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Species Identification and Seasonal Abundance of Red Spider Mite on Okra at Vidarbha Region of India

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ABSTRACT: *Tetranychus* mites have been reported to cause a loss of 20 to 45% in okra yield. Limited literature is available on red spider mite occurrence from the Vidarbha region at the same time species level identification of red spider mite from this region is unclear. On that note, during *Kharif*, 2017 and 2018 a study on seasonal abundance of red spider mite on okra was conducted in three different agro-ecological zones of Vidarbha region (viz., Akola, Gadchiroli and Nagpur) of Maharashtra, India and simultaneously field collected red spider mite was identified at the species level by both morphological and molecular techniques. The study revealed red spider mite species, *Tetranychus macfarlanei* as a predominant species on okra in the Vidarbha region, and to our best knowledge, this is the first report from these locations. The higher mite population was recorded at Akola location with 66.4 mites/2.5 cm^2 leaf area during 38th MW of *Kharif* 2018 while the least mite population was recorded at Nagpur location (4.9 mites/2.5 cm^2 during 42nd MW of *Kharif*, 2017). At Gadchiroli and Nagpur, *T. macfarlanei* showed a significantly positive correlation with maximum temperature while, a significant but negative correlation with relative humidity and rainfall.

Keywords: *Tetranychus macfarlanei, Kharif,* red spider mite, Vidarbha, species identification, seasonal abundance.

INTRODUCTION

Okra [*Abelmoscus esculentus* (L.) Moench] is an economically important vegetable in India. India occupies the first position in okra production with 62% of the share in the world's production (Anonymous, 2018). Okra is reported to infest mainly by six mite pest species, viz., *Tetranychus urticae* Koch, *T. macfarlanei* Baker and Pritchard, *T. ludeni* Zacher, *Brevipalpus phoenicis* (Geijskes), *Polyphagotarsonemus latus* Banks and *Aceria lycopersici* (Wolffenstein) (Prasad and Singh, 2011). Red spider mite, *Tetranychus* sp. (Tetranychidae: Arachnida) represents one of the most important groups of phytophagous mites. Highly polyphagous nature, high developmental rate and fecundity, dispersion to new host plants, arrhenotokous parthenogenesis, rapid resistance development to pesticides, very small size make spider mites a potential risk to agriculture. In India, the average yield loss to vegetable crops due to mite infestation was estimated to be 9.15-100% (Vinothkumar *et al*., 2009). Red spider mites typically colonize the undersurface of leaves, suck cell sap, remove the chlorophyll, produce typical speckling and stippling appearance of leaves, leading to yellowing and bronzing of leaves. Webbing causes reduction in photosynthetic activity and transpiration, stunting of plant growth, premature leaf drying followed by leaf fall and ultimately death of the whole plant (Demirel and Cabuk, 2008; Varadaraju, 2010).

Taxonomic identification of red spider mites is more ambiguous or indecisive and hence, probabilities of misidentification would be high even for an expert. An integrative approach of using morphological and molecular data would greatly resolve the instances of synonymy and misidentification of mite species. *ITS2* sequence divergence may be used to differentiate between closely related species, complexes of species and even populations (Navajas *et al*., 2001). Haque, (2007) studied the effect of temperature and relative humidity on the biology of red spider mites and identified 30 to 35°C as the most favorable temperature for the survival of mites. As the abiotic factors have a great effect on the biology and eventually on the population growth of mites it is desirable to have the basic knowledge of the seasonal incidence of spider

mites in relation to weather parameters in a particular locality which will be helpful in the development of the suitable management program against this pest.

MATERIAL AND METHODS

A laboratory experiment was conducted at Dr. PDKV, Akola and All India Network Project on Agricultural Acarology (AINPAA), Bangalore to identify red spider mite species collected from three different agro-ecological zones of the Vidarbha region (Akola (Plateau assured rainfall), Gadchiroli (Eastern Vidarbha high rainfall) and Nagpur (Central Vidarbha moderate rainfall)) on okra. For morphological characterization, few male and female mites from each location were killed and preserved in 80% ethyl alcohol. Permanent mounting slides of male and female mites were prepared by using Hoyer's media as a mounting media. The slide-mounted mite specimens were examined under Nikon eclipse 50i phase contrast compound microscope and different characters such as dorsal striation, legs chaetotaxy, the position of female tarsus I duplex setae, genital flap and pre-genital area of female and male aedeagus were studied by following the taxonomic key given in Gupta and Gupta (1994). For molecular characterization mite samples from three locations were taken separately; killed in absolute ethanol and stored in a -80°C freezer for 2 days before DNA isolation. CTAB method detailed by Rogers and Bendich (1994) followed in Genomic DNA extraction. Molecular characterization of mites was done at the ITS2 region and DNA amplification and sequencing was done with the primers mentioned below (Navajas *et al*., 1998):

