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Demographics and developmental biology of mites infesting mulberry

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Abstract
The study was conducted from March to December 2019 to know the species composition of mites infesting bush and tree type mulberry gardens. Observations were recorded at fortnightly intervals. The study revealed the association of two phytophagous mites *viz.*, *Polyphagotarsonemus latus* (Banks), *Tetranychus truncatus* (Ehara) and three predatory mites *viz.*, *Neoseiulus longispinosus* (Evans), *Euseius ovalis* (Evans), *Amblyseius* sp. In both tree and bush mulberry, *P. latus* was observed only in the upper canopy (top 6-7 leaves). *P. latus* infested foliage becomes rigid or bronzed and assumes a shrivelled and scorched aspect. The apical leaves were heavily damaged, grow distorted and die; similarly in case of the buds. Blistering, crinkling and development of dark colour follows mite attack. Total population of *P. latus* (2cm^2 leaf area) attained peak in November and August on bush and tree mulberry, respectively. Total population of *P. latus* showed significant positive correlation with RH on both bush and tree type of mulberry. The total developmental period for *P. latus* and *T. truncatus* ranged from 3.24 to 3.91 days and 9.40 to 9.52 days, respectively.

Keywords: *Polyphagotarsonemus latus*, *tetranychus truncatus*, mulberry, demographics, biology

Introduction
The increase in productivity of silk per unit area can be achieved by suitable production technologies, combined with eco-friendly management of pests and diseases of mulberry and the silkworm. Poor yield of mulberry is attributed to a number of factors, of which losses inflicted by insect pests has been considerable. Though the frequent leaf picking and pruning of the shoot restrict the pest build up, many of them still find enough time and place on mulberry for feeding and breeding. Over 300 insect and non-insect pests have been reported to attack mulberry (Srinivasgowda 2004) [19]. Mite infestation in mulberry adversely affects the economic characters of silkworm and cocoon.

The importance of mites as crops pests is noteworthy. Narayanaswamy *et al* (1996) [12] reported fifteen species of mites on mulberry, of which seven species were reported from India *viz*., *Aceria mori* Keifer, *Eotetranychus orientalis* (Klein), *Tetranychus equitorius* McGregor, *T. ludeni* Zacher, *T. neocaledoinicus* Andre, *T. telarius* Linnaeus and *T. urticae* Koch. *Tetranychus truncatus* Ehara was first described from mulberry in Japan (Ehara 1956) [6]. Bolland *et al* (1998) [4] reported 62 host plants for *T. truncatus* and its distribution is restricted to Asian countries. *T. truncatus* was recorded from the North Western Himalayan regions of Jammu and Kashmir and Himachal Pradesh on *Dahlia* sp. (Rather 1983) [16]. It was reported on both wild and cultivated species of mulberry in Karnataka (Srinivasa *et al* 2012) [21]. Though *Polyphagotarsonemus latus* (Banks) was reported on jute as a serious pest since 1940, it was reported on mulberry only in 2002 (Chauhan *et al* 2002) [5]. *P. latus* is a major pest in chilli and also known to attack more than 250 species of plants (Rajlakshmi *et al* 2009) [14]. It is popular known as yellow mite, broad mite, chilli mite, *etc*.

Material and Methods
Seasonal incidence
Fortnightly collection of mites was made from March to December during 2019 in Bengaluru (Location 1) (12.8691° N, 77.5342° E) and Chikkaballapura (Location 2) (13.2781° N, 77.9096° E) districts, where mulberry is cultivated as bush and tree, respectively. Fortnightly collection of mites was also done in bush type of mulberry garden at GKVK campus (Location 3). In each location five mulberry gardens with V-1 variety were selected and each garden was sub-divided into three sub-plots.

In each sub-plot, five plants were selected and again each plant canopy was divided based on number of leaves as upper (6-7), middle (8-14), lower (>14). From each canopy level, three leaves were selected and put into polyethylene bags and sample number was labelled accordingly. The collected samples were taken to the laboratory and observations were recorded with a stereo-zoom microscope for eggs and active stages (protonymph, deutonymph and adults of *T. truncatus*; larvae and adults of *P. latus*) at three spots in each leaf (base, middle and tip) using $2cm^2$ window for *T. truncatus* and $1cm^2$ for *P. latus*. To have uniformity for comparison, the counts were converted to 2 cm^2 for *P. latus* population.

