Pest Management in Horticultural Ecosystems
Vol. 27, No.2 pp 225-231 (2021)

Temperature dependent development, reproduction and population performance of *Tetranychus hirsutus* Zeity & Srinivasa (Acari: Tetranychidae), on medicinal herb, *Gymnema sylvestre*

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ABSTRACT: Rearing temperature conditions showed profound influence on the biology, reproduction and demography of the mite, *Tetranychus hirsutus*. The time required for the development of female mite from egg to adult was 10.12 to 22.51 days at the rearing temperature conditions of 20°C - 32°C. At 25°C, each mated female laid 76 eggs over 20 days and produced a female-biased progeny of 8:1 sex ratio. Also, higher Net Reproduction Rate of 67.83 female off-springs/female/generation, Mean Generation Time of 24.44 days, Doubling Time of 7.91 days and a weak population progression of 0.076 female offsprings/female/day were observed. The adaxial surface feeding by the mite caused characteristic yellowish to white specks on the leaves of the medicinal herb *Gymnema sylvestre*. As the population explodes, mites colonize the lower surface (abaxial) also, caused extensive feeding damage with profuse webbing. Mite-infested leaves showed reduction in chlorophyll (15.89%), carbohydrate (90.90%), protein (69%) and flavonoid (17.60%) content, while alkaloid content was found increased (14.30%).

Keywords: *Tetranychus hirsutus, Gymnema sylvestre,* life history, demography and qualitative damage, temperature.

INTRODUCTION

Spider mites are the important pests of agricultural crops, damaging all types of economically important cultivated crops, including cereals, pulses, millets, vegetables, plantation, ornamental and medicinal plants (Vacante 2015). In India, Gupta and Karmakar (2010) reported that spider mites are the major pests of medicinal plants. While Sharma and Agarwal (2010) opined that two-spotted mite *Tetranychus urticae* Koch would be a new threat to medicinal herbs in the country. Apart from bronzing, webbing and defoliation they cause chlorophyll depletion, necrosis and ultimately death as consequence of direct damage while, decreased photosynthesis and transpiration are results of indirect damage (Uddin *et al.,* 2015). In 2016, Zeity *et al*. discovered a new spider mite species *Tetranychus hirsutus* damaging the wild medicinal creeper *Gymnema sylvestre* in Bangalore in the South Indian state of Karnataka. *G. sylyestre* grown in the herbal garden is widely used in the treatment of *Diabetes mellitus*, hence known by its Sanskrit name Madhunashini. Regular damage by this spider mite on *Gymnema* was observed in the herbal garden of the Agricultural University campus at Bengaluru, where no control measures are followed owing to the medicinal use of this plant in herbal preparations.

Temperature is the most crucial environmental factor affecting development and reproduction of poikilothermic organisms like spider mites. Hence, we felt it is necessary to study mite's biological characteristics at different temperature conditions for the construction of developmental curves which can be used for the predictions of developmental time as function of temperature. This kind of predictions can be made as components of population dynamic models to study the population fluctuation of pests. Thus, our study generated data on the development and population performance of this mite under constant temperature conditions between 20°C and 32°C in the laboratory.

Comparable to the indirect damage of spider mites to the cultivated crops (Badawy *et al.,* 2010), qualitative damage due to mite feeding on medicinal plants is important and significant too. Except for the reports of spider mite records by Saini and Reddy (2013), Ahalya and Mikundan (2009) and Karmakar *et al.* (2014) on medicinal plants from India, no attempt has been made so far on systematic biochemical analysis of medicinal plants damaged by the mites. Hence present study was conducted to ascertain the effect of mite feeding damage on the leaf biochemical constituents of medicinal importance. Thus, the present study aims at determining the effect of different constant temperature conditions on the development and demography of *T. hirsutus* and its qualitative damage on the host plant *G. sylyestre* for the first time after its discovery.

MATERIALS AND METHODS

Life history of mite: Developmental biology of the mite was studied at four different constant temperatures conditions *viz.,* 20±1°C; 75-85% RH, 25.3°C; 67-77% RH, 30±1°C; 64-73% RH and 32±1°C; 62-70% RH conditions, with light-dark conditions of 14h -10h in BOD incubator. A cohort of eggs laid on the leaf of *Gymnema* was transferred individually using a fine camel hair brush onto 30 separate 1.5 cm × 1.5 cm fresh leaf disks kept on wet foam placed in 9 "×6 " size rearing polyethylene trays. Development of the mite from egg hatching to adult emergence was recorded. Duration of different developmental stages, namely, larva, protonymph and deutonymph was recorded.