5'-ATATGCTTAAATTCAGCGGG-3'-(Forward)
5'-GGGTCGATGAAGAACGCAGC-3'-(Backward)

Polymerase chain reaction (PCR) amplification was carried out for ITS2 locus with 25 µl reaction mixture with the thermal profile as follows: -

Initial denaturation – 95°C for 1min,
Denaturation – 94°C for 1 min,
ITS2 primers annealing - 48°C for 0.45 min,
Primer elongation - 72°C for 0.52 min,
Final elongation – 72°C for 5 min,

Hold the sample for infinity at 4°C with 35 thermal cycles. PCR products of the expected size (~600 bp) were extracted from 1.5% agarose gel (Fig. 1). The PCR products were used for sequencing. Sequencing was done by outsourcing (Sci. Genom Lab. Pvt. Ltd., Cochin). Total of 3 DNA sequences submitted in the NCBI GenBank.

The accession number to the submitted sequences was obtained from NCBI (National Centre for Biotechnology Information). Simultaneously, live as well as alcohol preserved mite samples were sent for species confirmation to the experts in Tetranychid mite taxonomy: - Dr. N. Srinivasa, Co-ordinator, AINPAA, Bangalore and Dr. K. Ramaraju, Head, Plant Protection, Tamil Nadu Agricultural University (TNAU), Coimbatore.

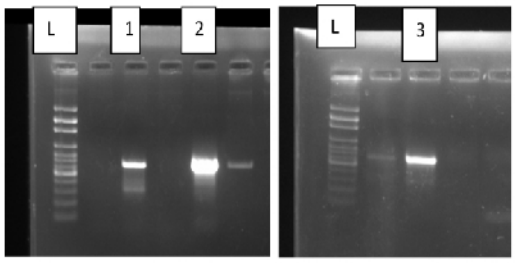


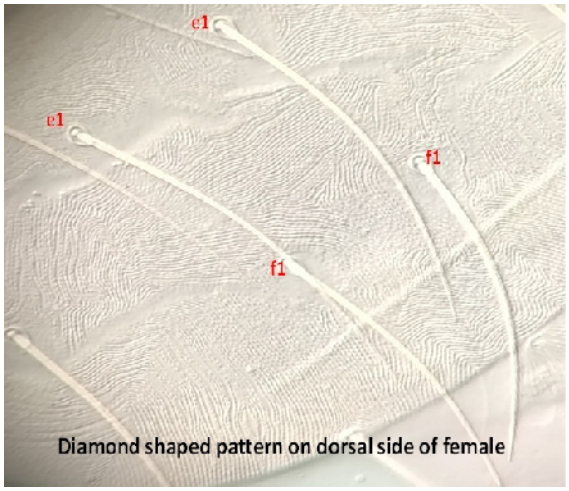
Fig. 1. PCR products of expected size (~600 bp) extracted from 1.5% agarose gel (L- Ladder, 1 – Akola, 2- Nagpur, 3- Gadchiroli).