Developmental Biology

Maintenance of stock culture under laboratory conditions

The *T. truncatus* mites collected on mulberry during the survey from each location were reared by placing mulberry leaf on water-soaked sponge sheets in trays. The mites were allowed to lay eggs and colonize for 10 to 15 days. Later adult female and males were picked individually and mounted on the glass slide separately for species confirmation, after which the culture from different locations were pooled and used as stock culture for the study of developmental biology. The leaf was maintained in turgid condition by watering daily and leaves were changed periodically as and when they got dried up (Anuradha, 2013). To prolong the freshness of the mulberry leaves, blotting paper was placed on the sponge. The maintenance of stock culture for

P. latus was initially tried with detached leaf technique on three different hosts namely chilli, French bean and mulberry (V-1 and MR-2) but culture was not established. Finally, *in vivo* culture was established on potted plants of V-1 mulberry plant in green house.

Study of developmental biology under laboratory conditions

Biology of *T. truncatus* was studied by adopting detached leaf technique (keeping the ventral surface upwards) in the laboratory on mulberry leaf (V-1 variety) at room temperature (23.72℃ to 24.8℃) and relative humidity (61.56% to 54.1%). Hundred gravid females of *T. truncatus* along with twenty-five males were released on fresh leaf bits of mulberry maintained on sponge sheet in a plastic tray. They were allowed to lay eggs for 3-4 hours and then males and females were removed. Observations were recorded once in every three-hours. Soon after hatching the larvae were immediately transferred individually to fresh leaf bits of 2 cm^2 placed on a sponge sheet in a separate tray. Fifty such leaf bits containing the larvae of *T. truncatu*s were maintained to study the developmental biology. The duration of egg, larval, nymphal stages and quiescent stages were recorded. The observations on size and colour of all the stages and other morphological characters were recorded stage-wise.

Hundred females and fifty males were released on the tender mulberry leaf and left for oviposition until 60- 70 eggs were obtained. Due to high mobility of *P. latus* vaseline was smeared on the edges of the leaf to reduce the mobility. Due to difficulty in transferring the eggs, larvae immediately after hatching, individual larvae were transferred to fifty different leaf bits of $1cm^2$ size. The leaf bits were placed on a sponge sheet with blotting paper in a separate tray. Observations were recorded once in three hours interval. The duration of developmental stages (egg, larvae and quiescent) were recorded until the adult emergence. Observations on colour, size and other morphological characters of all the stages were also recorded.

Results and Discussion

Incidence of *P. latus, T. truncatus* and predatory mites in mulberry gardens

The *P. latus* (eggs and active stages) and predatory mites / $2cm^2$ leaf area reached maximum peak in November {(112.65±15.73), (4.16±0.34)}. The total population of *T. truncatus* (eggs and active stages) / $2cm^2$ leaf area established rapidly with highest population in June (14.56±5.36) at Location 1(Fig.1a).

Total population of *P. latus* / $2cm^2$ leaf area attained a peak in September (18.07±5.84).

T. truncatus total population (eggs and active stages) / $2cm^2$ leaf area was found to be lowest in May (16.25±5.66). The population showed the rise and fall between June (19.08±5.85) and August (19.55±5.87), then onwards the population decreased by September (18.32±5.82) and it remained steady from October (16.84±5.71) to December (16.79±5.12). The total population of predatory mites (eggs and active stages) / $2cm^2$ leaf area was observed from July (2.76±0.42) which showed a sudden rise in August (3.20±0.50) and it gradually declined reaching lowest levels in November (2.07±1.00), becoming nil in December at Location 2 (Fig.1b)

P. latus reached peak levels in August (120.00±8.33) maximum peak in November (123.80±6.95). The total population of *T. truncatus* (eggs and active stages) / $2cm^2$ leaf area was found to be lowest in April (9.08±3.82). The population doubled in May (14.16±3.41) and started to oscillate between May to August (18.53±4.56) and August to November (19.16±4.92), followed by a decline in December (14.38±8.12). The total population (eggs and active stages) of predatory mites / $2cm^2$ leaf area was observed from April (2.14±0.20). The population reached a peak in June (2.97±0.69), preceded by a decline in May (2.11±0.29). It again showed a decline in July (2.45±0.50) followed by increase in August (2.59±0.60). The population again decreased and reached minimum levels in October (2.11±0.24), followed by a rapid increase in November (2.84±0.72) and slight increase in December (2.52±0.45) at Location 3 (Fig. 1c)

Total population of *P. latus* revealed, highly significant positive correlation with relative humidity and significant negative correlation with wind speed. *T. truncatus* total population revealed the non-significant negative correlation with maximum temperature and wind speed and non-significant positive correlation with minimum temperature, relative humidity and rainfall. Correlation of the total population of predatory mites revealed significant negative correlation with maximum temperature, non-significant negative correlation with minimum temperature, wind speed and non-significant positive correlation with relative humidity and rainfall at Location 1 (Table1).

