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RESEARCH ARTICLE

Feeding impact of *Cisaberoptus kenyae* Keifer (Acari: Eriophyidae) on photosynthetic efficiency and biochemical parameters of *Mangifera indica* L.

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ABSTRACT: *Cisaberoptus kenyae* Keifer, the mango leaf coating mite is a widely distributed eriophyid species in the tropics, infesting all varieties of mango trees. The mite produces a white colored leaf coating on the adaxial surface of mango leaves, which extends to the entire leaf lamina in severely infested leaves. The present paper discusses the feeding impact of the mite on the photosynthetic pigments, and other biochemical parameters of mango leaves. Biochemical studies enabled to record a significant decrease in the chlorophyll and carotenoid pigments in mite infested mango leaves when compared to the uninfested leaves. The percentage loss in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was observed to be 65.80-84.64, 76.57-95.29, 68.69-87.13 and 39.31-56.75 respectively. Analysis of photosynthetic efficiency by measuring chlorophyll fluorescence (F_v/F_m) using the Handy PEA Hansatech Instrument pt. Ltd, UK revealed a decreased value, falling in the range of 25.15-28.43%. Mite infestation induced a significant loss in total carbohydrate also (57.86-80.58%) when estimated through Anthrone's method. Contrary to the above, a significant increase was observed in the proline and total phenol concentrations in mite infested leaves. An increase from 2.9578±0.36 to 5.2611± 0.61 µg/g was observed in the concentration of proline in mite infested leaf tissue. Total phenol content showed an increase from 55.14± 1.72 to 81.16± 0.85 mg/g in mite infested leaves. Results showed a statistical significance ($p<0.05$), confirming that infestation by *C. kenyae* induced severe stress, leading to enhanced production of defense compounds like proline and total phenol, thereby adversely affecting the photosynthetic efficiency and primary metabolite production of the host, *M. indica*.

Keywords: Carotenoids, chlorophyll, *Cisaberoptus kenyae*, *Mangifera indica*, proline.

INTRODUCTION

Eriophyids represent a highly host specific group of phytophagous mites, infesting almost all categories of economic crops and inducing diverse forms of plant abnormalities. A good number of these mites are designated as notorious pests of fruit crops. *Mangifera indica* is prone to attack by a variety of pests, comprised mainly of insects and mites. Of the major group of mite pests attacking mango foliage, *Cisaberoptus kenyae* of the family Aberoptidae has been recognized as a common species which induces a prominent leaf coating on mango leaves (Keifer, 1966). The species is commonly called as the mango mite of the tropics and it enjoys a wide distribution in all mango cultivated regions. The mite initiates the leaf coating as a white powdery substance secreted at the leaf petiole and which gradually extends as silken strands across the laminar area on the adaxial surface. When fully formed, the leaf coating appears as an ashy white colored membrane on the upper surface and in severely infested leaves, the entire leaf lamina is covered by the membrane. Under high magnification, the membrane appears as a web formed by crude silken strands. The mite lives gregariously under the web which affords protection to its various life stages viz. the egg, nymph I, quiescent stage I, nymph II, quiescent stage II, adult male and adult females (protogyne and deutogyne).

Cisaberoptus kenyae was known as a silver blotch mite (Sternlicht and Goldenberg, 1976), it causes damage in the epidermal tissue that leads to necrosis and subsequent secretion of a milky white substance from the midrib or veins. The leaf coating produced by the mite was found to cause leaf decline, but without inducing any mechanical damage (Hassan and Keifer, 1978). Mite infestations were found relatively higher on majority of the mango trees in India, especially in South India where all varieties showed the presence of leaf coating. The level of infestation and population density of *C. kenyae* had a direct correlation with the varietal difference of mango trees as evidenced through population studies carried out in Kerala on five mango varieties (Ramani and Haq, 1989). Detailed studies were undertaken on the incidence of the mite and its natural enemies (Ramani, 1991). Developmental parameters of the mite were also elucidated on mango leaves from Kerala (Ramani and Haq, 1991). Studies on monthly fluctuations in the population density of the mite, susceptibility of common varieties of mangoes to mite infestation as well as per cent of leaf area coated by the mite on different mango varieties etc. were performed in North India at Varanasi, Uttar Pradesh (Rai et al., 1993). Investigation on seasonal incidence and control measures using chemical and botanical acaricides against *C. kenyae* infesting mango cultivars such as Neelum, Bangalora, Malgoa, Rumani and Sappattai was carried out in Tamil Nadu, South India (Umapathy and Rajendran, 1999). Population studies of the species were carried out

on mango trees in Egypt also, for a period of two years (Al-Azzazy, 2005). Detailed studies were undertaken on the seasonal distribution pattern and population density of the mite on two mango varieties such as Hindi and Alphonso cultivars in Egypt (Abou-Awad et al., 2009), the authors collected data also on the control aspects of the mite employing natural enemies like the predatory mites as well as acaricides. The effect of feeding activity of the mite on the macro and micronutrient levels of mango leaves was studied also in Egypt (Abou-Awad et al., 2012).