Reproduction: One quiescent deutonymph female and two males were released (to ensure mating) separately onto each of 30 leaf discs. After the emergence of the female on individual leaf disc, preoviposition, oviposition & postoviposition periods and number of eggs laid daily by the female was recorded at 24 hours interval from emergence till the female stopped laying eggs and died naturally. The ovipositing female as carefully transferred onto fresh leaf disc every day. The eggs laid daily on each of these leaf bits were reared till the emergence of adults, and the sex of the emerging adult was recorded.

Population performance or demography: Age-specific life table of *T. hirsutus* was constructed separately at four different constant temperatures and compared to know the influence of the temperature. Demographic characteristics such as average age of parenthood in days (T), no. of female off-springs/female/generation (the average number of newborn females produced by a female during its entire lifetime (R_o)), Gross Reproduction Rate (GRR) - as no. of female off-springs/female/day, Intrinsic Rate of Natural Increase (r_m) - as no. of female off-springs/female/day (maximal rate of increase by the combination of food, temperature, quality of food, *etc.*) and Doubling Time in days (DT) were calculated following the procedure suggested by Birch (1948) and Atwal & Bains (1974) as below;

Net Reproductive Rate (R_o)= $\Sigma\, l_x m_x$

Mean Generation Time (T)= $\frac{\Sigma\, xlxmx}{Ro}$

Finite Rate of Increase in number (λ)= $\text{anti ln}\,[\frac{\log eRo}{T}]$

Intrinsic Rate of Natural Increase (r_m)= ln (λ)

Doubling time, DT = $\frac{\ln 2}{rm}$

where,

l_x = proportion of females alive at age interval x

m_x = number of female off-springs produced by the surviving female at the age interval x

Statistical analysis and interpretation of data

Data recorded on the development and reproduction attributes were expressed as mean ± SE. Data in respect of total development (female and male) were analyzed and compared using Student's 't' test. Life cycle data were analyzed following One-way ANOVA (Post Hoc analysis)

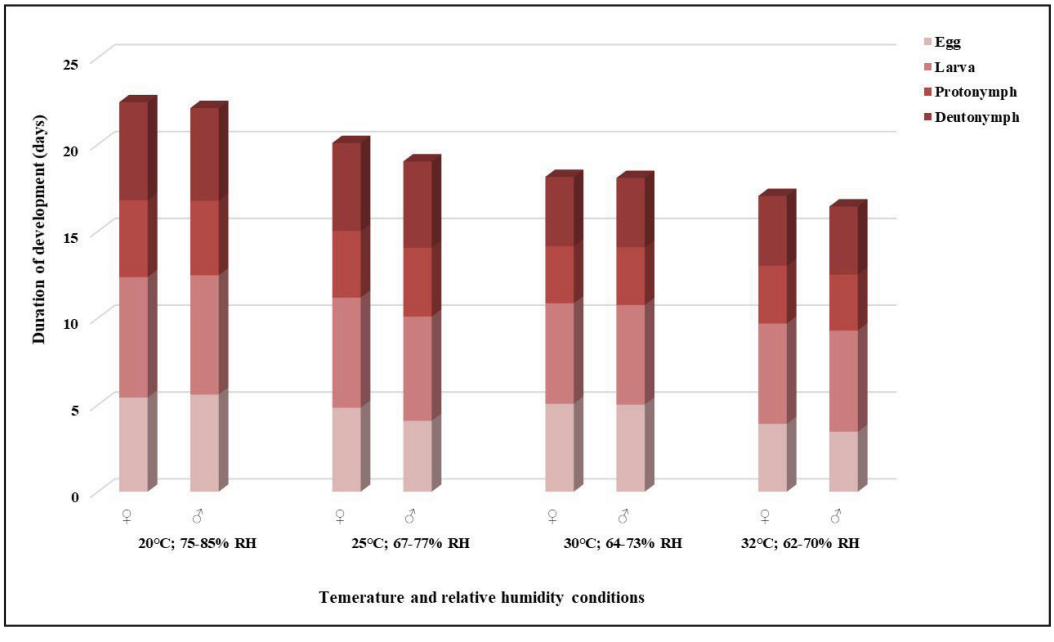

Fig. 1. Development of *Tetranychus hirsutus* on *Gymnema sylvestre* at different constant temperatures in the laboratory

(P=<0.05) using the software SPSS 23. Demographic parameters were computed using the formulae and expressed as mean±SE determined by bootstrapping method and subjected to Post Hoc analysis to compare across different constant temperature conditions.