The total population of *P. latus* showed significant negative and positive correlation with temperature and relative humidity. The total population of *T. truncatus* revealed its non- significant negative correlation with temperature and highly significant positive correlation with relative humidity beside non-significant positive correlation with wind speed and rainfall. The total predatory mite population had a non-significant negative correlation with temperature, non-significant positive correlation with relative humidity, wind

speed and rainfall at Location 2 (Table1).
The total population of *P. latus* showed significant negative and positive correlation with temperature and relative humidity, respectively. The total population of *T. truncatus* revealed its non-significant negative correlation with temperature, wind speed, rainfall, while it had non- significant positive correlation with relative humidity. The total population of predatory mites showed a non-significant negative correlation with temperature, rainfall. While, non-significant positive correlation with relative humidity and non-significant negative correlation with wind speed was observed at Location 3 (Table1).
The results obtained are in concurrence with the studies of Chauhan *et al* (2002) [5] regarding the fluctuation in the *P. latus* population during the entire study period. Rajalakshmi *et al* (2009) [14] reported that *P. latus* population in mulberry had a positive correlation and non-significant positive correlation with RH and rainfall, respectively. The studies of Ghose *et al* (2018) [7] on bell pepper pests, showed their non-significant negative correlation with temperature (maximum, minimum) and wind speed. Bathari *et al* (2016) [3] showed that the *P. latus* incidence in capsicum had a non-significant negative correlation with temperature (maximum and minimum). The significant positive correlation of *P. latus* incidence with relative humidity was also reported by the studies of Ahuja (2000) [1] on sesame crop and Kavitha *et al* (2007) [9] in Jatropa and by Srinivasulu (2000) [20] in chilli for relative humidity and maximum temperature. The results obtained on correlation of predatory mites with relative humidity are in line with the results of Ghosal *et al* (2004) [8], Pokle (2016) [13] on *Avicenia alba* and tomato crop, respectively.

Developmental Biology of Phytophagous Mites

Developmental biology of *P. latus* under laboratory conditions (mean temperature of 23.72℃ to 24.8 and mean RH of 61.56% to 54.1%)

The life cycle parameters of *P. latus* which includes egg, larva, quiescent1 and adult stages are detailed below. The description of the stages are also furnished in the subsequent paragraphs:

Egg: The egg was found to be oval in shape with 8 to 10 rows of tubercles arranged longitudinally. The colour of the egg remained white from the time of egg laying upto hatching. The eggs were found attached to the base of the midrib of mulberry leaf. The morphometric parameters were recorded through calibrated ocular micrometer. The length and breadth were found to be 119.50±6.29 µm and 70.38±11.47 µm, respectively (Table 3). The incubation period for male and female was found to be 40.05±0.53 h and 41.33±0.18 h, respectively (Table 2). The present results are on par with the findings of Chauhan *et al*. (2002) [5] on mulberry.

Larva: The larva of the yellow mite was found to be spindle shaped, with milky white colour and longitudinal mid-dorsal white band. It has three pairs of legs. The length and breadth of the mite were found to be 216.53±17.36 µm and 130.76±5.55 µm, respectively (Table 3). The duration of the male and female larva was found to be 22.68±0.84 h and 22.37±1.48 h, respectively (Table 2). The present results are supported by the findings of Wuryantini *et al*. (2014) [24] on orange, mandarin, tangerine and that of Rai *et al.* (2007) [15] on chilli.

Quiescent 1: Quiescent 1 was found to be resting stage in the life cycle of mite, it is also called as chrysalis. The quiescent is spindle shaped and pointed at both ends. The longitudinal mid- dorsal white band is reduced and having an hour glass shape. The male carries the female quiescent before it becomes adult female. This behaviour is known as copulatory guarding. The length and breadth of the quiescent was found to be 286.14±15.50 µm and 130.38±6.26 µm, respectively (Table 3). The duration of male and female quiescent stage was found to be 15.08±0.21 h and 15.28±1.36 h, respectively (Table 2). This stage of the mite was found to have the shortest duration in the life cycle.