A present study was undertaken to gather knowledge on the extent of damage induced by the mite on the physiological parameters of the host, such as the cellular damages, photosynthetic pigment concentration and photosynthetic efficiency, and other biochemical components like total carbohydrate, phenol and proline contents.

MATERIAL AND METHODS

Collection of leaf samples for biochemical estimation

Leaf samples required for biochemical estimation were collected randomly from the mango variety, 'Rumani' cultivated in the premises of the Department of Zoology, University of Calicut. Both the control and experimental leaves were collected from the same host plant. The leaves were collected randomly from different heights of the tree, the uninfested leaves were treated as control while mite infested leaves showing the presence of leaf coating and harboring a population range of 88-144 mites/ cm^2 area were considered for the experiments.

Qualitative estimation of feeding damage

Assessment of visual damage symptoms

Visible symptoms of damage induced by the mite on the foliage of the mango variety, *M. indica rumani* were traced based on the presence, nature and extent of leaf coating and other associated symptoms developed on the leaves.

Elucidation of cellular levels of leaf damage

Anatomical features of uninfested and mite infested leaves of *M. indica* were studied by examining stained sections and comparing the cellular characteristics of both samples. For making sections of uninfested and infested leaf tissues, the leaf samples collected from the host plant were brought to the laboratory and thoroughly cleaned with distilled water for further processing. Thin sections of mite infested and uninfested leaves were made using a sharp blade, then were placed in separate cavity blocks. Sections of both leaf samples were dehydrated in alcohol series and stained in safranin. After proper staining, the sections were destined in distilled water and then on microscope slides using glycerin. The mounted slides were examined using a microscope to observe the cellular features for making a ready comparison of the cellular damage induced by the mite. Appropriate photographs were taken using a microscope (Labomed Lx 400) and presented.

Quantitative estimation of damage

Estimation of photosynthetic pigments

Concentration of photosynthetic pigments like chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids present in the uninfested and mite infested leaf tissues was estimated using the method of Arnon (1949) and Coombs et al. (1985). One gram of the fresh leaf tissue, representing the uninfested and mite infested tissues separately, was cut into small pieces and placed into separate specimen bottles (for control and experimental samples) containing 10 ml of absolute ethanol and stored in the dark for two weeks. One ml of the filtered extract was then diluted with 6 ml of absolute ethanol and the absorbance of the chlorophyll solution was measured using a UV-spectrophotometer (Shimadzu) at 645, 663, and 470 nm (at 750 nm also for making correction for impurities) against the solvent blank (absolute ethanol). The amounts of chlorophylls and carotenoids present in the mite infested and uninfested leaf samples were calculated and expressed in mg/g fresh weight of the leaf tissue, using the formula of Arnon (1949), as given below. The experiment was repeated 9 times to get concordant results. The data were statistically analysed using SPSS Statistics (IBM version 19) and the values were expressed as Mean ± SEM.

- 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 (A663A750)] × 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).
- Based on the above equations, calculations were made and the data were presented in µg/g tissue.

Measurement of photosynthetic efficiency

Photosynthetic efficiency of the mite infested and uninfested mango leaves was analysed with the help of a portable fluorescence monitoring system (Handy PEA, Hansatech Ltd., Vorfolk, UK) by measuring chlorophyll fluorescence. Prior to the fluorescence measurements, a circular surface of the upper face of the leaves of both the control and experimental categories 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 analysed using independent-sample's T-test, following SPSS version 16.0.

Estimation of total carbohydrates

The amount of total carbohydrates present in mite infested and uninfested mango leaf samples was estimated using Anthrone's method (Hedge and Hofreiter, 1962). The leaf samples were thoroughly cleaned, a 500 mg of each leaf sample (control and experimental separately) was homogenized with 5 ml of 2.5N HCl. The homogenate was then kept in hot water bath for about 3 hours and then each sample was made up to 25 ml and centrifuged at 2000 rpm for about 20 minutes. 0.1 ml of each supernatant was taken in a test tube and made up to 1.0 ml with distilled water. To each test tube, 4.0 ml of cold Anthrone's reagent was added and heated for 8 minutes and then cooled to room temperature. The dark green color developed was read in a UV-spectrophotometer at 630 nm against glucose as standard. The amount of total carbohydrates present in each leaf sample was calculated separately for mite infested and uninfested samples following the equation given below:

$$\text{Amount of carbohydrates present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of the test sample}} \times 100$$

