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STUDIES ON LEAF DAMAGE INDUCED BY *OLIGONYCHUS COFFEAE* NIETNER (ACARI: TETRANYCHIDAE) ON *MALUS SYLVESTRIS* (L.) MILLER

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ABSTRACT

The feeding impact of the spider mite pest, *Oligonychus coffeae* Nietner on the leaves of the European crab apple (*Malus sylvestris* (L.) Miller) was analysed through visual assessment of feeding symptoms and by estimating and comparing the quantitative changes in the photosynthetic pigments and photosynthetic efficiency of mite infested and uninfested leaves. The visible symptoms induced by the species comprised of the development of chlorotic spots or patches, necrotic spots, etc. and heavily infested leaves presented a bleached, sunken and crinkled appearance. Results of quantitative analysis of the photosynthetic pigments enabled to record significant loss in chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids in the tune of 61.69 ± 0.081%, 68.21 ± 0.073%, 63.52 ± 0.012% and 55.42 ± 0.064% respectively ($p < 0.01$ level). Analysis of photosynthetic efficiency by measuring the value of Fv/Fm ratio (a parameter to measure chlorophyll fluorescence for denoting the PSII system of leaves) using the Handy Photosynthetic Analyzer revealed a lowered value for infested leaves (0.587 ± 0.015) when compared to the uninfested leaves (0.823 ± 0.003). The result when statistically analyzed was found significant at $p < 0.01$ level, thereby clearly establishing that infestation by *O. coffeae* would drastically affect the photosynthetic efficiency and hence the vigor of *M. sylvestris.*

INTRODUCTION

The tea red spider mite, *Oligonychus coffeae*, is a severe pest of agricultural, plantation, ornamental and fruit crops, inducing damage symptoms such as chlorotic spots, bronzing, crinkling and drying of leaves (Cranham, 1966; Jeppson *et al*., 1975; Banerjee and Cranham, 1985). The mite shows severe infestation on the fruit crop, *Malus sylvestris*, the commonly called European crab apple. The fruits of *M. sylvestris* are recognized as rich source of moisture, dietary fiber and nutrients and the consumption of which imparts immunity to the human body (Considine, 1982; Aziz *et al*., 2013). The leaves are used as natural medicines for treatment of various diseases. In the present study, the impact of *O. coffeae* infestation on the *M. sylvestris* was assessed by recording the visual symptoms of damage as well as by quantifying the photosynthetic pigments like the chlorophyll and carotenoids and measuring and comparing the chlorophyll fluorescence emission of mite infested and uninfested leaves. Chlorophylls, as the key pigments, play a direct role in the conversion of solar energy to biochemical energy (Peto *et al*., 1981; Demiral and Türkan, 2005) and chlorophyll fluorescence has been used extensively as a fast and non-intrusive tool for elucidating the structural and functional aspects of photosynthetic apparatus in plants (Strasser *et al*., 2000). Chlorophyll fluorescence kinetics, especially the ratio of the maximal variable fluorescence to the maximum level of chlorophyll fluorescence (Fv/Fm), is directly related to the PS II and hence can be used as an index for rating the photosynthetic efficiency of plants.

MATERIALS AND METHODS

Collection of the leaf samples

Random samples of mite infested leaves were collected fortnightly, from the host tree, *M. sylvestris* (of a height of around 2.5 meters) growing in the Botanical garden of the Calicut University Campus, Malappuram Dt., of Kerala, during the period of December 2014 to May 2015.

Assessment of leaf damages

Assessment of leaf damage due to the feeding activity of the mite, *O. coffeae* was carried out by adopting both qualitative and quantitative methods.

Qualitative methods of damage assessment

Leaf damage induced by *O. coffeae* was assessed by examining the infested leaf samples under a Stereo-zoom microscope (Model No: MVNSZ – 405, Macro Vis, USA), and recording data on the mode of infestation by the mite, its feeding activity,

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damage symptoms produced etc. For recording the progressive symptoms of damage induced by the mite, three categories of leaves were considered viz. leaves showing low level of mite infestation (with 5-10 adult females/leaf), moderate level of mite infestation (10-20 adult females/ leaf) and high level of mite infestation (> 20 adult females/leaf). Data on the damage symptoms developed on the above three categories of leaves were recorded separately and photographs were taken with a digital camera (LEICA DFC 295) attached to a stereozoom Microscope (LEICA S8 AP0).

