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STUDY TO ASSESS THE DAMAGE POTENTIAL OF *TETRANYCHUS URTICAE* KOCH. ON PAPAYA, *CARICA PAPAYA* L.

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Abstract

A preliminary study was conducted to assess the damage potential of *Tetranychus urticae* Koch on *Carica papaya* L.The study was conducted during the period of March 2019 to February 2020. Papaya plants growing in different localities particularly from Makkarapparamb panchayath of Malappuram district, Kerala was taken for the investigation. The preliminary studies revealed that most of the papaya plants were found to be infested with the two spotted spider mite, *T. urticae* . The mite feeding caused significant damage symptoms like the presence of white spots or patches and curling of the leaves. The damage potential of *T.urticae* was analysed both qualitatively and quantitatively. Qualitative studies of mite injured leaves disclosed damage to the mesophyll layer. Quantitative estimation of chlorophyll (chlorophyll a - 54.18%, chlorophyll b - 50.72% and total chlorophyll - 53.16%), total carbohydrate (5.31%) and total protein (5.83) showed a decrease in their concentration while the concentration of proline (38.31%) and total phenol (33.58%) recorded an increase in mite infested leaves.

Keywords **:** *Spider mite, T. urticae, Damage potential, Carica papaya, Chlorophyll estimation*

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Introduction

Tetranychus urticae , commonly called as the two spotted spider mite is a notorious pest of many agricultural and horticultural crops. It has been recorded from most countries in Europe, Asia, Africa, Australia, The Pacific and Carribean islands. The species was reported for the first time from USA (Tuttle and Baker, 1968) and it has been recognized as the most polyphagous species of spider mite with worldwide distribution on more than 1200 species of host plants (Dermauw et al., 2013). T. urticae feeding has shown to produce a set of damages in host plants ranging from mere mechanical injuries to miscellaneous physiological alterations. This spider mite is well known to cause significant reduction in leaflet chlorophyll content, rate of photosynthesis and fruit size (Sances et al., 1982; Van de Vrie., 1983).The host plant, Carica papaya (Papaya) is a major fruit crop of most tropical countries being rich in nutrients and thought to have a range of health benefits in addition to its use in a number of culinary ways. In the present study, the feeding nature and extent of damages caused by T. urticae on papaya is elucidated through field observations and a series of laboratory experiments.

Materials and Methods

Collection and identification of mite specimens

The study was conducted at different localities particularly from Makkarapparamb panchayath of Malappuram district, Kerala during the period of March 2019 to February 2020 where Papaya plants are growing intermittently. The mite infested and un infested leaves of the host plant were plucked and kept in zip lock polythene bags, labelled and transported to the laboratory for further analysis.All the Laboratory observations were made under a Binocular Stereo Zoom Microscope (Labomed CZM 4 Binocular Zoom Stereo Microscope). Collected mite specimens of both male and female individuals were preserved in 70% alcohol. Permanent slides were prepared by mounting these specimens in Hoyer's medium for further identification procedures and identified with the help of identification keys, appropriate literature and also with the help of experts in the field. Photographs of the infested and un infested leaves, various life stages of the mite were taken using Olympus DP22 digital camera for microscopes.

Assessment of feeding damage:

The potential of T. urticae to feed on the plant cells leading to the development of significant damage symptoms were assessed through qualitative and quantitative analysis.

A. Qualitative estimation:

The qualitative measures included the assessment of visible symptoms developed on the mite infested leaves as well as the cellular damages examined through leaf sectioning method. Visible damage symptoms induced by *T. urticae* on papaya leaves were assessed both under field and laboratory conditions. Confirmation of field studies were done in the laboratory by rearing different life stages of mite and the symptoms developed were observed following their feeding. In the leaf sectioning method thin leaf sections were stained with safranin and mounted on glycerine for further microscopic observations. Photographs of the stained sections of the infested and un infested leaf tissues were taken. In order to assess the impact of feeding of the mite on the tissues, the comparison of the cellular features of both infested and un infested leaves were done.