Estimation of total phenol

The concentration of total phenol present in mite infested and uninfested mango leaves was estimated using the method of Malick and Singh (1980). 1.0 g each of thoroughly cleaned leaf sample was ground in a mortar with 10 ml of 80% ethanol and then was centrifuged at 10,000 rpm. To the residue, 5.0 ml of 80% ethanol was added, ground and centrifuged. The supernatant was evaporated to dryness and to the dried residue, 5.0 ml of distilled water was added and mixed well. From the above solution, 1.0 ml was pipetted out and to which 2.0 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added. To the above mixture, 20% solution of sodium carbonate was added and kept in a water bath for 1 minute, cooled to room temperature and then the OD of the solution was read at 650 nm in a UV-spectrophotometer against tannic acid used as standard for the reaction. The total phenol content of the leaves was estimated following the equation given below and expressed in µg phenol/g sample.

$$\text{Concentration of sample} = \frac{\text{Standard concentration} \times \text{Sample OD}}{\text{OD of standard}} \times \frac{5 \times 1}{1\ \text{gm}}$$

Estimation of proline

The feeding impact of *C. kenyae* on the production of stress amino acids, like proline in mango leaves, was assessed using the method of Bates et al. (1973). The experiment was conducted by homogenizing 0.5 g of thoroughly washed leaf samples (mite infested and uninfested samples separately) in 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through a glass fibre filter and to 2.0 ml of the filtrate, 2.0 ml of glacial acetic acid and 2.0 ml of acid ninhydrin reagent were added. The solution was heated for one hour in a hot water bath and the reaction was terminated by keeping the test tubes in an ice bath for 5 minutes. To each of the ice-cold test tube, 4.0 ml of toluene was added and stirred well for 20 seconds. The separated toluene layer was taken out and read at 520 nm in a UV- spectrophotometer (Shimadzu). The concentration of proline was determined from the standard curve and calculations were made following the equation given below and expressed on a fresh weight basis.

$$\text{µg moles per gm tissue} = \frac{\text{µg proline ml} \times \text{ml toluene}}{115.5} \times \frac{5}{\text{gm sample}}$$

RESULTS

Results of field observations revealed infestation by *C. kenyae* on almost the mango trees grown/cultivated in the Calicut University Campus and adjacent localities, as evidenced through the presence of ashy-white coating developed on the upper surface of leaf lamina (Figs 1A,B). The newly sprouted leaves were found devoid of mite infestation. The mite was found to induce the development of a white powdery substance, initially on the leaf petioles of *M. indica*. Subsequently, with the population growth and progressive feeding activity of the life stages of the mite, the infested leaves developed ashy –white colored silken strands which gradually became hardened as a silver colored membrane, covering the entire leaf lamina including the mid rib and veins (Fig. 1C). All life stages of the mite such as the egg, nymphal stages, quiescent stages and adult (male and females) were found to enjoy a secluded habitat underneath the leaf coating (Figs 1D-F) and the areas covered by the leaf coating turned into brown-black colored. All the active life stages sucked out the plant sap, resulting in the color change, loss of vigor, browning, blackening, drying up and defoliation. Bronzing and necrosis were also common on the adaxial leaf surface where the foliar surface completely turned into black colored and quite often became dry.

The population density of the mite showed variation and the maximum population size was evident during the summer months. During the present study, leaf samples with a population density of 88-144 mites/cm^2 were considered for biochemical estimations. Heavy mite infestation was common during the summer months. However, during monsoon season, the leaf coatings were very closely adhered to the leaf surface and relatively low populations of *C. kenyae* were observed on wet leaves. Severely infested leaves appeared brown-black colored, especially under the leaf coating, which often turned to dried patches along the mid rib and side veins.

Results of histological studies disclosed reduced number of chloroplasts in the palisade and spongy tissues of the mesophyll in mite infested leaves. The cell size in the spongy layer of mesophyll tissue was also found reduced. The control leaf showed tightly packed, elongate cells with dense chloroplast in the palisade layer (Figs 2A-C), while the mite infested leaf showed loosely arranged and less elongate palisade cells with scanty amount of chloroplast or quite often with a total absence of chloroplast also. The cuticle and upper epidermis of mite infested leaf were disrupted occasionally, and the underlying palisade