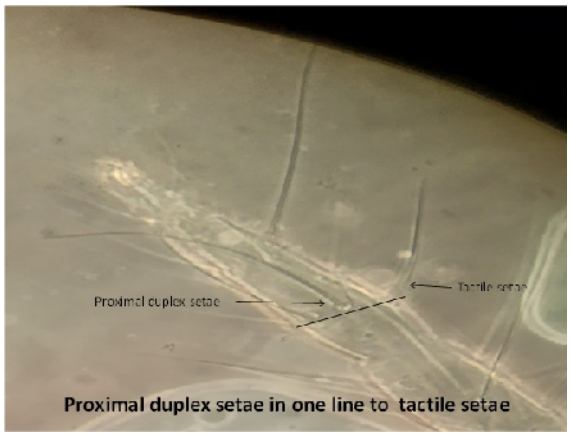
Seasonal abundance of red spider mite in relation to weather parameters was studied on okra crop which was grown on the experimental plots, at Akola, Gadchiroli and Nagpur. Okra (var. *Arka Anamika*) crop raised on 10 × 10 m area with 60 × 45 cm spacing and with all agronomic practice, except pesticide spray. The crop was sown from the last week of June to the first week of July. Observations on the red spider mite were recorded from the initiation of pest incidence at Akola (weekly), Gadchiroli and Nagpur (fortnight), till the crop maturity. Ten plants were randomly selected and three leaves from the basal, middle and top canopy of each plant were observed for red spider mite. Spider mite population was counted on the ventral surface of leaf on 2.5 cm^2 leaf area with the help of a foldable magnifier hand lens (10 x). The weekly meteorological data were obtained from the agro-meteorological observatory. The influence of key meteorological parameters on pest incidence was worked out through simple correlation studies (Gomez and Gomez, 1984). Multiple regression equations were also developed.

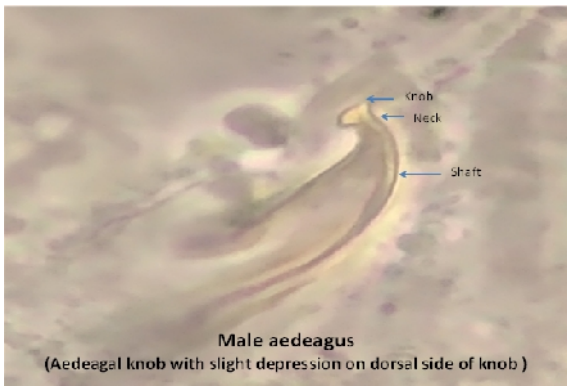
RESULTS AND DISCUSSIONS

A. Identification of red spider mite at the species level

Slide mounted mite specimens were examined for the characters such as the thumb-claw process of pedipalp, tarsal claw and empodium, duplex setae on tarsi I and II and structure of aedeagus (Plate 1).







(a) Plicated female genitalia (b) Diamond shaped pattern on doesal side of female (c) Proximal duplex setae in one line to tactile setae (d) Male aedeagus (Aedeagal knob with slight depression on dorsal side of knob).

Plate 1: Morphological characterization of *Tetranychus macfarlanei.*

After following the taxonomic key, given by Gupta and Gupta (1994); Zeity *et al.*, (2017) no significant taxonomic difference was seen and all the three mite populations were found to be belonging to species, *Tetranychus macfarlanei*.

Three rDNA sequences of ITS2 region of red spider mite (555-626 bp) are registered under National Center for Biotechnology Information. Accession numbers awarded from GeneBank i.e. MN073196 (Nagpur), MN073080 (Akola) and MN073186 (Gadchiroli)) revealed the occurrence of the same species in all three locations of Vidarbha on the okra crop-ecosystem, which belongs to genus *Tetranychus* and species *macfarlanei.* Earlier, Gupta and Gupta (1994) in their work on a review of Indian Tetranychidae, described *T. macfarlanei* along with distinct morphological characters. Bennur, (2015) in Kerala identified *T. macfarlanei, T. truncatues* and *T. okinawanus* based on the empodium, male aedeagus, duplex setae, chaetotaxy, etc.

B. Seasonal incidence of Tetranychus macfarlanei in relation to weather parameters

Incidence of red spider mite, *T. macfarlanei* on okra initiated during 32nd MW at all three locations and was recorded throughout the crop season thereafter, till the crop maturity. During the study, the range of maximum temperature and relative humidity in locations understudy during the peak period was found in the range of 30.9-34.2°C and 74-89%. At, Akola, the peak of the mite population was observed during 36th MW (17. 6 mites/2.5 cm^2) and 38th MW (66.4 mites/2.5 cm^2). Maximum temperature, minimum temperature, morning and evening relative humidity and rainfall prevailed during this period was 32.3°C, 23.8°C, 89%, 62% and 9.7 mm and 33.3°C, 23.1°C, 85 %, 52 % and 62.4 mm respectively for *Kharif*, 2017 and 2018 (Fig. 2). At Gadchiroli, spider mite population showed a peak on 42nd MW, (15.5 and 19.9 mites/2.5 cm^2) with maximum temperature, minimum temperature, morning relative humidity, evening relative humidity and rainfall prevailed during this period in the range of 30.9°C, 24.1°C, 89%, 75%, 0.0 mm and 34.2°C, 21.7°C, 74%, 69 %, 0.0 (mm) respectively during the 2017 and 2018 (Fig. 3). At Nagpur, the red spider mite population reached its peak of 4.9 and 13.5 mites/2.5 cm^2 during 42nd MW and 38th MW with maximum temperature, minimum temperature, morning relative humidity, evening relative humidity and rainfall prevailed during this period was in the range of 32.7°C, 21.1°C, 76%, 52% and 9.6 mm; 33.5°C, 23.5°C, 77%, 67%, and 44.6 mm respectively during 2017 and 2018 (Fig. 4).

