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SEASONAL INCIDENCE OF ECTOPARASITIC MITES AND GREATER WAX MOTH IN *APIS MELLIFERA* L COLONIES

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ABSTRACT

The incidence of ectoparasitic mites *Varroa destructor* (Anderson and Trueman), *Tropilaelaps clareae* (Delfinado and Baker) and wax moth *Galleria mellonella* (L.) on the honey bee *Apis mellifera* L. was assessed during 2019-20 at the Krishi Vigyan Kendra, Morena, Madhya Pradesh. The results revealed that *V. destructor* infestation was at minimum (4%) in the first fortnight of July 2019 and maximum (14%) in the first fortnight of September 2019 with the visual examination method. There existed a negative correlation with the brood area. *Tropilaelaps clareae* infestation was minimum (6 mites/ colony) in the second fortnight of November 2019 and maximum (22 mites/ colony) during the first fortnight of October 2019, as assessed by the sugar dusting method. The infestation revealed a negative correlation with colony strength. *Galleria mellonella* incidence was at maximum (176 larvae, 72 pupae, and 17 adults/ colony) in the second fortnight of September 2019 and minimum (5 larvae, 5 pupae, and 2 adults/ colony) in the first fortnight of September, August and August 2019, respectively. The number of pupae and brood area was found significantly negatively correlated (r= -0.534); while the number of adults vs. colony strength and brood area were significantly negatively correlated (r= -0.499 and r= -0.607, respectively).

Key words: *Apis mellifera*, *Tropilaelaps clareae*, *Varroa destructor*, *Galleria mellonella*, seasonal incidence, brood area, colony strength, correlation coefficients, honey store, pollen area

Honey bees are beneficial creatures playing a pivotal role in the pollination of a wide variety of crops and help in maintaining biological diversity (Johannesmeier and Mostert, 2001). These are attacked by many diseases and enemies, which cause weakness of colonies and ultimately low honey production. These include mites, diseases, insect pests, wasps, ants, *Apis dorsata*, lizards, bee-eater birds, etc. Among the ectoparasitic mites, *Varroa destructor* (Anderson and Trueman) is a brood pest of European honey bee *Apis mellifera* L. causing loss of >50% of colonies (Martin et al., 2012; Nazzi et al., 2012). The infestation by *Tropilaelaps clareae* (Delfinado and Baker) causes irregular pattern of sealed and unsealed brood as found with all brood diseases. Modern beekeeping with *A. mellifera* in tropical and subtropical Asia frequently encounters infestation with ectoparasitic mite *T. clareae*. Greater wax moth *Galleria mellonella* L. is the most destructive insect pest of hives and damages the combs causing heavy losses (Williams, 1997; Milam, 1970). Beekeepers should be aware of these enemies and protect their colonies to get the maximum benefits. As only a few attempts have been made to observe the seasonal incidence of parasitic mites and wax moth infesting *A. mellifera*, the present study was conducted.

MATERIALS AND METHODS

The present study was carried out in the apiary at the RVSKVV- Krishi Vigyan Kendra, Morena (26.47°N, 77.98°E, 177 masl), Madhya Pradesh, during 2019-20. Observations were recorded at fortnightly intervals; for colony strength, three randomly selected colonies were observed for the number of frames covered with bees, with both the faces of bee frames taken into account. The brood area and pollen area were recorded in terms of capped brood and pollen comb cells with the help of a wire grid frame (brood measuring frame) by placing it on the hive frame and the number of squares of wire grid covering the brood and pollen area was counted. This provided the brood and pollen area in inch^2 which was converted into cm^2 after multiplying by a factor of 6.45. Honey stores were estimated visually on the assumption that one Langstroth frame sealed with bee wax from both sides of the frame contains 1800-2000 g of honey. The incidence of ectoparasitic mites *V. destructor* and *T. clareae*) in brood and adults was recorded in three selected colonies of *A. mellifera* by

sugar dusting/ visual examination method (Asha et al., 2013; Poonia et al., 2014).

In the visual examination method, 30 brood cells in each selected colony were diagnosed and identified based on their physical appearance with the help of a lens and in the sugar dusting method; 15 g of powdered sugar was kept over the top bar of every bee frame and was gently dusted along with the cells with a bee brush. Mites falling after dusting were collected on the sticky paper screened at the bottom board and were counted after 24 hr. For *G. mellonella* five colonies of equal bee strength were selected randomly and were equalized concerning all colony parameters. All the bees were brushed off the frames and were held against the sunlight and observations were recorded on the number of larvae, pupa, and adults. The experiment was conducted in randomized block design and all the research data were square-root transformed as per the method described by Gomez and Gomez (1986). The data were subjected to statistical analysis by adopting ANOVA as described by Fisher (1958) and by comparing the treatments by using F-test. Data on weather parameters such as temperature, relative humidity, and rainfall was obtained from the Agrometeorological Unit, Zonal Agriculture Research Station, Morena. Correlation coefficients of incidence with weather factors were worked out.