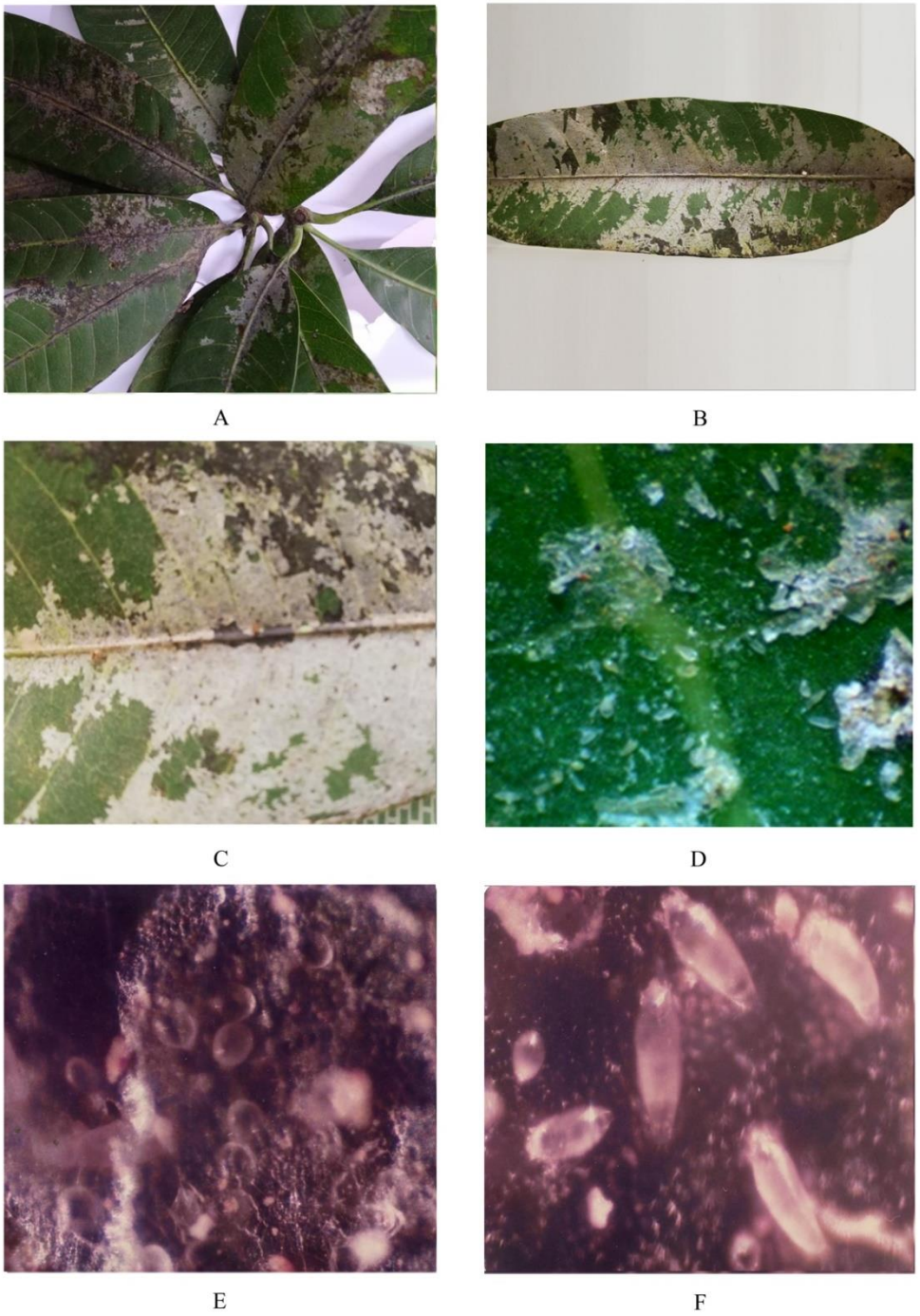

Figure 1. A) Twig of *Mangifera indica* L. showing infestation by *Cisaberoptus kenyae* Keifer, B) A single leaf showing the ashy white coating, C) Damage on the adaxial surface including midrib, D) A colony of *Cisaberoptus kenyae*, showing the different life stages exposed after removing leaf coating, E) An enlarged view of eggs laid under the leaf coating, F) Enlarged view of different life stages.

tissue also presented brown colorations and symptoms of drying up of leaves (Fig. 2D). Spongy layer of mesophyll showed very low amount of chloroplast and often appeared shrunken and irregular with larger intercellular spaces (Figs 2E,F).

Results of biochemical estimation of photosynthetic pigments revealed a significant reduction ($p<0.05$) in the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid pigments in mite infested mango leaves. As presented in Table 1, the mean concentrations of chlorophyll a, b and total carotenoids in mite infested mango leaves were 872.017 µg/g, 438.89 µg/g and 1426.66 µg/g respectively when compared to 3519.07 µg/g, 3119.79 µg/g and 6456.65 µg/g of the uninfested mango leaves. Thus, the percentage loss was 75.22±9.42, 85.93±9.36 and 77.91±9.22 in chlorophyll a, chlorophyll b and total chlorophyll respectively (Table 1), which showed a significant ($p<0.05$). Similarly, a significant reduction ($p<0.05$) in the carotenoid pigments also was recorded during the study which was accounted to 2125.72 µg/g in mite infested leaves against 4090.62 µg/g in uninfested leaves (Table 1), thereby resulting in a percentage loss of 48.03 ±8.72 ($p<0.05$) (Table 1).