Quantitative methods of damage assessment

Quantitatively, the leaf damage was assessed by estimating the amounts of major photosynthetic pigments and by measuring the loss in chlorophyll fluorescence emission by the mite infested leaves, and thereby assessing the photosynthetic efficiency of the leaves. Freshly collected middle aged leaves showing heavy infestation by *O. coffeae* were considered for quantitative assessment, after careful removal of all the mite specimens, with moistened cotton. Uninfested leaves (control) collected randomly from the same tree were also subjected to quantitative assessment, for comparing the results.

Estimation of Photosynthetic Pigments

The amounts of major photosynthetic pigments viz. Chlorophyll (chl *a*, chl *b* and total chlorophyll) and carotenoids present in the uninfested and heavily infested leaves of *M. sylvestris* were estimated following the method of Arnon (1949). Known weight of freshly collected leaf tissue (0.5gm) was taken and ground to a fine pulp in a mortar (kept in a tray containing ice cubes) by adding 10 ml of 80% acetone as the extraction medium. Precautions were taken to avoid exposure of the extract to light. Then the homogenate was centrifuged at 5,000 rpm for 10 minutes in a cooling centrifuge at 4^{o}C and the supernatant was transferred to a volumetric flask. The process of extraction with 80% acetone was repeated until the residue became colorless. The final volume of the supernatant was made up to 50 ml with 80% acetone in a standard flask. The absorbance was read at 663 nm, 645 nm, and 470 nm, (also at 750 nm to make correction for impurities) against the solvent blank (80% acetone), using a UV visible spectrophotometer. The amounts of chlorophyll and carotenoids present in the leaves were calculated and expressed in mg/g fresh weight of the leaf tissue. The experiment was repeated 10 times to get concordant results. The data were statistically analyzed using student's *t* test with IBM SPSS Statistics (Version 19) and the values were expressed in Mean ± SEM.

Calculations

Mg chlorophyll a = [12.7 (A663- A750) - 2.69 (A645- A750)] × V/ (W×1000)
Mg chlorophyll b = [22.9 (A645- A750) - 4.68 (A663- A750)] × V/ (W×1000)
Mg total chlorophyll = [20.2 (A645- A750) + 8.02 (A663- A750)] × V/ (W×1000)
Mg carotenoids = [1000 (A470) + 3.27 {(chlorophyll a) – (chlorophyll b)}] × V/ (W×229× 1000)

Where A is the absorbance, V is the volume, and W is the fresh weight of tissue extracted (g).

Measuring Photosynthetic Efficiency

For measuring chlorophyll fluorescence, the samples (n = 10) of uninfested and heavily infested middle aged leaves were collected in every 2 weeks. The Photosynthetic efficiency was measured at room temperature on freshly collected fully expanded leaves by using the portable fluorescence monitoring system (Handy PEA, Hansatech Ltd., Vorfolk, UK). Prior to the fluorescence measurements, a circular surface of the upper face of the leaves was dark adapted for 15-20 minutes using the dark adaptation clips. Data on general parameters like F_0 (minimum/initial fluorescence), Fm (maximum fluorescence), Fv (variable fluorescence) etc. were recorded separately for uninfested and mite infested leaves. The values of Fv/Fm (where Fv = Fm - F_0), a parameter commonly known as maximum quantum yield of primary photochemistry or maximal electron transport rate (ETR) of PS II of both uninfested and infested leaves were recorded separately. The data were statistically analyzed using student's *t* test and the values were expressed as Mean ± SEM.

RESULTS

The results of field sampling conducted during the present study revealed that the incidence of the *O. coffeae* initiated on the leaves of *M. sylvestris* during November, 2014. Symptoms of heavy infestation by the mite could be recorded during the period of January to April, 2015. Mite infestation was found more confined to the middle aged leaves when compared to the newly sprouted and older leaves. On microscopic examination of infested leaves, the mite was found to colonize the upper surface of the leaves of *M. sylvestris*, where the adult females predominated, exhibiting intense feeding activity (Fig.1).

