-May	1647.61±139.26	2562.08±211.32	4.22±2.03	1.88±0.82	0.00±0.00	1.00±1.22	5.16±2.50	5.33±3.39
15-May	953.88±188.72	2394.38±636.13	3.66±3.34	1.00±0.40	0.33±0.40	0.00±0.00	7.50±3.14	4.50±3.02
1-Jun	811.98±192.98	1956.50±474.59	4.33±1.08	3.00±1.24	1.00±1.22	0.00±0.00	2.50±0.61	2.00±0.93
15-Jun	1117.46±34.81	2049.66±360.29	1.88±1.18	1.66±0.62	0.00±0.00	0.66±0.81	2.16±0.20	3.00±1.76
1-Jul	1119.43±309.46	1546.56±485.85	2.33±1.64	1.11±0.36	0.00±0.00	0.00±0.00	2.00±0.70	1.83±1.13
15-Jul	985.41±206.43	1453.40±445.45	2.00±1.43	1.00±0.47	0.00±0.00	0.00±0.00	0.66±0.54	0.50±0.35
1-Aug	890.81±187.62	1173.90±285.03	0.22±0.13	0.66±0.47	0.66±0.81	0.00±0.00	0.33±0.20	0.50±0.35
15-Aug	654.31±69.63	1434.76±392.41	0.22±0.13	0.55±0.27	0.00±0.00	0.00±0.00	0.66±0.20	1.16±0.88
1-Sep	788.33±135.19	1509.30±372.29	0.33±0.00	0.77±0.54	0.00±0.00	0.00±0.00	0.50±0.35	0.66±0.54
15-Sep	433.58±25.54	801.23±207.59	0.11±0.13	0.22±0.13	1.00±1.22	0.00±0.00	0.33±0.40	0.50±0.35
1-Oct	720.85±111.76	1229.24±128.54	0.11±0.13	0.11±0.13	0.00±0.00	0.66±0.81	0.33±0.20	0.16±0.20
15-Oct	933.70±79.04	1480.79±128.60	0.22±0.13	0.33±0.23	0.00±0.00	0.00±0.00	0.83±0.20	0.50±0.35
1-Nov	797.47±136.84	1286.25±214.65	0.44±0.13	0.33±0.23	0.33±0.40	0.00±0.00	1.16±0.20	1.00±0.70
Mean	1068.43	1735.95	2.13	1.40	0.42	0.36	2.88	3.01
LSD (p=0.05)	740.85	954.89	3.34	1.75	NS	1.46	4.55	4.08

[Mean±S.E.m]*

Table 2: The correlation coefficient between various *Tropilaelaps clareae* (Delfinado and Baker) parameters and worker brood rearing and ambient weather parameters in different bee strength *Apis mellifera* Linnaeus colonies

Parameters	8-10 bee-frame strength *A. mellifera* colonies				18-20 bee-frame strength *A. mellifera* colonies			
	Worker brood rearing	Ambient temperature (^{0}C)		Mean ambient RH (%)	Worker brood rearing	Ambient temperature (^{0}C)		Mean ambient RH (%)
		Max	Min			Max	Min	
T. clareae population in worker brood	0.74*	-0.02^{ns}	-0.25 ^{ns}	-0.19 ^{ns}	0.79*	-0.06^{ns}	-0.27^{ns}	-0.15^{ns}
T. clareae population on adult bees	0.44*	-0.04 ^{ns}	-0.15 ^{ns}	-0.04 ^{ns}	0.49*	-0.15^{ns}	-0.26^{ns}	0.14 ^{ns}
T. clareae fall	0.80*	0.00 ^{ns}	-0.26 ^{ns}	-0.29 ^{ns}	0.77*	-0.04^{ns}	-0.26^{ns}	-0.13^{ns}

Significant at 5 per cent level; ns: non-significant

Conclusions

The present examination unmistakably demonstrated that there was most extreme mite increase during dynamic brood raising time of the honey bee which was additionally shown as a little parasitic mite population on adult bees during the spring brood raising indicated negligible phoresis. Further, in twofold strength (18-20) honey bee colonies wherein brood raising had been higher than in low strength (8-10) honey bee colonies, the parasitic mite population had been the lowest in the brood of double strength (18-20) colonies which could be credited to high adult honey bee population (more caretaker honey bees in a hive) brought about more Mite fall which brought about low rate of the mite in the brood.

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