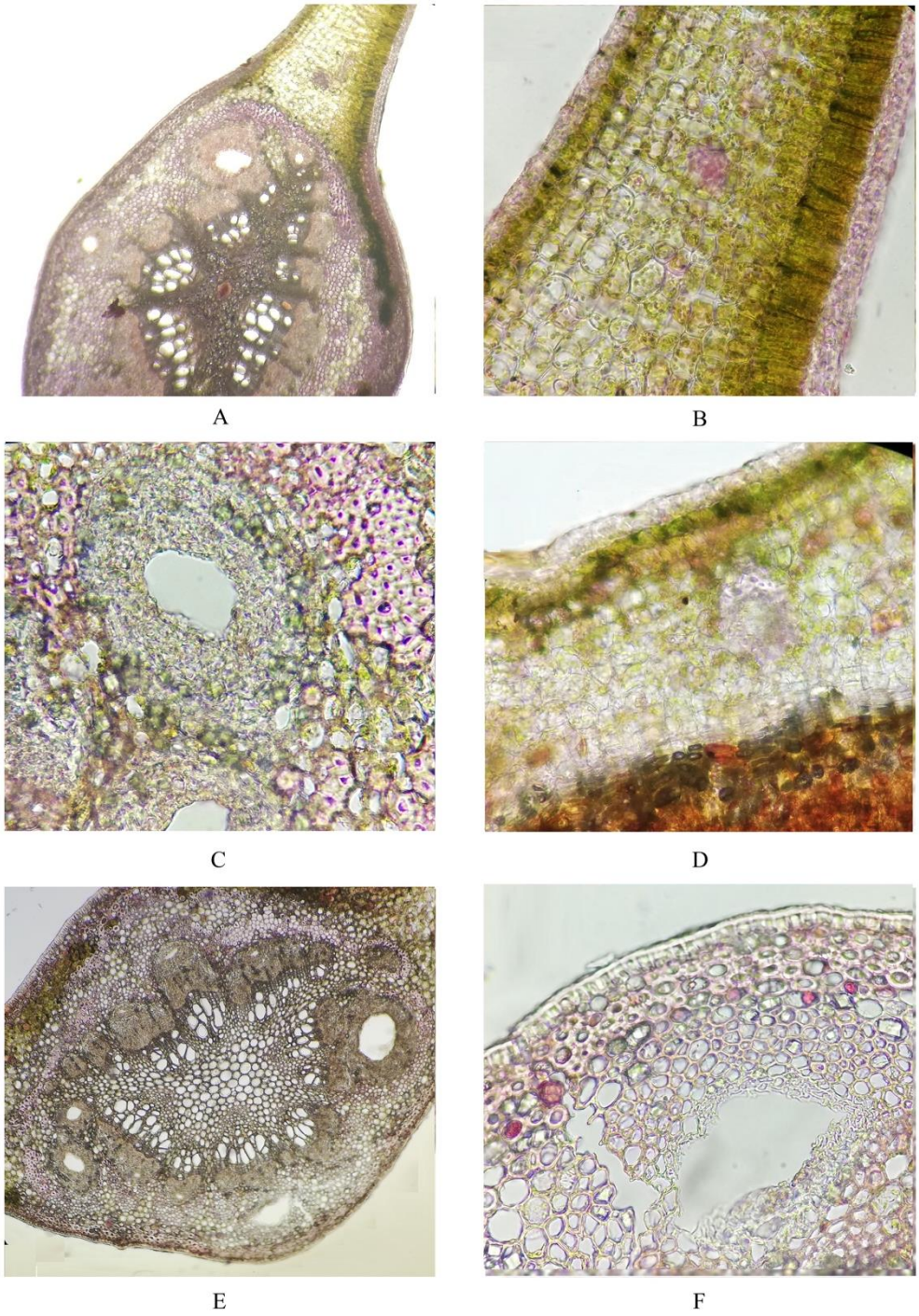

Figure 2. A-C) Uninfested mango leaves showing the normal densely packed, chloroplast rich palisade tissue, spongy mesophyll and vascular bundles, D-F) Mite infested mango leaves showing drying symptoms on cuticle, epidermis and palisade tissue, reduced number of chloroplast, irregular & shrunken spongy mesophyll tissue with larger intercellular spaces.

