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REMOVAL OF SAFRANIN –O FROM AQUEOUS SOLUTION BY ADSORPTION ONTO CARBONIZED SPENT COFFEE GROUND

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Research Article

FEEDING IMPACT OF THE VEGETABLE MITE, *TETRANYCHUS NEOCALEDONICUS* ANDRÉ (ACARI: TETRANYCHIDAE) ON *MENTHA ROTUNDIFOLIA* L

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ABSTRACT

The vegetable spider mite, *Tetranychus neocaledonicus* (André, 1933) has been recognized as a highly polyphagous pest, infesting 432 species of host plants (Bolland *et al.*, 1998) of varied economic categories. During the present study, the mite was found to infest the medicinal as well as spice crop, *Mentha rotundifolia* L. Samples of mite infested and healthy leaves of *M. rotundifolia* were collected from the Botanical Garden of the Calicut University Campus and the extent of damage induced by *T. neocaledonicus* was estimated by assessing the loss of chlorophyll, carotenoids and photosynthetic efficiency. Apart from the above, the host plant stress induced by the mite infestation was also quantitated by estimating the levels of phenol in infested and healthy leaves of *M. rotundifolia*. Arnon's (1949) method was followed for estimating the chlorophyll and carotenoid pigments and the photosynthetic efficiency was measured using the Handy Photosynthetic Efficiency Analyser. Total phenol content of the mint leaves was estimated with Folin-Ciocalteau reagent. Results of the study enabled to record a significant quantitative reduction ($P<0.01$) in chlorophyll a (31.22%), chlorophyll b (34.42%), total chlorophyll (32.31%) and carotenoids (24.48%) owing to mite infestation. A significant reduction ($P<0.05$) in the photosynthetic efficiency of the leaves was also evidenced, by recording the F_v/F_m value of 0.8256 for uninfested leaves against a reduced value of 0.5754 for mite infested leaves. The study enabled to record a significant increase ($P<0.01$) in total phenol content of infested leaves, which showed an increase of 50.7%.

INTRODUCTION

*T. neocaledonicus*has been recognized as a highly polyphagous species of spider mite included under the category of major pests (Jeppson, *et. al*. 1975).The species enjoys a pantropical distribution (Seeman and Beard, 2005) in 62 countries, exhibiting an extremely wide host range of 432 plants. The mite lives in colonies, under a silken protective web which they spin on the underside of the host leaves. Heavy infestation by the species is generally confined to the lower surface of host leaves when compared to the upper surface (Sangeetha and Ramani, 2007) where they feed on the plant sap by puncturing the cells with its stylet like chelicerae. The present host plant, *M. rotundifolia* forms a new host for the species, and which is well known for its medicinal properties. The leaf extracts of mint plants are very effectively used for respiratory ailments, stomach aches, irritable bowel syndrome, etc. Mint is known to possess both insecticidal and acaricidal properties (Sertkaya, *et al*. 2010, Kumar *et al*., 2011) and the essential oils from *M. rotundifolia* possess antioxidant and antimicrobial activities also. In the present study, infestation by *T. neocaledonicus* on mint leaves leads to the development of varying visible abnormalities like chlorosis, yellowing, leaf curling, bronzing, etc. apart from various biochemical alterations, leading to qualitative and quantitative depreciation of economic value. The present study, therefore is an attempt to elucidate the biochemical alterations induced by infestation of *T. neocaledonicus* on mint leaves by analyzing the amount of photosynthetic pigments like chlorophyll a, b total chlorophyll, carotenoids, total phenols etc And by measuring the photosynthetic efficiency based on chlorophyll fluorescence.

MATERIALS AND METHODS

Collection of samples

Leaf samples of *M. rotundifolia* were collected for a period of around of 60 days, from June-July, 2015 from the potted plants cultivated in the Botanical Garden of the Calicut University Campus, Malappuram District, Kerala (N: 11.1382028º E: 75.88933229º), India. While collecting, leaf samples of two categories viz. the uninfested (=healthy) and mite infested

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(leaves infested with *T. neocaledonicus*) were collected separately, with the help of a blade/scissors and put in separate polythene bags for transportation to the laboratory for subsequent biochemical analysis.