B. Quantitative estimation:

Quantitative analysis of mite infested and un infested leaves were made through estimation of various biochemical parameters. In the present study, quantitative estimation of chlorophyll a, chlorophyll b and total chlorophyll of mite infested and un infested leaves were performed to assess the impact of mite feeding on the photosynthetic efficiency of the plant. Other parameters like total carbohydrate, total protein, total phenol and proline were also estimated through standard procedures to understand the extent of stress caused by the mite population on the host plant.

The chlorophyll content was estimated according to the method of Arnon (1949). Determination of total carbohydrate is done by Anthrone Method (Hedge and Hofreiter., 1962). Estimation of total protein in mite infested and un infested papaya leaf was done by Lowry's method (Lowry et al., 1951). The amount of total phenols in the plant extracts were estimated by the method proposed by Mallick and Singh (1980). Free proline contents present in the mite infested and un infested samples were estimated following the method of Bates et al. (1973).

Results and Discussion

Qualitative assessment of feeding damage induced by *T. urticae* on Papaya plant:

Qualitative assessment of leaf damage was made under field conditions based on the visual symptoms developed. Confirmation of the field studies was made in the laboratory by rearing the mites on host leaves and thereby producing colonies with different life stages (Fig: 1). Presence of white spots or patches was found to be the major visible symptom caused by the feeding activity of *T. urticae*. Along with

this, yellowing of leaves and appearance of necrotic spots also occurred in the advanced stages of infestation. The leaf may also get wrinkled or curled in severe cases of infestation. The active life stages of the mite exhibited active feeding by sucking the leaf sap with their needle like piercing mouthparts. This activity results in the removal of sap and as a result small chlorotic spots formed at each feeding site. Continued feeding causes the joining of chlorotic spots to form white patches and later the leaves turn yellow. (Fig:2). This supports the earlier findings on the general symptoms of spider mite infestation (Gupta, 1985). Density of mites declines on damaged leaves while the density found to be increasing in fresh and uninfected leaves. The results of field observations revealed that the matured leaves are more preferable to *T.urticae* over tender leaves. Well established colonies were a rare scene in tender leaves. This result is in concordance with the report of Dhooria (1985), Sobha and Haq (1999) where they reported that the leaves that were too old or too young are preferred less by spider mites when compared to the middle-aged leaves. Mite density was lower on young leaves when compared to mature leaves as the young leaves appear to be protected by a high concentration of mono and polyphenols although they contain more nutrients than the mature leaves (M Kielkiewicz and M Van de Vrie 1990). In the present study, *T. urticae* infestation was found to be mainly confined to the lower surface of the leaf lamina. They were found densely populated near the midrib and veins. Adjacent to the petiole, the midrib is comparatively more thickened which provide ample space for the mite to establish large colonies. The space available in regions like this provides shelter for their oviposition from direct exposure to sunlight. Veins and veinlets also provide firm grip to these mites during oviposition (Banu and ChannasBasavanna, 1972; Dhooria, 1982). They were rarely found on the upper surface and fringe regions of the leaves. This indicates the selectivity of mites in areas of the leaves for their establishment and further propagation. The Females usually prefer the underside of the leaves for egg laying since the eggs are better protected. This observation agrees with the studies of Gimenez - Ferrer et al. (1994). They were found in colonies under the elaborate silk webbings. They spin fine, loose webs over the surface of leaves. The intensity of webbing is influenced by leaf surface texture.

The colony consisted of all life stages such as eggs, larva, nymphs, adult males and females and quiescent stages. Many cast skins can also be found in the leaf lamina. Males were very rare in the colonies as compared to the number of females. Webbing protects the eggs and immatures as they remained totally confined within the canopy of the webbing. It also safe guard the viability of eggs, as their removal often leads to low hatchability rate of these eggs (Hazan et al., 1974). The deposited eggs were found to be entangled in the web along with the faecal pellets. Egg cases and moulting skins of various developing stages were also found attached

to the webs on the leaf surface. These observations seem to support earlier findings (Sumangala and Haq, 2000; Reddall et al., 2004; Sangeetha and Ramani, 2007).