When the leaf chlorophyll fluorescence parameters such as F_0 (minimum/initial fluorescence), *Fm* (maximum fluorescence), *Fv* (variable fluorescence) etc, which were measured separately for uninfested and mite infested mango leaves, a reduction was observed in the *Fv*/*Fm* values in mite infested leaves (0.6188±0.0133) when compared to those of uninfested leaves (0.8457±0.012) (Table 2). The percentage loss in chlorophyll fluorescence was recorded as 25.15-28.43 (Table 2) owing to infestation by *C. kenyae*.

A significant reduction ($p<0.05$) in total carbohydrate was also observed during the study in mite infested mango leaves. The mean concentration of total carbohydrate in uninfested leaves was 85.48±0.88 mg/g while it was 26.31 ±0.87mg/g. (Table 3) in mite infested leaves, which could account for a mean percentage loss of 69. 22±11.36. Unlike the depleting effect on various biochemical parameters like the photosynthetic pigments and total carbohydrate, feeding activity of *C. kenyae* enhanced production of stress factors like proline and phenolic compounds as observed during the study (Table 3). Mite infested leaves

Table 1. Changes in the concentration of photosynthetic pigments (µg/gm tissue) in the leaves of *Mangifera indica* L. due to feeding impact of *Cisaberoptus kenyae* K. (Mean ±SEM)*

Photosynthetic pigments	Concentration in uninfested mango leaf tissue (µg/g)	Concentration in mite infested mango leaf tissue (µg/g)	Loss in concentration of pigments (µg/g)	Per cent loss of pigments
Chlorophyll a	3519.07±39.27 a	872.02±2.26 b	2647.05±37.1	75.22±9.42
Chlorophyll b	3119.79±51.94 a	438.89±3.28 b	2680.9±48.66	85.93 ±9.36
Total Chlorophyll	6456.65±61.47 a	1426.66±4.77 b	5029.99±56.7	77.91 ±9.22
Total Carotenoids	4090.62±8.52 a	2125.72±1.09 b	1964.91±7.43	48.03 ±8.72

*Different letters in each row indicate significant differences - p<0.05.

Table 2. Changes in the values of F_v/F_m and photosynthetic efficiency induced by the feeding activity of *Cisaberoptus kenyae* K. in the leaves of *Mangifera indica* L. (Mean ±SEM)*

F_v/F_m value in uninfested mango leaf tissue	*Fv*/*Fm* value in mite infested mango leaf tissue	Reduction in *Fv*/*Fm* value	Per cent loss in photosynthetic efficiency
0.8456±0.01158 a	0.6188±0.01304 b	0.2268±0.00146	26.79 ±1.64

*Different letters in each row indicate significant differences - p<0.05.

Table 3. Changes in the concentration of various biochemical constituents induced by the feeding activity of *Cisaberoptus kenyae* K. in the leaves of *Mangifera indica* L. (Mean±SEM)*

Biochemical parameters	Concentration in uninfested mango leaf tissue (µg/g)	Concentration in mite infested mango leaf tissue (µg/g)	Loss/increase in concentration (µg/g)	Per cent loss/increase in concentration
Total carbohydrates	85.48± 0.88 a	26.31±0.87 b	-59.17±0.01	33±11.36
Total Phenol	55.14±1.72 a	81.16±0.85 b	+26.02±0.87	47.18 ±5.06
Proline	2.96±0.36 a	5.26±0.61 b	+2.68±0.25	77.70 ±6.94

*Different letters in each row indicate significant differences - p<0.05.

showed a mean percentage increase of 47.18 ±5.06 in phenolic compounds when compared to that of the uninfested leaves (Table 3). A significant increase was observed in the concentration of proline also in mite infested leaves. Results of the study disclosed a mean concentration of 2.96±0.36 µg/g of proline in uninfested leaves whereas mite infested leaves showed an enhanced level, reaching a mean value of 5. 26±0.61 µg/g which could account to a mean percentage increase of 77.7±6.94 (Table 3).

DISCUSSION

Cisaberoptus kenyae was designated as the mango leaf coating and webbing mite or blotch leaf miner mite (Keifer, 1966) infesting under the epidermis of the upper surface of young leaves, raising the epidermal cells, and inducing the development of curling and browning spots on the undersides of mango leaves (Abou-Awad et al., 2009). In the present study, infestation of C. kenyae was readily identified under field conditions by the presence of ashy-white membranous coating on the adaxial surface of mango leaves. However, no curling or spotting symptoms were noticed on mite infested leaves. All life stages of the mite were found to colonize the upper leaf surface, enjoying the microhabitat available underneath the leaf coating secreted by the mite and the upper cuticle. Leaves of all mango varieties examined showed mite infestation, though population differences could be observed depending upon varietal difference (Ramani and Haq, 1988; Abou-Awad et al., 2009).