Weather conditions during the month of September and October were found to be more congenial for the population growth of mites on the okra crop. Amongst all three locations, the highest mite population was observed at the Akola location. Previous workers support the results of the study; Nath *et al.*, (2011) studied the impact of weather parameters on major insect pests of okra at Uttar Pradesh and reported the highest mite (*Tetranychus* sp.) count of 37.89 mites/ leaf during the 40th SMW of *Kharif*, 2005 and 40.83 mite/ leaves in 39th SMW of *Kharif*, 2006.

Siddhapara, (2015) noticed a higher spider mite population (8.49 to 15.23 mites/2 cm^2) during the 2nd week of August to 2nd week of October with a peak population during the month of September in JAU, Junagadh, Gujarat. Singh *et al*., (2018) observed maximum populations of *T. urticae* in 42nd MW with 14.25 mites/2.5 cm^2 during Zaid and *Kharif*, 2015 on okra crop at SKUAST, Jammu, India.

At Akola, data showed a non-significant positive correlation of red spider mite population with maximum temperature, minimum temperature, morning relative humidity, and rainfall, while, a non-significant and negative correlation with evening relative humidity. Data showed a positive and significant correlation of red spider mite at Gadchiroli and Nagpur with maximum temperature (r = 0.80 and 0.63) while, a non-significant negative correlation with minimum temperature (r = -0.40 and 0.44) respectively. A significant but negative correlation was observed between red spider mite and morning relative humidity (r = -0.88 and – 0.70), evening relative humidity (r = -0.81 and -0.58) and rainfall (r = -0.65 and -0.74) respectively in Gadchiroli and Nagpur (Table 1). From the linear regression equations, it could be inferred that with an increase in maximum temperature, relative humidity (morning), relative humidity (evening) and rainfall by 1 unit there was a proportionate increase of 2.70 and 1.72 percent and a decrease of 0.85 and 0.65, 0.86 and 0.32 and 0.17 and 0.11 percent in mite population observed respectively at Gadchiroli and Nagpur.