Damage symptoms induced by *Oligonychus coffeae* on the leaves of M*alus sylvestris*

Figure 1 Actively feeding adult females of *Oligonychus coffeae*

Figure 2 Leaf with chlorotic patches and faecal matter

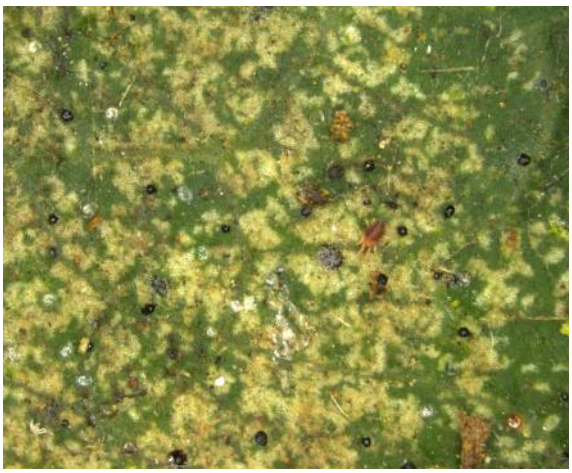


Figure 3 Moderately infested leaf with various life stages of *O. coffeae*

Figure 4 Moderately infested leaf with various life stages of *O. coffeae*

Figure 5 Sunken and crinkled appearance of the leaf surface with numerous chlorotic spots

Figure 6 Mite infested leaf showing sunken crinkled appearance

Figure 7 Dark brownish and cracked appearance of severely infested leaf

All life stages of the mite were found to colonize under a silken web, along the midrib, veins and veinlets of the leaves and also along the front marginal portions on the upper surface of infested leaves. During heavy infestation, presence of the various life stages of the mite could be noted on the lower surface of the leaves also. In the initial stages of infestation, the females were found to commence their feeding activity by staying at localized areas and as a result of feeding, the chlorotic spots were developed which appeared as white dots. At each feeding site, 5 - 6 chlorotic spots (1 - 2 mm in diameter) were found produced by each actively feeding adult female. This was followed by the deposition of eggs and in several instances, 10 - 15 freshly laid eggs were visible adjacent to the feeding spots, covered under the silken web.

On moderately infested leaves, due to continuous feeding activity of the developing stages of the mite, the chlorotic spots were found extended, and quite often merged together to form chlorotic or white patches of 3 - 4 mm diameter (Fig. 2). Later, these patches coalesced together to give a pale green or yellowish colouration, thereby imparting a bleached appearance to the infested area (Fig. 3). Subsequently, small necrotic spots of 2 - 3 mm diameter were observed on the bleached surface of the infested leaves. Moderately infested leaves harboured more number of developing stages of the mite (Fig. 4) when compared to the leaves which showed low degrees of mite infestation. The adults, nymphal stages and larvae were found actively feeding on the leaf by piercing the leaf tissue with their cheliceral stylets and sucking out the plant sap containing the various cellular components of the leaf. Quite often, the mite infested leaves presented localized shrunken and crinkled appearance and such leaves, up on

microscopic examination, showed large number of eggs, egg cases and newly hatched larvae of the mite (Figs. 5 & 6). Along with the life stages of the mite, moulting skins and faecal pellets were also observed on the surface of the leaves (Fig.6). The infested leaves also showed the accumulation of dust particles which were found entangled among the silken web.