Stained sections of infested and infested leaves of *C. papaya* acknowledged the various cellular damages caused by the feeding. Mite infested leaves displayed significant transformations from the normal leaf anatomy. The number of chloroplasts were diminished leading to the reduction in photosynthetic efficiency. The consumption of the contents of the mesophyll cells by the *T.urticae* has been confirmed from the destruction of the mesophyll layer. They are not found to damage epidermis and cell wall. The stylets of mites actually pass between the epidermal cell without penetrating them. Histological studies on injured leaves enclosed many empty spaces in the spongy parenchyma and pallisade parenchyma and found to have only few chloplasts per cell. (Fig:4). It could be clearly evidenced form comparing an un infested leaf wiih out such changes on their leaf tissues (Fig:3).

1: Qualitative feeding damages by *T. urticae*

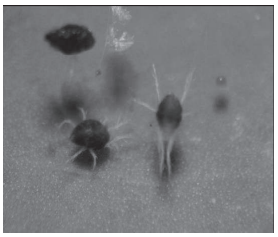

Fig. 1 : Colony of *T. urticae*

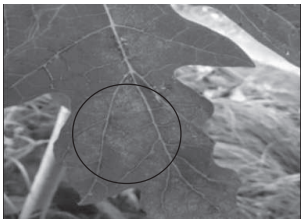

Fig. 2 :Infested *C. papaya* leaf with white patches

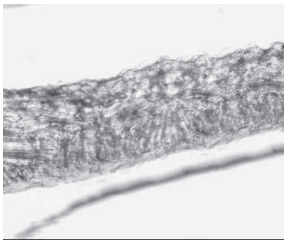

Fig. 3: Cross section of un infested **C. papaya**

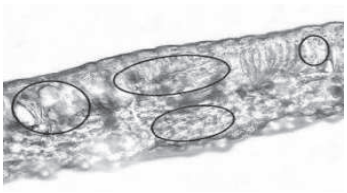

Fig. 4 :Cross section of *T.urticae* infested *C. papaya* leaf showing chloroplast loss in mesophyll layer

Quantitative studies on leaf damage caused by mite feeding:

Chlorophyll:

One of the most important parameters that determines the photosynthetic efficiency of a plant is the chlorophyll content of the leaves. Chlorophyll contents of the mite infested leaves showed a depletion in concentration because of the feeding activity of mite, T. urticae (Fig:5). In the present study, the concentration of chlorophyll a decreased from 1.47 ± 0.044 mg/g tissue to 0.67 ± 0.041mg/g tissue, chlorophyll b decreased from 1.68 ± 0.034 mg/g tissue to 0.82 ± 0.052 mg/g tissue and the total chlorophyll decreased from 3.27 ± 0.110 mg/g tissue to 1.53 ± 0.038 mg/g tissue. The respective loss in photosynthetic pigments could be recorded as 54.18% for chlorophyll a, 50.72% for chlorophyll b and 53.16% for total chlorophyll (Table:1). Highly significant negative correlation was recorded between mite population and photosynthetic pigments. Mite infested leaves were found to harbour cast skins, egg cases and faecal pellets under the silk webbing. Dead mites may remain on the leaf for long time entangled in the webbing. This fostered the settling of dust particles on the leaf surfaces and this coating of dust dissuaded the absorption of light by the chlorophyll molecules that left unfed by the mites. Thus, pressures a retardation of photosynthesis which is a vital process of plants (Sumangala and Haq, 2000).