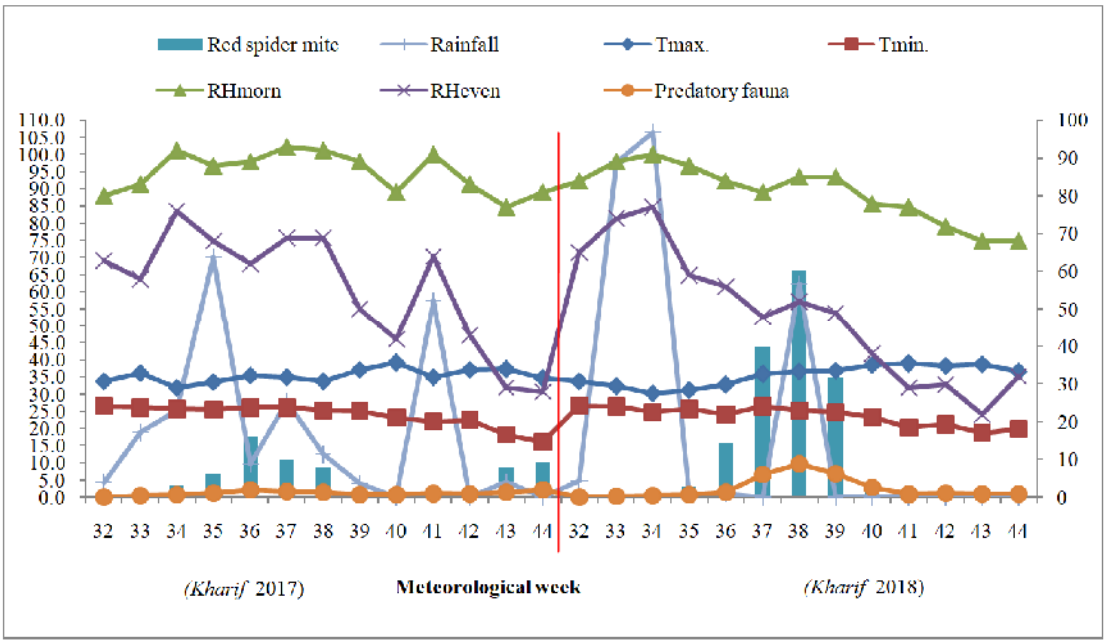


Fig. 2. Seasonal incidence of red spider mite at Akola.

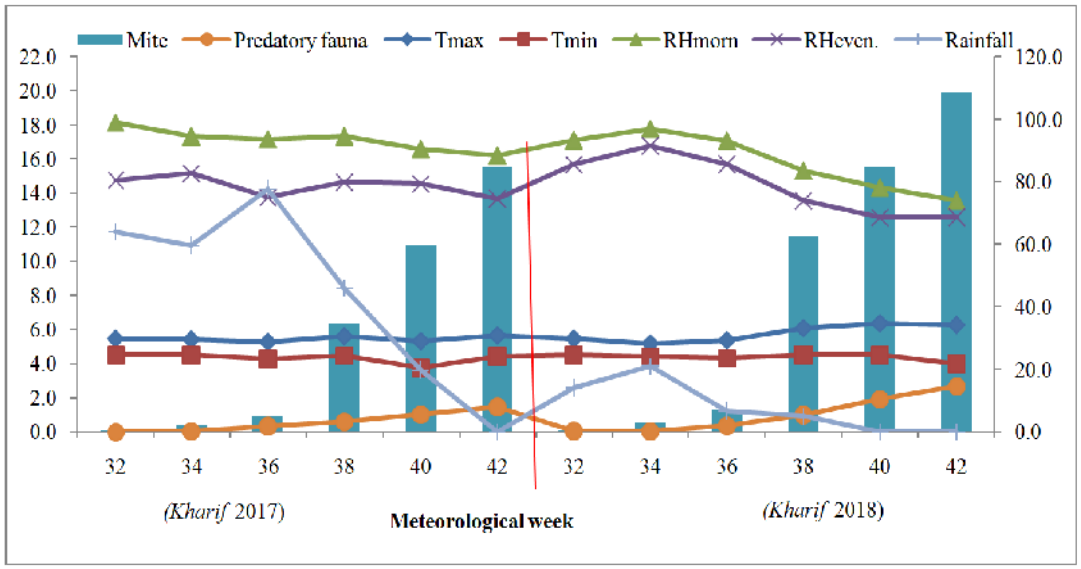


Fig. 3. Seasonal incidence of red spider mite at Gadchiroli.

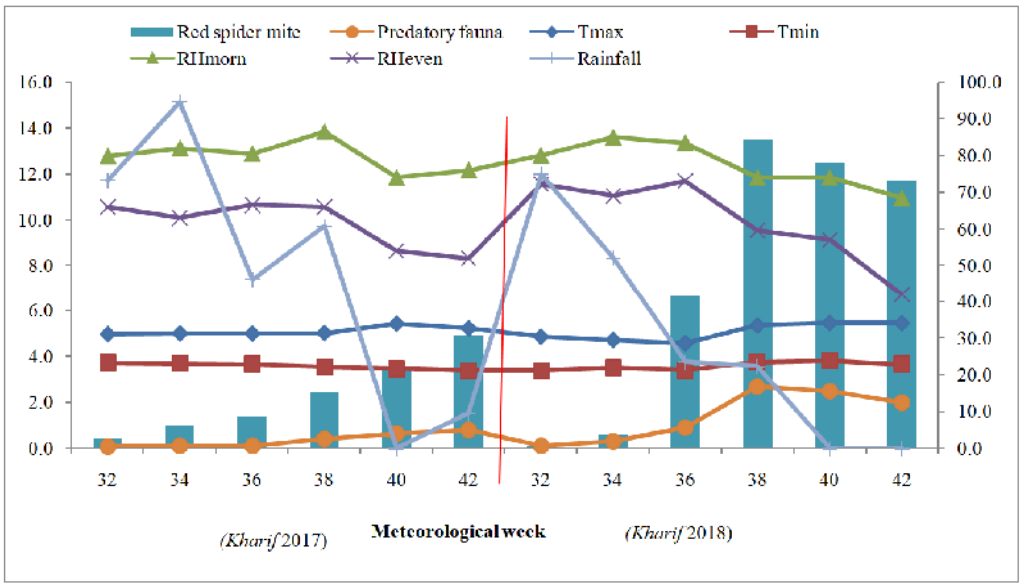


Fig. 4. Seasonal incidence of red spider mite at Nagpur.