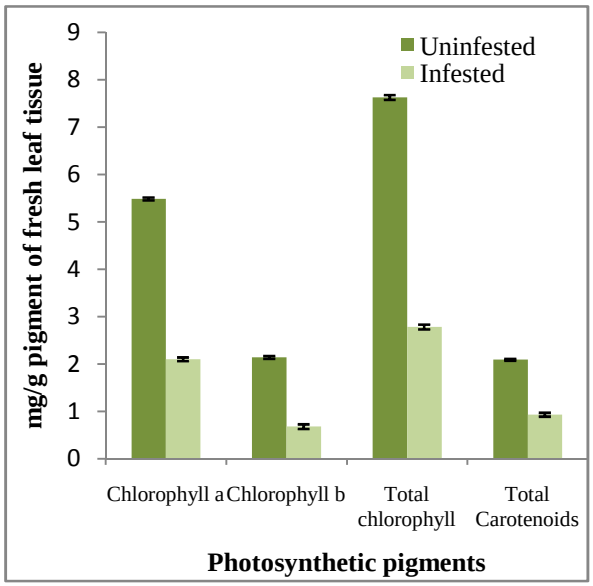
Fv/Fm in infested leaves was found to be very low during the heavy infestation period of January to April (Graph. 3). Statistical analysis of the data on chlorophyll fluorescence using t-test were found highly significant at $p < 0.01$ level.

Table 1 Changes in the amounts of photosynthetic pigments (mg/gm tissue) in the leaves of *Malus sylvestris* due to infestation by *Oligonychus coffeae.*

Sl. No.	Photosynthetic pigments	Uninfested leaf tissue	Infested leaf tissue	Loss in pigment	% loss in pigment
1	Chlorophyll *a*	5.489 ± 0.031	2.103 ± 0.053	3.386 ± 0.032	61.69 ± 0.081
2	Chlorophyll *b*	2.142 ± 0.009	0.681 ± 0.048	1.461 ± 0.037	68.21 ±0.073
3	Total chlorophyll	7.631 ± 0.014	2.784 ± 0.062	4.847 ± 0.062	63.52 ± 0.012
4	Total Carotenoids	2.093 ± 0.017	0.933 ± 0.032	1.160 ± 0.041	55.42 ±0.064

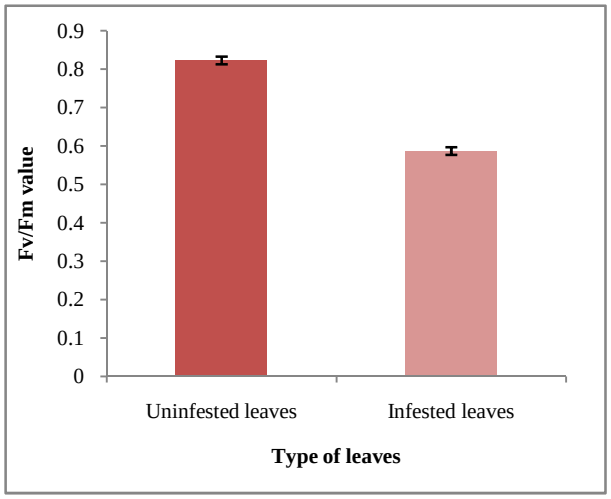
On heavily infested leaves, the necrotic spots were found coalesced to give a brownish appearance to the leaf surface and severely injured areas appeared as more dark brownish in colour and later these portions of the leaf became dried and cracked, especially along the front marginal regions of the leaf (Fig.7). Heavily infested leaves carried almost all life stages of *O. coffeae viz.* egg, larva, nymphs, quiescent stages and adults in large numbers along with their moulting skins and faecal pellets.

Results of quantitative estimation of photosynthetic pigments revealed an average loss of 3.386 ± 0.032, 1.461 ± 0.037, 4.487 ± 0.062 and 1.160 ± 0.041 mg/g fresh leaf tissue for Chl *a*, Chl *b*, total Chl and carotenoids respectively (Table.1; Graph.1). The per cent loss in amounts of Chl *a*, Chl *b*, total Chl and carotenoids recorded during the study were 61.69 ± 0.081%, 68.21 ± 0.073%, 63.52 ± 0.012% and 55.42 ± 0.064% respectively. The data obtained on the loss in photosynthetic pigments up on statistical analysis using t-test were found significant at $p < 0.01$ level.