Table 1: Quantitative estimation of chlorophyll content (mg/g tissue) in mite infested and un infested leaves of *C.papaya* under the attack of *T. urticae*

Chlorophyll	Milligram chlorophyll per gram tissue		Loss in chlorophyll	% chlorophyll loss
	Un infested	infested		
Chlorophyll a	1.47 ± 0.044	0.67 ± 0.041	0.79 ± 0.019	54.18 ± 1.919
Chlorophyll b	1.68 ± 0.034	0.82 ± 0.052	0.85 ± 0.071	50.72 ± 3.561
Total Chlorophyll	3.27 ± 0.110	1.53 ± 0.038	1.74 ± 0.018	53.16 ± 0.800

n=5

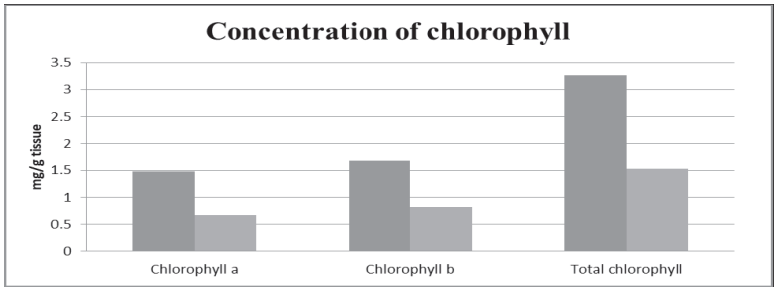

Fig 5 : Concentration of chlorophyll (mg/g tissue) in mite infested and un infested leaves of *C. papaya* under the attack of *T. urticae*

The amount of total carbohydrate was also found to be decreased on mite infested leaves when compared to un infested leaves.(Fg:6) The mean content of total carbohydrates in un infested leaf samples was 91.46 ± 0.076 mg/g tissue whereas that of mite infested leaf samples was 86.60 ± 0.027 mg/g tissue. The per cent decrease in total carbohydrate content was 5.31%. (Table:2) This is in support of some earlier studies (S.Ghoshal, 2013).

Table 2 : Quantitative estimation of total carbohydrate content in mite infested and uninfested leaves of *C. papaya* under the attack of *T. urticae*

Milligram total carbohydrate/ gram tissue		***Loss in total carbohydrate***	***% loss in total carbohydrate***
Un infested	***infested***		
91.46 ± 0.076	86.60 ± 0.072	4.86 ± 0.098	5.31 ± 0.103

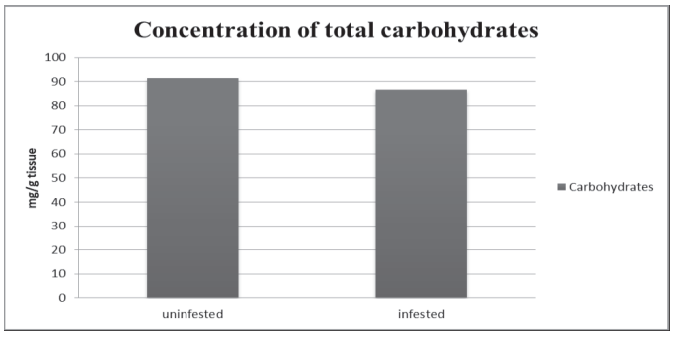

Fig 6: Concentration of total carbohydrate (mg/g tissue) in mite infested and un infested leaves of *C. papaya* under the attack of *T. urticae*

Total protein:

The results of biochemical analysis revealed a slight reduction in the total protein content in mite infested leaves. (Fig :7) The concentration of total protein was found to be reduced from 13.84 ± 0.017 mg/g tissue to 13.03 ± 0.013 mg/g tissue. The estimation suggests a decrease of 5.83%. (Table:3) This observation is in conformity with the findings of S. karmakar (2019) and Zukova (1963).