RESULTS AND DISCUSSION

Varroa destructor infestation depicted in Table 1 reveal that it was maximum i.e., 14% in the first Fortnight of September, followed by 11% in the first fortnight of October 2019. The infestation was invariably observed ranging from 4% in the first fortnight of July to 8% in the second fortnight of September 2019. Brood was free from mite infestation in the rest of the months. When it was measured by sugar dusting on top bars of bee frames, a similar trend was seen. The data on the correlation of *V. destructor* incidence with colony and weather parameters showed a significantly negative correlation with brood area {r= -0.522 (visual examination) and r= -0.548 (sugar dusting method)}. During the period of absence of *V. destructor*, it was observed that colony strength and brood area were considerably high. The present results derive support from earlier observations with temperature and rainfall which reported a positive correlation (Deosai and Chhuneja, 2012; Asha et al. 2013; Poonia et al. 2014). Incidence of *V. destructor* was at its peak during September and October corroborating with the results of Fries et al. (1994), Tibor and Szabo (2003) and DeGrandi-Hoffman and Curry (2004).

Seasonal incidence of *Varroa* spp., in *A. mellifera* colonies showed that the maximum infestation occurs during April (12.10± 0.57) in 2008-09; Sharma et al. (2011) observed maximum incidence during November in the Shivalik hills of Himachal Pradesh. Although the peak infestation was noticed in October (72.00± 0.86), November (80.10± 1.58) and December (72.50± 1.27) during 2009-10 (Sharma et al., 2011). Poonia et al. (2014) observed the maximum number of mites in the second fortnight of May (38 and 51 mites/ hive) which showed a significant positive correlation with maximum (r= 0.659) and minimum (r= 0.648) temperature; however, a negative correlation was observed with relative humidity (r= -0.416) and sunshine hours (r= -0.023), whereas rainfall was non-significantly correlated (r= 0.019). It was suggested that during the summer months, when the temperature is high and flower availability is less, the mite population increases. Sharma (2010) while screening *A. mellifera* against *Varroa* reported infestation at Nauni varying from 0.70± 0.15 to 12.10± 0.57. Rinkevich et al. (2017) observed that *V. destructor* can cause devastating losses through direct feeding, vectors for transmitting diseases, and increasing pathogen and parasite susceptibility.

The incidence of *T. clareae* reveals that the brood infestation was noticed initially at 3% in the first fortnight of September 2019. Colonies were invariably infested from 2% in the second fortnight of September to 13% in the first fortnight of November 2019. Colonies were found free from infestation during the remaining months. A similar trend was observed with sugar dusting on top bars of bee frames. The brood infestation was observed to be at minimum of 6 mites/ colony in the second fortnight of November and the maximum was in the first fortnight of October 2019 (22 mites/ colony). Colonies were invariably infested with *T. clareae* from 15 mites/ colony in the first fortnight of September to 19 mites/ colony on the second fortnight of October 2019. The correlation of incidence showed a significant negative correlation with colony strength (r = -0.671) and relative humidity (r= -0.515) by visual examination method; and also showed a significant negative correlation with colony strength (r = -0.552) and brood area (r= -0.591).

The incidence of *T. clareae* in *A. mellifera* had been reported to vary greatly (Camphor and Martin, 2009). The present findings agree with those of earlier workers-Chahal et al. (1986) who observed two peaks period of *T. clareae* infestation i.e. February to May (33.7-51.7%) and September to November (26.8-42.0%), coinciding with a peak of brood rearing activity in Ludhiana, Punjab. Aggarwal and Kapil (1998) reported a high

Table 1. Incidence of *V. destructor*, *T. clareae,* and *G. mellonella* in *A. mellifera* colonies and colony parameters (July 2019- February 2020)

Months	Fort-night	Incidence of *V. destructor*		Incidence of *T. clareae*		Incidence of *G. mellonella*			Colony parameters	
		Visual examination method (%)	Sugar dusting method (no./ colony)	Visual examination (%)	Sugar dusting method (no./ colony)	No. of larvae/ colony	No. of pupae/ colony	No. of adults/ colony	Colony strength (bee frames)	Brood area (cm^2)
July, 2019	1	4.00 (2.12)*	10.00 (3.24)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.20	1590.59 (39.89)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.50	1849.51 (43.01)
August	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	10.00 (3.24)	5.00 (2.35)	2.00 (1.58)	5.20	2042.29 (45.2)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	27.00 (5.24)	8.00 (2.92)	4.00 (2.12)	5.90	1829.87 (42.78)
September	1	14.00 (3.81)	29.00 (5.43)	3.00 (1.87)	15.00 (3.94)	5.00 (2.35)	37.00 (6.12)	5.00 (2.35)	5.50	1770.67 (42.09)
	2	8.00 (2.92)	19.00 (4.42)	2.00 (1.58)	18.00 (4.3)	176.00 (13.29)	72.00 (8.51)	17.00 (4.18)	5.00	1630.33 (40.38)
October	1	11.00 (3.39)	35.00 (5.96)	9.00 (3.08)	22.00 (4.74)	138.00 (11.77)	56.00 (7.52)	15.00 (3.94)	4.30	1419.17 (37.68)
	2	6.00 (2.55)	18.00 (4.3)	10.00 (3.24)	19.00 (4.42)	87.00 (9.35)	39.00 (6.28)	19.00 (4.42)	3.60	1190.88 (34.52)
November	1	0.00 (0.71)	0.00 (0.71)	13.00 (3.67)	17.00 (4.18)	37.00 (6.12)	13.00 (3.67)	11.00 (3.39)	3.30	1610.21 (40.13)
	2	0.00 (0.71)	0.00 (0.71)	8.00 (2.92)	6.00 (2.55)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	3.70	2361.30 (48.6)
December	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	4.90	3290.11 (57.36)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	6.80	3920.49 (62.62)
January, 2020	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	8.00	4123.63 (64.22)
	2	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	8.40	3409.71 (58.4)
February	1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	7.30	2513.59 (50.14)
	2	5.00 (2.35)	13.00 (3.67)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	6.20	1990.34 (44.62)
CD (p=0.05)		0.12	0.28	0.17	0.29	0.27	0.25	0.37	0.06	0.62