Male adult: The adult was found to have the prominent four pairs of spider like legs, it is highly motile compared to adult female. It carries the female quiescent. The colour of the male adult is golden yellow in colour. The length and breadth were found to be 188.84±7.55 µm and 124.30±5.87 µm, respectively (Table 3).

Female adult: The female adult has four pairs of legs and is found to be less motile as compared to the male. The body of the mite appears glossy with golden yellow colour because of sclerotization. Depression was noticed on the dorsal side of the body. The length and breadth were found to be 194.45±8.62 µm and 115.69±10.50 µm, respectively (Table 3).
Incubation period accounts for 51.47 per cent of the total developmental period and quiescent is of shortest duration. The developmental duration of female is slightly more than that of male which may be due to development of reproductive organs. Among the developmental stages, length and breadth was found to be highest in quiescent and larval stage, respectively.
The total developmental period of the *P. latus* was found to be 77.81±0.88 h (3.24±0.04 days) and 78.98±2.71 h (3.91±0.11 days) for male and female, respectively (Table 3). The female biased sex ratio of 2.98:1 was recorded. These results are supported by the findings of Verirera and Chiavegato (1999) [22] on lemon and Shukla and Radadia (2018) [18] on chilli.

Developmental biology of *T. truncatus* under laboratory conditions (mean temperature of 23.99 °C to 24.60 °C and mean RH of 75.09% to 72.55%)

The life cycle of *T. truncatus* includes egg, larva, quiescent 1, protonymph, quiescent 2, deutonymph, quiescent 3 and adult.

Egg: The eggs were laid near the midrib, newly laid eggs were round in shape and translucent in colour and colour turned to creamish white by the time of hatching. The incubation period was found to be 106.87 h and 105.90 h for male and female, respectively (Table 2). The length and breadth of the egg were found to be equal (142.15 µm) (Table 3).

Larva: The newly hatched larvae were pale yellow in colour with three pairs of legs. The larval duration was 18.58±1.58 h and 18.88±1.13 h for male and female, respectively (Table 2). The length and breadth of larvae were found to be 143.38±16.32 µm and 131.15±14.39 µm, respectively (Table 3).

Quiescent 1: It is the resting stage between larva and protonymph. The duration was found to be 16.50±2.71 h and

16.45±1.86 h for male and female, respectively (Table 2). The length and breadth of quiescent 1 were found to be 143.53±16.06 µm and 135.15±14.39 µm, respectively (Table 3).

Protonymph: It was yellow-orange in colour. It has four pairs of legs. The duration was found to be 19.00±4.11 h and 19.65±3.25 h for male and female, respectively (Table 2). The length and breadth of protonymph were found to be 183.84±22.26 µm and 156.15±25.63 µm, respectively (Table 3).

Quiescent 2: It is the resting stage between protonymph and deutonymph stages**,** the duration was found to be 21±1.81 h and 20.32±3.49 h for male and female, respectively (Table 2). The length and breadth of quiescent 2 were found to be 222.30±28.89 µm and 189.99±26.81 µm, respectively (Table 3).

Deutonymph: It was orange in colour, male or female could be distinguished during this stage due to differences in abdomen shape. The duration was found to be 17.50±h and 17.13±h for male and female, respectively (Table 2). The length and breadth were found to be 285.37±25.24 µm and 232.30±29.85 µm, respectively (Table 3).

Quiescent 3: It is the resting stage between deutonymph and adult, the duration of quiescent 3 was found to be 26.25±h and 30.19±h for male and female, respectively (Table 2). The length and breadth were found to be 382.29±25.63 µm and 206.15±17.13 µm, respectively (Table 3).

Male: They were orange- red in colour, abdomen was wedge shaped. The length and breadth were found to be 287.68±23.86 µm and 182.30±20.11 µm, respectively (Table 3).

Female: They were orange- red in colour, abdomen was round and broad in shape. The length and breadth were found to be 373.06±42.64 µm and 219.99±24.25 µm, respectively (Table 3).