Table 3: Quantitative estimation of total protein content (mg/g tissue) in mite infested and un infested leaves of *C. papaya* under the attack of *T. urticae.*

Milligram total Protein/ gram tissue FE		*Loss in total Protein*	*% loss in total Protein*
Un infested	*infested*		
13.84 ± 0.017	13.03 ± 0.013	0.80 ± 0.018	5.78 ± 0.130

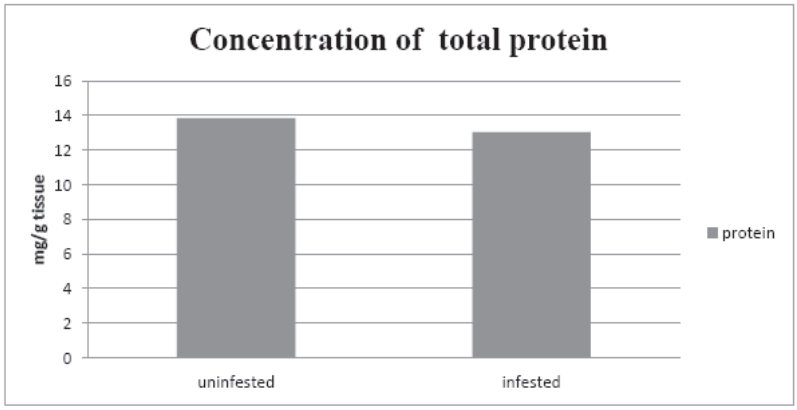

Fig7:Concentration of total protein (mg/g tissue) in mite infested and un infested leaves of *C. papaya* under the attack of *T. urticae*

Total phenol:

Phenol is an extensively studied molecule in the context of plant stress physiology. A strong correlation between the accumulation of phenolic compounds and abiotic stress tolerance have been reported from many plants. Phenolic synthesis and accumulation is a general stress response to environmental stresses including pathogen attack and mineral deficiencies. Any kind of mechanical damage can also lead to synthesis of polyphenols. Phenols are secondary metabolites that help the plants to cope up with environmental constraints (Oszmanski, 1995). In the present investigation, in the mite infested leaves, a significant increase in the 0246810121416uninfestedinfestedmg/g tissueConcentration of total proteinprotein concentration of total phenol was observed when compared to that of the un infested leaves. (Fig:8) Concentration of total phenol in un infested leaves was recorded as 19.34 ± 0.021 mg/g tissue, while that in infested leaves was 25.83 ± 0.019 mg/g tissue. An increase of 33.58% was found in mite infested leaves of *C. papaya.* (Table:4) Being an antioxidant, increased phenolic concentration is associated with enhanced stress tolerance. A significant increase in phenol concentration was reported by Ananthakrishnan et al. (1992) in Manihot esculenta, castor and eucalyptus due to mite infestation and they concluded that the increase concentration of phenolic

compounds induces resistance in host plants against herbivory. Deposition of phenolic compounds in plant tissues is reported to be one of the reasons behind photosynthetic suppression (Puchalska, 2006).

Milligram total Phenol/ gram tissue		***Loss in total Phenol***	***% loss in total Phenol***
Un infested	***infested***		
19.34 ± 0.021	25.83 ± 0.019	6.49 ± 0.012	33.58 ± 0.088

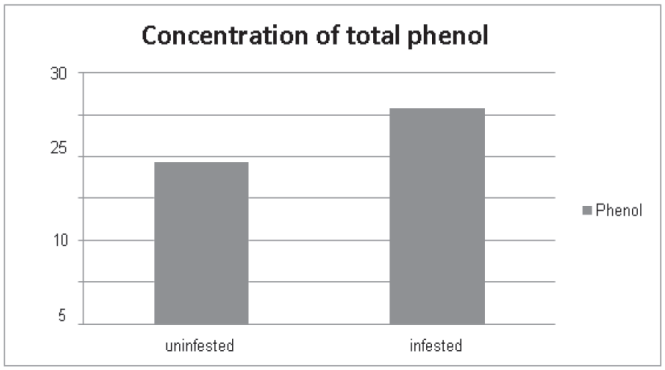

Fig:8: Amount of total phenol (mg/g tissue) in mite infested and un infested leaves of *C. papaya* under the attack of *T. urticae*