The regression equation showed 63 and 39, 78 and 49, 65 and 33 and 42 and 54 percent of fluctuation in mite population contributed individually by maximum temperature, morning relative humidity, evening relative humidity and rainfall at Gadchiroli and Nagpur respectively. Multiple regressing equations showed that meteorological factors together contributed 89 and 63 per cent variability in mite population respectively for Gadchiroli and Nagpur (Table 1).

Table 1: Correlation and regression analysis of red spider mite in relation with weather parameters.

Year	Location	Factors	r value	R^2	Linear equation	Multiple equation
Kharif 2017-18	Akola	Temp. (Max)	0.09	—	—	
		Temp. (Min)	0.20	—	—	
		RH (Morn.)	0.10	—	—	
		RH (Even.)	- 0.01	—	—	
		Rainfall (mm)	0.06	—	—	
	Gadchiroli	Temp. (Max)	0.80**	0.63	Y= -75.69+2.70*Tmax.	Y=106.3-0.71*Tmax- 0.11*R Hmorn. -0.81*RHeven-0.14*Rainfall (R^2= 0.89)
		Temp. (Min)	-0.40	—	—	
		RH (Morn.)	-0.88**	0.78	Y=83.57-0.85*RHmorn.	
		RH (Even.)	-0.81**	0.65	Y=74.33- 0.86*RHeven.	
		Rainfall (mm)	-0.65*	0.42	Y=11.36-0.17*Rainfall	
	Nagpur	Temp. (Max)	0.63*	0.39	Y= - 49.83+1.72*Tmax.	Y=5.67+0.65*Tmax-0.37*R Hmorn + 0.17*RHeven-0.08*Rainfall (R^2=0.63)
		Temp. (Min)	0.44	—	—	
		RH (Morn.)	-0.70*	0.20	Y=56.26-0.65*RHmorn.	
		RH (Even.)	-0.58*	0.33	Y=24.48-0.32*R Heven.	
		Rainfall (mm)	-0.74**	0.54	Y=9.14 - 0.11*Rainfall	

(*significant at p = 0.05; **p = 0.01)

At Gadchiroli and Nagpur locations, the maximum temperature had a positive and significant correlation with spider mite while relative humidity (morning and evening) and rainfall had a negative but significant correlation. These findings are in line with the following workers Jadhav, *et al*., (2017); Naga *et al.*, (2017); Singh *et al.*, (2018) have documented the significant positive correlation between mite population and maximum temperature, whereas a negative correlation with morning, evening relative humidity; and rainfall. Contrary to these Mandal *et al*., (2006) at Pusa, Bihar reported that *T. telarius* on okra showed a non-significant negative correlation with the maximum temperature, a significant positive association with relative humidity and a non-significant positive correlation with rainfall. Regression analysis explained 78-85 percent variability in *T. telarius* due to meteorological parameters during *Summer*, 2000 and 2001. Chinniah *et al.*, (2009) inferred from regression equation data that, the increase in temperature by 1°C resulted in a proportionate increase in spider mite population in the brinjal ecosystem by 1.18 per cent; one unit increase in relative humidity and rainfall resulted in a decrease in mite population by 0.22 and 0.19 percent. Monica *et al.*, (2014) reported 73.50% variability in mite population contributed by weather factors.

CONCLUSION

This study identified and confirmed *T. macfarlanei* as the predominant species of red spider mite and registered it as the first record of such kind of species on okra in Vidarbha to our best knowledge. The peak of the mite population occurred during September and October. Akola location showed a relatively higher mite population (66.4 mites/2.5 cm^2). At Gadchiroli and Nagpur locations, maximum temperature has a positive and significant correlation with spider mite while relative humidity (morning and evening) and rainfall had a negative but significant correlation.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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