The total developmental period of the *T. truncatus* male and female was found to be 225.70±7.44 h (9.40±0.31 days) and 228.53±6.43 h (9.52±0.27 days), respectively. The female biased sex ratio of 2.58:1 was recorded (Table 2). Incubation period forms 47.30 per cent of total developmental period, quiescent1 is found to have shorter duration among the developmental stages. Total developmental period for female is slightly more than that of male. Adult female is found to have longer length among the developmental stages, whereas highest breadth is seen in deutonymphal stage. The present results are in concurrence with the findings of Sakuwarin *et al*. (2003) [17] and Win *et al.* (2018) [23] on mulberry.

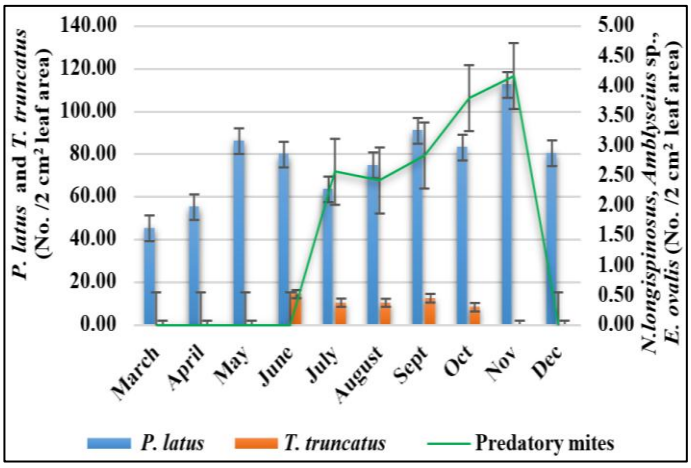

Fig 1 (a): Location 1

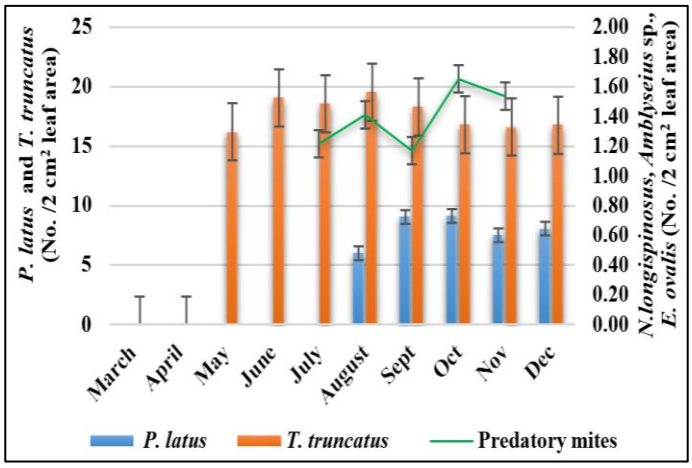

Fig 1 (b): Location 2

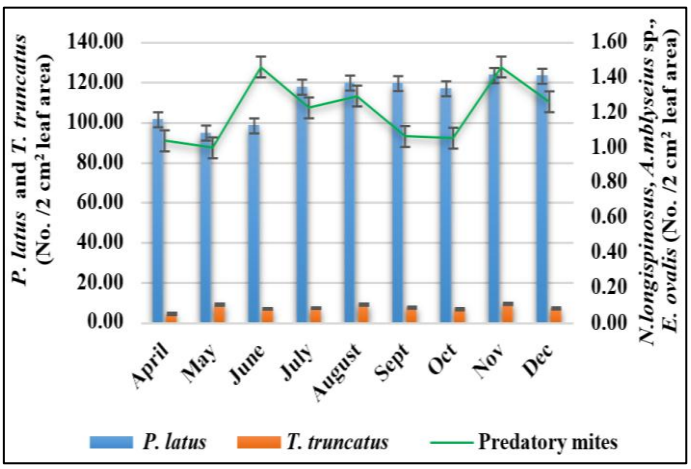

Fig 1 (c): Location 3

Fig 1: Incidence of mite species in mulberry gardens during-2019

Table 1: Correlation between incidence of mites (eggs and active stages) and weather parameters at different locations during-