Proline:

Concentration of proline also showed an increase in mite infested leaves when compared to that of un infested leaves. (Fig:9) The mean concentration of proline in un infested leaf samples was 0.89 ± 0.016 mg/g tissue, whereas in mite infested leaves reaching 1.23 ± 0.012 mg/g tissue. Proline content was also found to exhibit an increase of 38.31% in the host plant by the attack of T.urticae. (Table:5). Like that of phenol, the proline concentration was also found to be high in infested leaves as they are sensitive indicators of biotic stress. Stressful conditions induce the overproduction of proline in the plant tissues which in turn provides stress tolerance to the plants. Acting as a reactive oxygen species (ROS) scavenger and as an osmolyte, it is thought to impart protection against environmental stresses. Spider mite infestation induces oxidative stress and increased production of molecules like proline and phenol (Farouk and Osman, 2012).

Table 5 : Quantitative estimation of total proline content (mg/g tissue) in mite infested and un infested leaves of C. papaya under the attack of T. urticae

Milligram total Proline/ gram tissue		***Loss in total Proline***	***% loss in total Proline***
Un infested	***infested***		
0.89 ± 0.016	1.23 ± 0.012	0.34 ± 0.023	38.31 ± 3.109

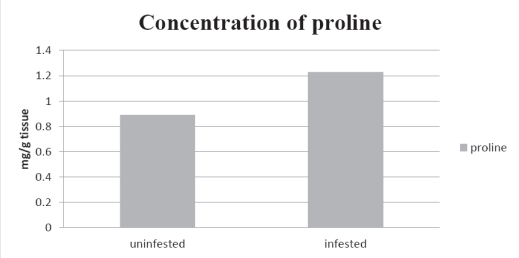

Fig:9: Amount of total proline (mg/g tissue) in mite infested and un infested leaves of *C.papaya* under the attack of *T. urticae*

Conclusion

T.urticae, commonly called as the two spotted spider mite belonging to the family Tetranychidae was found to be a potential pest of *C. papaya*. Spider mite colonies consisting of all life stages i.e. egg, larva, protonymph, deutonymph, adults and quiescent stages were found to be established on the lower surface of leaves, especially in regions close to the midrib and veins. Mature leaves harboured high populations of *T.urticae* than the tender leaves. Silk webbing was found to be an important character of *T.urticae.* Symptoms of feeding damage induced by the *T.urticae* on the host plant leaves include discolouration of leaves and wrinkled appearance. Spider mite feeding has shown to produce an array of damages in host plants ranging from mechanical damages to physiological changes. Histological studies revealed the destruction of mesophyll tissue – palisade and spongy parenchyma and alterations to stomatal apparatus. Biochemical studies performed for the quantitative estimation of damage caused by *T.urticae* on the chlorophyll content of leaves revealed a decrease in the concentration of chlorophyll (chlorophyll a -54.18% chlorophyll b - 50.72% and total chlorophyll -53.16%) suggesting that the mite feeding exerts a negative impact on the photosynthetic activity of the host plant. The extensive mite feeding has also observed to reduce the total carbohydrate content

in leaves of the host plant (5.31%). The total protein content of the mite infested and un infested leaves of C. papaya showed a decrease of 5.83%. Proline and phenol are components that plays a very important role in stress tolerance. Biochemical analysis of concentration of proline and total phenol exhibited an increase in their concentration (33.58% and 38.31% respectively) in mite infested leaves when compared to the healthy leaves suggesting the stress condition that the plant faces. Thus the concentration of various biochemical components were found to be altered due to mite feeding and it would definitely affect the health and productivity of the papaya plants. So, from the present study it is evident that the mite infestation can affect the plant both qualitatively and quantitatively and the damage due to their feeding was very severe leading to a loss in plant vigour. Though there are so many preventive measures and management strategies are in practice for their elimination, there is the need of adoption of more relevant methods like biological control measures for their effective control.

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