Estimation of Damage induced by T. neocaledonicuson mint leaves

Quantitative Estimation of Chlorophyll

The amount of chlorophyll (a,b and total) pigments present in mite infested and uninfested leaves of *M. rotundifolia* was estimated following Arnon's (1949) method. Known weights (W= 200 mg) of infested and uninfested leaves were taken separately and crushed in 80% acetone. The mixtures were centrifuged and the process was repeated for the pellet or precipitate. The volume of the total solution was noted as V (= 20 ml). The ODs of the test and control solutions were measured at 470nm, 645nm and 663 nm. Calculations were made as follows:

Mg chlorophyll a per g tissue = $[12.7(A_{663}) - 2.69(A_{645})] \times V/(1000xW)$
Mg chlorophyll b per g tissue = $[22.9(A_{645})-4.68(A_{663})] \times V/(1000xW)$
Mg total chlorophyll (a + b) per g tissue = $[20.2(A_{645}) +8.02(A_{663})] \times V/(1000xW)$
Mg Carotenoid per g tissue,
$= [1000(A_{470}) + 3.27\{(\text{chlorophyll a}) - (\text{chlorophyll b})\}] \times V/(W \times 229)$

Where,
A= Absorbance at wavelength mentioned
V= Final volume of chlorophyll extract in 80% acetone
W=Fresh weight of tissue extracted

Measurement of Photosynthetic Efficiency

The feeding impact of *T. neocaledonicus* was quantified by measuring the photosynthetic efficiency of the infested and uninfested mint leaves, using the Handy Photosynthetic Efficiency Analyzer instrument (Handy PEA Hansatech Ltd. Vorfolk, UK) Chlorophyll fluorescence transient of both the categories of leaves was measured at the room temperature on the fully expanded leaves. Data on general parameters like F_0 (minimum fluorescence), F_m (maximum fluorescence), F_v (variable fluorescence) etc. were recorded separately for uninfested and infested leaves. The values of F_v/F_m (where $F_v = F_m-F_0$), a parameter commonly known as the maximum quantum yield of primary photochemistry or maximal electron transport rate (ETR) of PS II of both uninfested and infested leaves were recorded.

Quantitative Estimation of Total Phenol content

Phenols, the aromatic compound with hydroxyl groups offer resistance to diseases and pests and which include an array of compounds like tannins, flavonols etc. Estimation of total phenol was carried out following Folin-Ciocalteau reagent. Phenol was extracted from 100 mg samples of uninfested and mite infested leaves separately in 10 ml of 80% ethanol and centrifuged. The solutions were then evaporated and the residues were mixed with 10 ml of distilled water. Aliquots of 0.2 ml, 0.4 ml, 0.6 ml and 0.8 ml for both uninfested and infested leaves were taken separately and were then allowed to react with 0.5 ml of Folin-Ciocalteau reagent and after 3 minutes, with 2 ml of 20% Sodium Carbonate and put in boiling water bath for 1 minute. The OD was measured at 650 nm of the molybdenum blue coloured solution (Malick and Singh, 1980).

The data obtained on the above studies were statistically analyzed using Student test with IBM SPSS Statistics (Version 19). Values were expressed as Mean ± SEM.