* Figures in parentheses square root √x+0.5 transformed values

infestation rate of *T. clareae* from March to April (21.75-29.80%) and October to November (13.80 &14.23%) at Hisar, Haryana. These findings corroborate with those of Nagaraja and Rajagopal (2003). Thakur et al. (2009) studied seasonal fluctuations in the population of *T. clareae* in *A. mellifera* colonies at Solan, Himachal Pradesh and made observations similar to the present. Padhi and Rath (2012) observed peaks in October followed by November. Sharma et al. (2011) observed peak in colonies during September-October in Himachal Pradesh. Their observations revealed that the *T. clareae* population peaked in September (56.00± 0.31) and October (43.00± 7.40).

The occurrence of *G. mellonella* (larval, pupal as well as adult)/ colony increased from the first fortnight of August till the second fortnight of September 2019; thereafter, started declining till first fortnight of November 2019 (Table 1); maximum larval counts (176 larvae/ colony) was in the second fortnight of September followed by 138 larvae/ colony in the first fortnight of October 2019; while the least of 5 larvae/ colony was in the first fortnight of September 2019. Maximum pupation (72 pupae/ colony) was in the second fortnight of September followed by 56 pupa/ colony in the first fortnight of October 2019, and the least of 5 pupa/ colony was in the first fortnight of August 2019. Maximum adults were observed (19 adults/ colony) in the second fortnight of October followed by 17 adults/ colony in the second fortnight of September 2019, 15 adults/ colony in the first fortnight of October, while the least of 2 adults/ colony was in the first fortnight of August 2019.

Correlation coefficients revealed that the larval counts are negatively correlated with colony and weather parameters, being non-significant. The correlation between the number of pupae and brood area was found significantly negative (r= -0.534). The correlation between the number of adults and colony strength was found negative and significant (r= -0.499) and negative and highly significant for the brood area (r= -0.607). This study on the incidence of larva, pupa, and adult of *G. mellonella* is in line with the other previous studies (Sharma et al., 2011). Raghunandan and Basavarajappa (2014) reported that the colonies with weak populations were more prone to *G. mellonella* infestation and it was more in the semi-arid region during summer (30.80% colonies) followed by the rainy season (23.40%). Kebede et al. (2015) carried out a cross-sectional study from April 28 to May 30, 2009, in four villages of Kafta Humera (Ethiopia) to determine the prevalence of wax moths in modern hive colonies. According to the study, the overall prevalence of *G. mellonella* larvae in modern bee hives was found to be 27.4% and the nature of severity was categorized into three groups. Swati and Verma (2016) studied *A. mellifera* pests in residential and migratory apiaries and its relation with weather parameters; occurrence of *G. mellonela* was significantly higher in migratory apiaries in all the blocks except in Morena and Joura. The incidence of wax moths in all the five blocks except in Morena and Joura was significantly higher (13.6) in migratory apiaries as compared to residential apiaries. The incidence of wax moth larvae was not correlated with the weather parameters except for minimum temperature (r= 0.758).

Sohail et al. (2017) studied the seasonal abundance of *G. mellonella* larvae in hives of honey bees located in district Sargodha, Punjab, Pakistan, and reported that the maximum moth abundance occurred during the regional dearth period, i.e. May-November. The peak abundance (14.8± 3.9 moth larvae/ hive) occurred in August. Usually, weak colonies succumb to moth damage first. The active period of this insect pest has been shown to occur from March to October in India (Garg and Kashyap, 1998) and can remain sustained in November (Brar et al., 1985). In the present study from July 2019 to February 2020, maximum moth abundance occurred from the second fortnight of September 2019 to mid-October, this may be due to increased relative humidity and temperature, unfavorable weather conditions, and scarcity of bee flora (Varshneya et al., 2008). Not surprisingly, wax moth abundance and the % of hives with moth-damaged combs were directly proportional to each other.

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AUTHOR CONTRIBUTION STATEMENT

N. conceived designed research, conducted experiments, and analyzed data. A SY guided and supervised the research. N and N.K. wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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