Physical damage by the mite

Healthy plants of *G. sylvestre* were maintained in the earthen pots in a polycarbonate house. Wild vines of *G. sylvestre* infested by *T. hirsutus* were collected and gently fastened onto healthy plants to facilitate the transfer of mites. Plants were observed every 24 hours for the resultant mite damage symptoms and the development of mite damage symptoms at 10-15 days intervals was photographed.

Estimation of phytochemicals

Chlorophyll content in healthy and mite-infested *G. sylvestre* leaves was estimated using the standard procedure (Arnon, 1949). One gram of fresh leaf sample was incubated overnight in a 1:1 mixture of Dimethyl sulfoxide and 80% Acetone. The supernatant of the extract was diluted, and Spectrophotometric absorbance (Hitachi U-2900) at 645 and 663 nm wavelengths was recorded. For estimating other biochemicals, healthy and mite-infested leaves were shade dried and powdered separately. The dry leaf powder was used for extraction by the Soxhlet apparatus using methanol. The extract was concentrated in a rotary flash evaporator and stored in airtight glass bottles till further use. Total carbohydrate content was estimated following the Anthrone method (Hedge and Hofretter, 1962) by recording the absorbance at 630 nm. Total protein content was estimated by Lawry's method (660nm), and total alkaloid content was estimated by the gravimetric method of Agarwal and Murali (2010). For total flavonoid content estimation, the method of Samata *et al.* (2012) was used by recording the absorbance at 510 nm, while for saponin content, absorbance recorded at 544 nm (Khan and Choudhary 2010). The biochemical data from healthy and mite-infested leaves were compared.

Table 1. Reproduction and demographic parameters of *Tetranychus hirsutus* on *Gymnema sylvestre* at different constant temperatures in the laboratory

Reproduction attributes	20°C; 75-85% (*n=17*)	25°C; 67-77% (*n=25*)	30°C; 64-73% (*n=21*)	32°C; 62-70% (*n=19*)
Pre- oviposition period (days)	2.77	2.08	1.52	1.63
Oviposition period (days)	22.36^{a}	20.32^{a}	13.60^{b}	8.75^{a}
Post-oviposition period (days)	3.32	1.84	1.32	2.25
Longevity of mated females (days)	28.45^{a}	24.24^{c}	16.44^{b}	12.63^{a}
Longevity of males (days)	30.33	23.00	15.00	9.50
Mean no. of eggs/ unmated female	26.00	79.40	71.80	20.13
Mean no. of eggs/ female	24.45^{a}	75.72^{c}	53.60^{b}	28.45^{b}
Mean no. of female offsprings/female	20.60^{a}	67.32^{c}	43.96^{b}	22.90^{a}
Mean no. of male offsprings/female	3.82	7.88	7.64	5.30
Sex ratio of progeny (♂:♀)	1:5.45^{b}	1:8.11^{c}	1:5.91^{b}	1:4.30^{a}
Demographic parameters				
Mean Generation Time (days)	39.26±0.12^{d}	24.44±0.09^{c}	18.03±0.15^{b}	15.66±0.14^{a}
Doubling time (days)	8.79±0.07^{d}	7.91±0.04^{c}	7.29±0.08^{a}	7.79±0.09^{b}
Net Reproduction Rate (No. of female offsprings/ female/generation)	21.26±0.04^{a}	67.83±0.18^{d}	45.47±0.25^{c}	22.95±0.14^{b}
Gross Reproduction Rate	28.96±0.07^{b}	72.02±0.15^{d}	47.32±0.24^{c}	26.92±0.12^{a}
Finite Rate of Increase (No. of female offsprings/female/day)	1.035^{a}	1.079^{b}	1.103^{d}	1.099^{c}
Intrinsic Rate of Natural Increase (No. of female off-springs/female/day)	0.034^{a}	0.076^{b}	0.098^{d}	0.094^{c}

n: numbers of mites observed; Mean values(±SE obtained by bootstrapping method) with same alphabetical superscript within the row are not significantly different as per Tukey's HSD test (p<0.05)