Feeding activity of eriophyid mites induces diverse types of toxemic and other non-distortive changes in their host plants (Oldfield, 1996), leading to the development of symptoms like galling, russeting, browning, bronzing, silvering, chlorotic spots, reddening and so on (Craemer et al., 1996; Rancic et al., 2006; Petanovic and Kielkiewicz, 2010a,b). Visible symptoms of such infestation could be associated with discolorations of plant organs, brown scarification, necrosis and destruction of buds, witches broom effect, distortion of veins, chlorotic spots, mottled appearance, presence of spotting on abaxial surface, rapid cell death and collapse of lower epidermal and mesophyll layers, development of lesions, failure of nutritive cell formation, premature defoliation etc. (Jeppson et al., 1975; Westphal, 1992; Cullen and Briese, 2001). In the present study, *C. kenyae* was found to induce damage symptoms such as brown to black coloration developed underneath the leaf coating and drying symptoms on the

foliar surface including the midrib and other veins. In severely infested cases, the entire leaf lamina was found to be covered by the coating with the upper surface completely turned black. Development of such brown-black colorations and subsequent drying up of leaves is a clear indication of chlorophyll loss resulted from the progressive feeding activity of the mite through leaf sap drainage. Further, the leaf coating on the upper leaf surface would hinder penetration of sunlight through the epidermal layer, which is the most essential factor for photosynthetic activity. Thus, the combined effect of chlorophyll destruction and prevention of direct entry of sunlight would impose an adverse impact on the photosynthetic efficiency of the host, *M. indica.*

Results of histological studies indicated that mite feeding would lead to a reduction in the chloroplast in the palisade and spongy tissues of the mesophyll. Additionally, the size of spongy cells also got reduced. Mite infested leaf sections showed the presence of larger intercellular spaces and often the inner mesophyll layer appeared shrunken and irregular. The cuticle and upper epidermis of mite infested leaf often showed signs of disruption and underlying palisade tissue became brown colored. This is a clear indication of drying up of leaves due to the removal of plant sap and the resulting water loss induced by the feeding activity of the mite. Thus the present study seems to support the earlier findings on similar cellular deformities leading to disruption of cuticle, punctured and collapsed epidermal cells, and reduced number of spongy and palisade parenchyma cells in mite infested leaves (Park and Lee, 2002; Sangeetha et al., 2011).

The photosynthetic efficiency of plants to a large extent is determined by the chlorophyll content of the leaves (Lahai et al., 2003; Netondo et al., 2004). Feeding by *C. kenyae* was found to induce significant reduction in photosynthetic pigments, resulting in respective per cent loss of 75.22±9.42, 85.93±9.36, 77.91±9.22 and 48.03±8.72 in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, thereby supporting earlier reports made on spider mites (Puchalska, 2006; Sangeetha and Ramani, 2011a-c), false spider mites (Prabheena and Ramani, 2013, 2014) and eriophyid mites (Nasareen et al., 2012). Chlorophyll a and b pigments in the leaf chloroplasts serve vital functions in photosynthesis, being instrumental in absorbing solar radiation, and through resonance transfer, channel the resulting excitation to the reaction centres, and thereby promoting release of electrons and setting up the photochemical process. Chlorophyll a is most essential for oxygenic conversion of light energy to the stored chemical energy (Gropper et al., 2009). Carotenoids also would help to channel photons unabsorbed by chlorophylls to the reaction centre for photosynthesis (Niyogi, 1999) and thus function as light-harvesting pigments. Hence, a significant reduction in the chlorophylls and carotenoids by *C. kenyae* would definitely impair the photosynthetic activity, leading to substantial decline in the vigor and biomass of *M. indica.* Thus the results of the study corroborate with the earlier findings made on another eriophyid species, *Acalitus hibisci* on its host, *Hibiscus vitifolius* where it induced a loss of 18.05% and 39.01%, respectively in chlorophyll a and b pigments (Chakrabarti et al., 1999). However, contradictory to the present finding on the reduced carotenoid concentration induced by *C. kenyae*, the feeding of *A. hibisci* enhanced production of carotenoids by 59.8%.