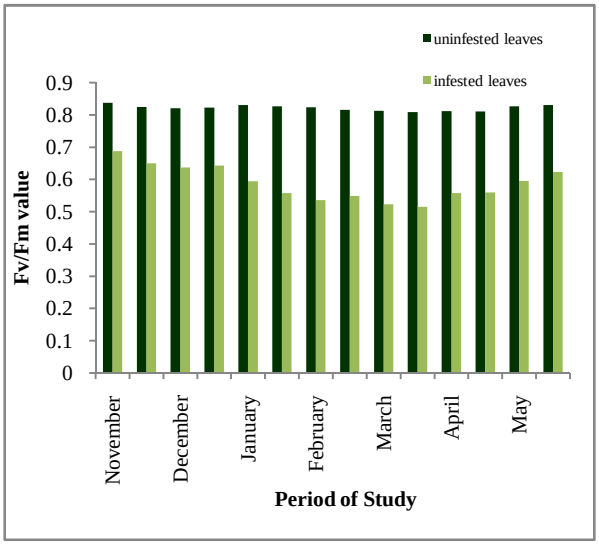


Graph 1. Changes in the amounts of photosynthetic pigments (mg/gm fresh tissue) in the leaves of *Malus sylvestris* due to infestation by *Oligonychus coffeae.*

The values of Fv/Fm recorded during the present study for uninfested and infested leaves of *M. sylvestris* were 0.823 ± 0.003and 0.587 ± 0.015 respectively (Graph. 2). The value of



Graph 2 Changes in Fv/Fm value (Mean ± SEM) in the leaves of *Malus sylvestris* due to infestation by *Oligonychus coffeae*



Graph 3 Changes in Fv/Fm values in the leaves of *Malus sylvestris* due to infestation by *Oligonychus coffeae* during the period of study

DISCUSSION

O. coffeae, the commonly called tea red spider mite is recognized as a cool weather pest, mainly of broad-leaved evergreens, and its incidence has been reported on 34 species of plants belonging to 15 families, distributed in 14 countries in

four continents (Borror, *et al*., 1989; Haque *et al*., 2007). In the present study, heavy infestation of *O. coffeae* was evident during the summer months, from January to April of 2015. This finding seems to disagree with the earlier findings that the species develop in to damaging populations during cooler months of the year and aestivate in the egg stage during summer months (Haque *et al*., 2007). However, the present result supports the earlier findings on the incidence of the species on tea, on which population of the mite showed an increase in response to warm weather and a decrease was experienced during rainy season (Roy *et al*., 2014). The present finding on the infestation of the species on *M. sylvestris* forms the first report of the species on this plant and hence serves to add a new host to the existing host range of the species.

In the present study, mite infestation was found confined to the upper surface of the leaves of *M. sylvestris*. Species of *Oligonychus* generally show a preference to occupy the upper leaf surface of their host plants. The various life stages of the species showed a preference to feed on the upper leaf surface of middle aged leaves of *M. sylvestyris.* This seems to support the earlier observations made on the preference of the species to the mature leaves of its host plants (Selvasundaram *et al.* 2003). In the present study, quite often mite infested leaves presented a more or less shrunken and crinkled appearance. On rose plant also, the species was reported to induce leaf deformity, and the deformed leaves would easily get abscissed (Haque *et al.* 2007). The crinkled appearance of the infested leaves was mainly due to water loss or increased transpiration rate through the feeding punctures created by the pest mites (Reddy and Baskaran, 2006).

During the initial stages of feeding, the species was found to induce the development of a number of chlorotic spots which up on continuous and intensive feeding, got coalesced to give a pale green or yellowish brown coloured appearance to the leaf surface. Similar damage symptoms were reported earlier on other host plants also owing to feeding activity of the species (Sangeetha and Ramani, 2011). During heavy infestation, moulting skin and faecal matter were deposited on the leaf surface, which in turn would negatively affect the normal photosynthetic processes of infested plants as observed earlier (Sumangala and Haq 2000; Reddall *et al.*, 2004). The impaired photosynthetic rate would enhance drying up of leaves, fecilitating the premature defoliation and thereby leading to drastic reduction in the yield and vigour of the plant (Reddy and Baskaran, 2006).