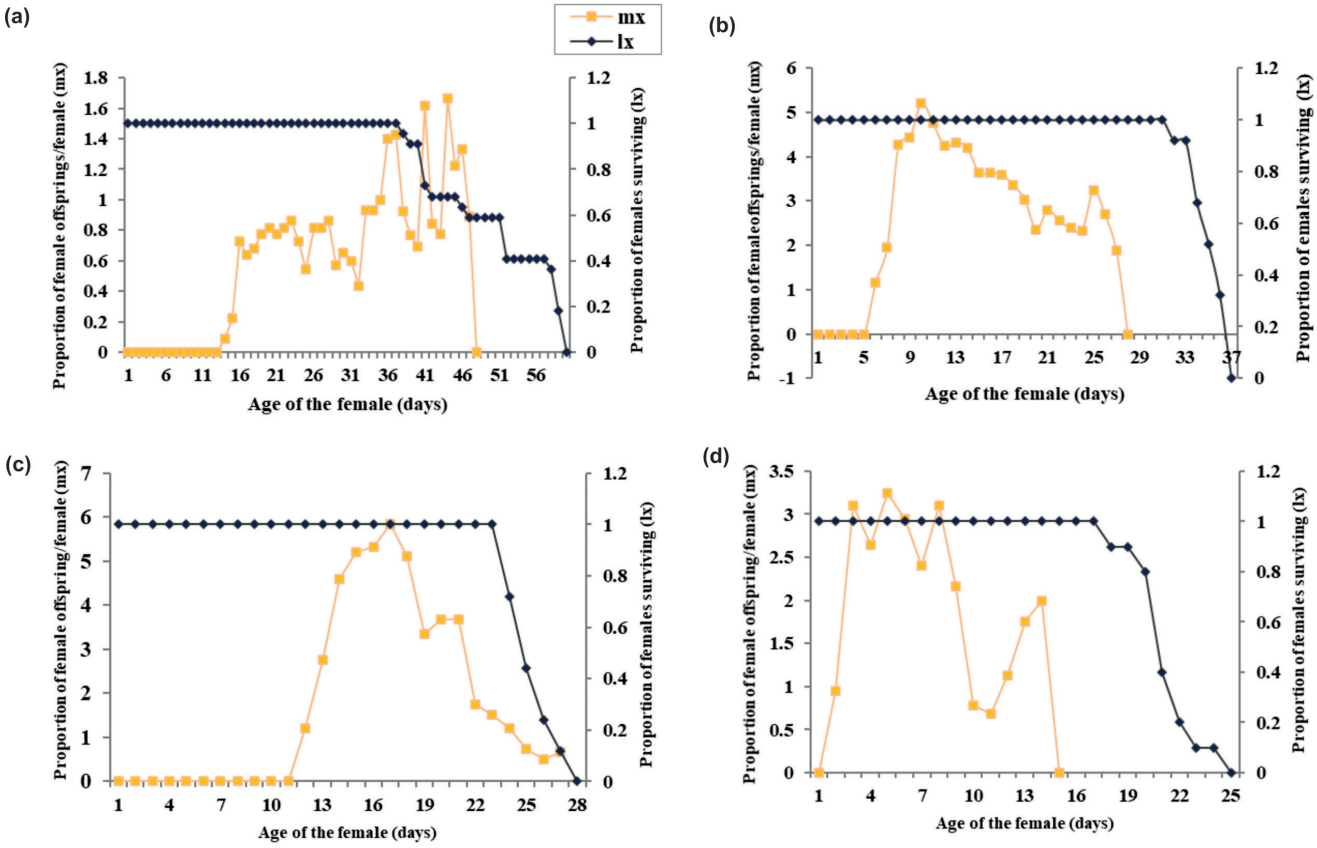

Fig. 2. Age specific survival and fecundity of *Tetranychus hirsutus* on *Gymnema sylvestre* at different temperature and humidity conditions: (a) 20°C; 75-85% RH, (b) 25°C; 67-77% RH, (c) 30°C; 64-73% RH and (d) 32°C; 62-70% RH

RESULTS

Life history

The average incubation period for female mites was 5.42, 4.83, 5.06 and 3.90 days at four temperature conditions between 20°C & 32°C, respectively. After 6.93, 6.34, 5.79 and 5.78 days, the female larva became protonymph, which developed into deutonymph in 4.42, 3.83, 3.28 and 3.33 days. Further, deutonymph required 5.65, 5.07, 3.99 and 4.02 days to develop into an adult. Developmental biology data of *T. hirsutus* on *G. sylvestre* at temperature range of 20°C - 32°C revealed mean developmental time (from egg to adult) as 22.51 days, 14.36 days, 12.15 days & 10.12 days for female and 18.59 days, 12.90 days, 10.68 days & 8.83 days for male (Fig. 1). At all the temperature-humidity conditions the male developed faster (8.83 to 18.59 days) than the female (10.12 to 22.51 days) and adult female lived longer.