2019

Weather Parameters		Location 1			Location 2			Location 3		
		P. latus / 2cm^2 leaf area	*T. truncatus* / 2cm^2 leaf area	Predatory mites / 2cm^2 leaf area	*P. latus* / 2cm^2 leaf area	*T. truncatus* / 2cm^2 leaf area	Predatory mites / 2cm^2 leaf area	*P. latus* / 2cm^2 leaf area	*T. truncatus* / 2cm^2 leaf area	Predatory mites / 2cm^2 leaf area
Temperature (℃)	Min	-0.303	0.405	-0.539	-0.093	-0.593	-0.485	-0.878**	-0.431	-0.401
	Max	-0.586	-0.468	-0.736*	-0.156	-0.417	-0.418	-0.887**	-0.403	-0.331
RH (%)		0.775**	0.557	0.629	0.520	0.791**	0.630	0.867**	0.415	0.431
Wind speed (km/h)		-0.694*	-0.107	-0.525	0.631	0.490	0.517	0.130	0.105	0.367
Rainfall (mm)		0.364	0.457	0.497	0.470	0.331	0.425	-0.014	0.185	-0.419

Predatory mites**:** *Neoseiulus longispinosus*, *Euseius ovalis* and *Amblyseius* sp.

* Significant at the 0.05 level (2-tailed).

#Active stages of *P. latus*- larva and adult; Active stages of *T. truncatus*- larva, protonymph, deutonymph and adult; Active stages of predatory mites- larva and adult

Table 2: Developmental biology of phytophagous mites on mulberry under laboratory conditions

Stage	*P. latus*		*T. truncatus*	
	Duration (h)*		Duration (h)*	
	Male	Female	Male	Female
Incubation Period	40.05±0.53	41.33±0.18	106.87±1.44	105.90±1.10
Larval Period	22.68±0.84	22.37±1.48	18.58±1.58	18.88±1.13
Quiescent 1	15.08±0.21	15.28±1.36	16.50±2.71	16.45±1.86
Protonymph	-	-	19.00±4.11	19.65±3.25
Quiescent 2	-	-	21.00±1.81	20.32±3.49
Deutonymph	-	-	17.50±5.09	17.13±4.86
Quiescent 3	-	-	26.25±3.65	30.19±3.49
Total developmental period	77.81±0.88	78.98±2.71	225.70±7.44	228.53±6.43
Total developmental period(days)	3.24±0.04	3.91±0.11	9.40±0.31	9.52±0.27
Sex ratio (Male: Female)	1: 2.98		1: 2.58	

*Mean of 50 individuals

Table 3: Morphometric parameters of Phytophagous mites raised on mulberry

Stage		*P. latus*		*T. truncatus*	
		Length (µ)* (Mean ± S.D)	Breadth (µ)* (Mean ± S.D)	Length (µ)* (Mean ± S.D)	Breadth (µ)* (Mean ± S.D)
Egg		119.50±6.29	70.38±11.47	142.15±12.45	142.15±12.45
Larva		216.53±17.36	130.76±5.55	143.38±16.32	131.15±14.39
Quiescent 1		286.14±12.50	130.38±6.26	143.53±16.06	135.15±14.39
Protonymph		-	-	183.84±22.26	156.15±25.63
Quiescent 2		-	-	222.30±28.89	189.99±26.81
Deutonymph		-	-	285.37±25.24	232.30±29.85
Quiescent 3		-	-	382.29±25.63	206.15±17.13
Adult	Male	188.84±7.55	124.30±5.87	287.68±23.86	182.30±20.11
	Female	194.45±8.62	115.69±10.50	373.06±42.64	219.99±24.25

*Mean of 25 individuals

Conclusions

Though two species of phytophagous mites were recorded throughout the study period on both the bush and tree mulberry in all the three locations, only *P. latus* exhibited the symptoms of damage only on the bush mulberry. Therefore, there is a strong relationship between the type of cultivation of mulberry crop and the incidence of the mite pest. Total population of *P. latus* (2cm^2 leaf area) attained peak in November and August on bush and tree mulberry, respectively, while total population of *T. truncatus* (2cm^2 leaf area) showed maximum incidence in June, November and August in Location 1, Location 2 and Location 3 respectively. Total population of *P. latus* showed significant positive correlation with RH in both bush and tree mulberry, whereas *T. truncatus* showed similar trend only on tree mulberry. Predatory mites showed significant negative correlation only with maximum temperature on bush mulberry. The total developmental period for *P. latus* (egg, larva, quiescent 1 and adult) ranged from 3.24 to 3.91 days and for *T. truncatus* (egg, quiescent1, protonymph, quiescent 2, deutonymph and adult) it ranged from 9.40 to 9.52 days, respectively.

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