Feeding activity of *C. kenyae* was also found to reduce photosynthetic efficiency of mango trees as evidenced during the study, based on measurement of chlorophyll fluorescence. A percentage loss of 25.15-28.43 was recorded in the chlorophyll fluorescence owing to infestation by *C. kenyae.* Being a very rapid, nondestructive, and early indicator of stress, chlorophyll fluorescence has been employed as a widely used parameter to evaluate impact of varied factors on photosynthetic efficiency of plants, even in the absence of visible symptoms (Iatrou et al., 1995). Emission of chlorophyll fluorescence is often negatively correlated with photosynthetic efficiency (Pereira et al., 2000) and *Fv*/*Fm* values are taken as an index to assess the potential quantum yield of PS II and thus the physiological state of photosynthetic apparatus of plants (Maxwell and Johnson, 2000; Pereira et al., 2000). Generally, *Fv*/*Fm* values are constant, falling in the range of 0.778 to 0.860 for healthy plants and reduced *Fv*/*Fm* values indicate severe abiotic or biotic stress conditions of host plants (Kawashima and Nakatani, 1998; Pospíšil et al., 1998; Schansker et al., 2005) which in turn would lead to a drastic reduction in chlorophyll content (Morales et al., 1991). A significant reduction by about 20% is suggestive of disturbance of PSII (Berova et al., 2007), which could have resulted from the destruction of photosynthetic pigments and feeding stress induced by herbivory. In the present study, a decrease of > 20% was recorded in the *Fv*/*Fm* value, thereby indicating a reduction in the photosynthetic efficiency, resulted from a decrease in chlorophyll content, owing to mite feeding. Various authors have established a close linkage between the chlorophyll reduction and the reduced photosynthetic activity of mite infested plants (Bondada et al., 1995; Haile and Higley, 2003). The feeding activity of arthropods is known to damage xylem or phloem (Welter, 1989), and which in turn often would affect other physiological functions of host plants like transport of water and sugars, stomatal aperture etc. and hence would lead to a decline in the photosynthesis of remaining leaf tissues also (Nabity et al., 2009). The sucking activity of *C. kenyae* has led to structural degeneration of chloroplast in the palisade and spongy layers of mesophyll tissue and which would have an adverse impact on the neighbouring tissues also, thereby culminating in significant loss in photosynthetic pigments and photosynthetic efficiency of the host as observed during the present study. Added to these, the leaf coating produced by the mite would hamper the entry of sunlight on the leaf surface and which in turn would aggravate loss in photosynthetic efficiency.

Sap sucking arthropods like the mites are known to induce a significant alteration in various metabolic products and such significant changes were induced by *C. kenyae* also in *M. indica.* Dependence of phytophagous mites on minerals and other phytochemical components of host plants to meet their nutritional and reproductive needs has been well established (Al-Azzazy, 2012). *C. kenyae* was also shown to induce a significant reduction in vari-

ous macro and micronutrients in two mango cultivars studied in Egypt (Abou-Awad et al., 2012). Furthermore, the present data confirmed that feeding activity of *C. kenyae* not only would affect the micro and macronutrient contents but also the photosynthetic pigment concentration, photosynthetic efficiency as well as the total carbohydrate content of its host, *M. indica.* In addition, it would also induce biotic stress, leading to enhanced production of stress factors like proline and phenols.

Herbivory is known to generate diverse types of defensive responses in plants which often would lead to a reduction in the expression of photosynthesis related genes (Kessler and Baldwin, 2002). Several such defensive compounds serve as biocides against herbivores and their production would destroy the normal photosynthetic or homeostatic functioning of plants (Zangerl et al., 2002). Phenolic compounds are produced as a part of defense-related system, which are designated as widely distributed secondary plant products to counteract stress conditions (Harborne, 1980). Phenol offers resistance to diseases and pests in plants and enhanced production of phenolics in economically important plants during pest attack was recorded and it was concluded that the increase in total phenols induced resistance in hosts against herbivory (Ananthakrishnan et al., 1992). Plants rapidly synthesise phenolic compounds and often polymerize them in the cell walls in order to defense against infection (Matern and Kneusel, 1988). Certain phenolic compounds serve to precipitate plant proteins so as to convert them as indigestible to herbivores. Mango leaves infested by *C. kenyae* showed a mean percentage increase of 47.18±5.06 in phenolic compounds when compared to that of the uninfested leaves, thereby supporting the earlier finding that the plant enhanced production of phenolic compounds to defense herbivory.

Proline, one of the basic amino acids is produced by plants to defend against herbivory which affords protection to cellular structure and cytoplasmic enzymes (Serrano and Gaxiola, 1994) by storing nitrogen and carbon sources. Proline plays a critical role in protecting plants under stress condition (Kuznetzov et al., 1999) as it moves between tissues, and promotes detoxification of reactive oxygen species and stabilization of cell membranes (Kavi Kishor et al., 2005). The highly enhanced levels of proline in mango leaves infested by *C. kenyae* as observed during the current study could be considered as a defense mechanism developed by the plant against the feeding stress induced by the mite. Degradation of photosynthetic pigments, destruction of chloroplast, reduction in chlorophyll fluorescence and net photosynthetic rate generally indicate the stress conditions faced by plants (Bounfour et al., 2002; Anitha and Ramani, 2016). The results of the present study strongly support the above by clearly establishing the extent of damage induced by the leaf coating mite by way of inducing depletion of photosynthetic pigments, decline of photosynthetic efficiency of the plant, decrease in total carbohydrate and by stimulating the production of defensive substances like proline and phenolic compounds.

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