Reproduction attributes

Mated females of *T. hirsutus* laid 24.45, 75.72, 53.60 and 28.45 eggs over an oviposition period of 22.36, 20.32, 13.60 and 8.75 days at the rearing temperatures of 20°C, 25°C, 30°C and 32°C, respectively and at the corresponding temperature conditions male to female ratios were 1:5.45, 1:8.11, 1:5.91 and 1:4.30, (Table 1). The age-specific survival pattern of mated females is shown in Fig. 2, with peak female offsprings production on the sixteenth, tenth, eighth and fifth day after emergence at the corresponding temperature conditions.

Demography

The important demographic parameter Intrinsic Rate of Natural Increase (r^m) of *T. hirsutus* estimated on *G. sylvestre* leaf disc under lab condition were 0.034, 0.076, 0.098 and 0.094 females/female/day at different constant temperatures. Net Reproduction Rate (Ro) was 21.26, 67.83, 45.47 & 22.95 female offsprings/ female/ generation and Mean Generation Time (T) was 39.26, 24.44, 18.03 & 15.66 days, respectively. Doubling time (DT) was 8.79, 7.91, 7.29 and 7.79 days at the corresponding lab rearing temperatures (Table 1).

Except for *the* host plant record (*G. sylvestre*) of this recently discovered mite by Zeity *et al.* (2016), no information is available on this mite species. Each mated female of *T. hirsutus* laid a maximum number of

Fig. 3. Physical damage of *Tetranychus hirsutus* on *Gymnema*

eggs (7.42) and reproduced with a high proportion of female individuals at its preferred rearing temperature of 25ºC. Also, showed the highest gross reproduction rate of 72.09 at this temperature, but with a high population progression character (r_{m} = 0.094 to 0.098) at the rearing temperatures of 30ºC and 32ºC.

Qualitative damage

The tetranychid mite species was initially found to feed on the upper surface of leaves, and as the population increased, mites moved on to the lower surface of the leaf. Feeding damage was evident 3 to 4 weeks after infestation, which was usually characterized by the appearance of yellowish spots on the leaves. With an increase in the mite population and the subsequent feeding damage, complete yellowing and drying of leaves were observed. The high mite population associated with damage was evident from a distance due to mite's profuse webbings (Fig. 3). The mites generally congregated on the webbings in large numbers to get dispersed to nearby plants. Severe mite infestation and damage caused complete drying, eventually leading to death of the plant.

Mite damage on *G. sylvestre* leaves showed reduction in major biochemicals; 16% reduction in chlorophyll, 91% in carbohydrates, 69% in proteins, 18% in flavonoids and a low of 3% reduction in saponin content. However, alkaloid content showed 14.30% increase upon mite feeding (Fig. 4). This increase in the concentration of secondary biochemical could be attributed to induce plant defense against feeding damage by mites.

DISCUSSION

The present study is the first of its kind on *T. hirsutus* mite's development and reproduction capabilities after the discovery of the mite on medicinal plant *G. sylvestre* in the wild by Zeity *et al*. (2016). The mite completed its development faster (8.83- 10.12 days to 18.59-22.51 days) as the rearing temperature increased from 20º to 32ºC. Also, showed the highest population progression characters (r_{m} = 0.094 to 0.098 and = 1.099 to1.103), lower Mean Generation Time (15.66 to 18.03 days) and lower Doubling Time (7.29 to 7.79 days) at the rearing temperatures of 30ºC and 32ºC. This can be ascribed to the expected economic damage of mite on the medicinal plant in India, particularly in the south Indian conditions, where the mean atmospheric temperature in most part of the year would be above 25, that would favor the multiplication of *T. hirsutus*.