Photosynthetic pigment degradation, chloroplast destruction, chlorophyll fluorescence diminution and net photosynthetic rate reduction are indicative of stress conditions in plants (Bounfour *et al.*, 2002). Similar sort of chlorophyll depletion was reported to be induced by the species on other host plants like Cassava (Sangeetha and Ramani, 2011). Chlorosis has been reported as a most obvious leaf injury and it is indicative of chlorophyll loss (Landeros *et al*., 2004; Sivritepe *et al*., 2009). In the present study, the leaf chlorophyll content showed a decrease in par with the increasing mite population and which resulted in the coalescence of chlorotic spots to form chlorotic patches and subsequently a imparting a bleached or bronzed appearance to the leaves. Similar findings were reported earlier in cases of spider mite infestation, in which increasing mite density enhanced the severity of mechanical damage to leaf chloroplast (Kolodoziej *et al.* 1979; Park and Lee, 2002; Sangeetha and Ramani, 2007). Destruction of chloroplasts and reduction of photosynthetic pigments would lead to reduced photosynthetic activities, thereby leading to poor physiological processes in mite infested plants. (Avery and Briggs, 1968; Sances *et al*., 1981; De Angelis *et al*., 1983; Welter *et al*., 1989; Francesconi *et al*., 1996). Feeding by *Tetranychus urticae* on red raspberry also was found to cause a decrease in net photosynthetic rate and stomatal conductance of the plant (Cameron *et al*., 1990).

In the present study, infestation by *O. coffeae* was found to induce significant reduction in the photosynthetic pigments like the chlorophyll and carotenoids. The photosynthetic efficiency was also found reduced by displaying lowered Fv/Fm values for mite infested leaves when compared to uninfested leaves. In healthy leaves, Fv/Fm ratio is in the range of 0.75 - 0.85 (Bolhar and Oquist, 1993). The low Fv/Fm value in the case of infested leaves indicates that the plant undergoes severe abiotic or biotic stress (Shigeto and Makoto, 1998). A reduction in the Fv/Fm value in infested plants is a reflection of the decreasing of ability of PS II activity (Schansker *et al*., 2005). The significant Fv/Fm decrease in the infested plants (by about 20%) is indicative of serious PSII disturbances (Berova *et al*., 2007). The efficiency of photosynthetic machinery is indicative of the function of chlorophyll pigments or chlorophyll 'a' fluorescence, which demonstrates the functioning of water oxidation and photosynthetic electron transport in photosystem II under stress condition (Gray *et al*., 1997). Thus, the present results suggest that mite infestation would drastically decline the photosynthetic activity of the plant, by creating a stressed condition. In photosynthesis, antenna pigments like Chl *a* and chl *b* in leaf chloroplasts absorb solar radiation, and through resonance transfer the resulting excitation is channelled to the reaction centre pigments, which release electrons and set in motion the photochemical process. Chlorophyll *a* is the most important pigment, and which is essential for the oxygenic conversion of light energy to the stored chemical energy (Groff *et al*., 1995). Carotenoids function as light-harvesting pigments by channeling photons unabsorbed by the chlorophyll molecule to the reaction centre for photosynthesis (Niyogi, 1999). Thus, a marked loss of both chlorophyll and carotenoid pigments as observed during the present study is a clear indication that feeding by *O. coffeae* induces severe damage to the photosynthetic machinery by creating biotic stress on *M. sylvestris.*

CONCLUSION

The tea red spider mite, *O. coffeae* was found to induce severe damages on the leaves of *M. sylvestris*. The visible symptoms of feeding damage include chlorosis, necrosis, bleaching, shrunken and crinkled appearance of the infested leaves. Besides the mechanical damage, the mite was found to induce quantitative changes in the photosynthetic pigments and chlorophyll fluorescence parameters also. Mite infestation was found to lead to significant reduction in the amounts of major photosynthetic pigments like chlorophyll and carotenoids. Further, the mite infested leaves presented a lowered value for Fv/Fm, indicating that the mite infestation would induce biotic stress, in turn leading to significant reduction in photosynthetic efficiency. Thus, infestation by *O. coffeae* would lead to drastic

reduction in photosynthetic efficiency and hence vigour of the fruit crop, *M. sylvestris*.

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