The feeding damage of *T. hirsutus* on *G. sylvestre* was characterized by greyish spots, yellowing and drying of leaves due to profuse webbing, covering the entire plant, ultimately leading to the death of the plant as the mite population and damage increased. The webbings with huge mite population congregate enabled the mites' dispersal to the adjacent plants. Infested leaves recorded 15.89 per cent reduction in chlorophyll content, 90.90 per cent in carbohydrates, 69 per cent in proteins and 17.60 per cent in flavonoids. Alkaloid content showed 14.30 per cent increase with mite feeding damage. Qualitative damage of this mite species on medicinal plants is reported for the first time through our study. As in the present study, feeding damage of *T. urticae* on eggplant revealed, significant reduction in protein content

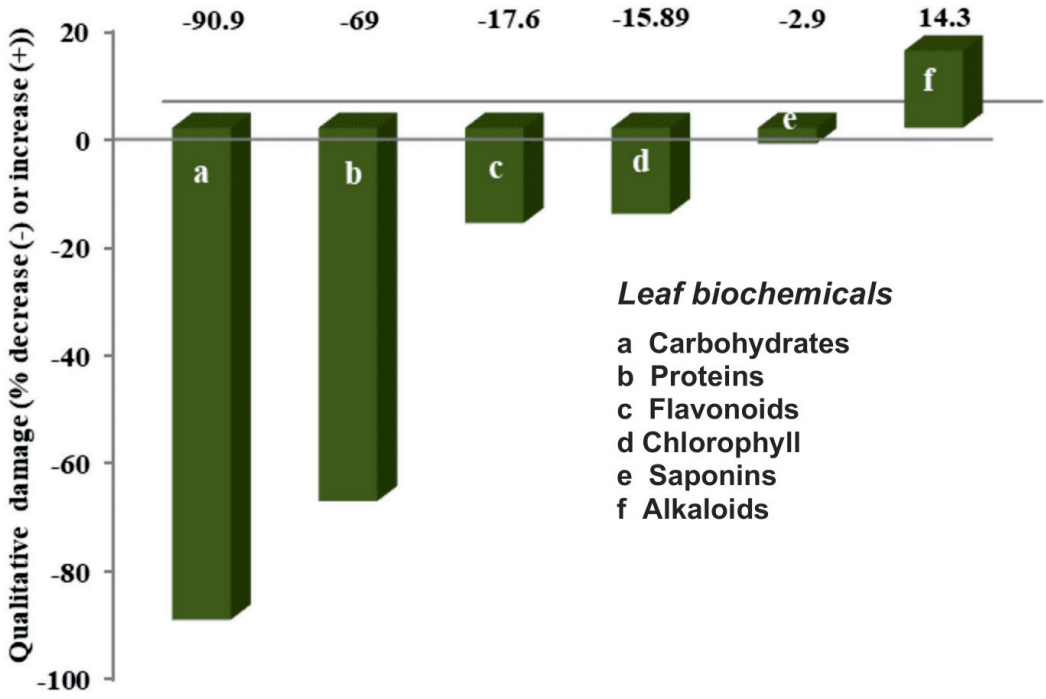

Fig. 4. Biochemical profile of *Gymnema sylvestre* leaf damaged by *Tetranychus hirsutus*

(48.33%), while total phenols (51.30%) and the activity of phenylalanine ammonia-lyase enzyme (51.86%) were found increased subsequent to the mite damage (Mutturaju, 2013). Increase in alkaloid content upon *T. hirsutus* mite feeding in our study might be attributed to the sequestration of secondary metabolites as a defensive strategy of the plants against mite feeding (Safeena and Srinivasa, 2021).

T. hirsutus being an emerging pest and *G. sylvestre* an exceptional anti-diabetic drug often used in pharmaceuticals, further study on the quantitative alteration of biochemical constituents resulting from feeding damage the mite needs more systematic investigation. High temperature favored development and reproduction of *T. hirsutus* alarms its intimidations to become a severe pests in this era of global warming. Hence, holistic management of mites has greater relevance especially in countries like India, where such mite damage is often ignored and application of acaricide on medicinal plants is neither recommended nor practiced. In such cases this kind of temperature dependent population studies are needed to forecast the future population dynamics to formulate the need of mite management decision.

ACKNOWLEDGMENT

Authors thank acarologists Dr. C. Chinnamade Gowda and Dr. B. Mallik, UAS, Bengaluru for their valuable suggestions and acknowledge the ICAR-AINP on Agril. Acarology for the laboratory facilities and logistics. Help of Dr. M. Vasundhara and Dr. Priyanka Reddy in the biochemical analysis is acknowledged.

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MS Received 01 October 2021
MS Accepted 25 November 2021