RESULTS

Quantitative Estimation of Chlorophyll:

Results of quantitative estimation of chlorophyll through Arnon's Method revealed a decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. The mean amount of chlorophyll a present in uninfested sample was 903.73± 1.08 µg/g tissue where as a lower concentration (621.63± 1.27 µg/g tissue) (Table 1, Fig. I) could be recorded in infested leaves Thus the percent loss in chlorophyll a owing to infestation by *T. neocaledonicus* as observed during the study was 31.22% (P<0.01). Similarly, chlorophyll b also was found decreased from 468.61± 1.4 µg/g tissue in uninfested leaves to 307.32± 1.07 µg/g tissue in infested leaves showing 34.22% (P<0.01) decrease. Loss in total chlorophyll content was found decreased from 1369.05± 0.84 µg/g tissue to 926.74± 1.12 µg/g tissue, showing 32.31% (P<0.01) decrease.

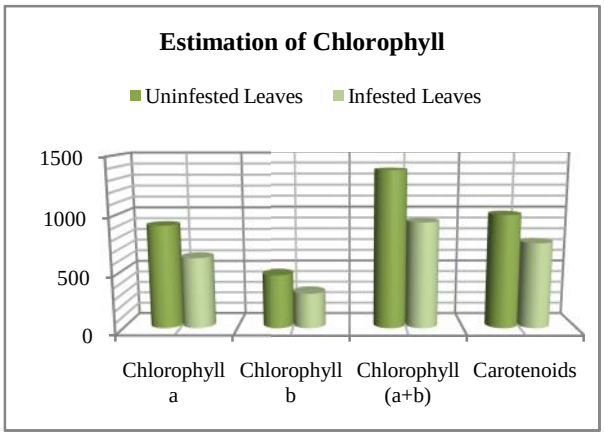

Fig. I. Quantitative changes in Photosynthetic pigments induced by *Tetranychus neocaledonicus* in the leaves of *Mentha rotundifolia*

Table 1 Quantitative changes in Photosynthetic pigments induced by *Tetranychus neocaledonicus* in the leaves of *Mentha rotundifolia*

Sl. No.	Chlorophyll	µg Substance/ g tissue of Uninfested Leaves	µg Substance/ g tissue of Infested Leaves	Loss (µg/ g tissue od leaves)	%Loss
1.	Chlorophyll 'a'	903.73 ± 1.08	621.63 ± 1.27	282.1 ± 0.19	31.22
2.	Chlorophyll 'b'	468.61 ± 1.4	307.32 ± 1.07	161.29 ± 0.33	34.42
3.	Total Chlorophyll	1369.05 ± 0.84	926.74 ± 1.12	442.31 ± 0.28	32.31
4.	Carotenoids	990.76 ± 1.53	748.28 ± 1.36	242.48 ± 0.17	24.48

Table 2 Changes in the chlorophyll fluorescence parameters induced by *Tetranychus neocaledonicus* in the leaves of *Mentha rotundifolia*

Sample Type.	Sl. No.	F_0	F_m	F_v/F_m	Mean F_v/F_m	P_{index}	T_{fm} (mS)	Area (bmS)
Uninfested Leaves	1.	532	2734	0.805	0.8256 ± 0.00612	0.697	200	25200
	2.	477	2871	0.834		1.244	170	27200
	3.	462	2799	0.835		1.293	160	26600
	4.	467	2847	0.836		1.297	170	25800
	5.	497	2734	0.818		0.856	180	23400
Infested Leaves	1.	922	1866	0.506	0.5754 ± 0.090341	0.065	220	7400
	2.	667	2861	0.767		0.580	200	20600
	3.	1320	1750	0.256		0.007	240	3600
	4.	761	2257	0.663		0.185	290	7200
	5.	753	2389	0.685		0.191	240	10000

The amount of carotenoids also showed a decrease from 990.76± 1.53 µg/g tissue in uninfested leaves to 748.28± 1.36 µg/g tissue in infested leaves, there by showing 24.48 % loss (P<0.01) loss.

Measurement of Photosynthetic Efficiency

The Photosynthetic efficiency when measured using the Handy Photosynthetic Efficiency Analyser enabled to record a mean F_v/F_m value of 0.8256 for uninfested leaves while that of mite infested leaves was 0.5754 (P<0.05). (Table 2, Fig. II)

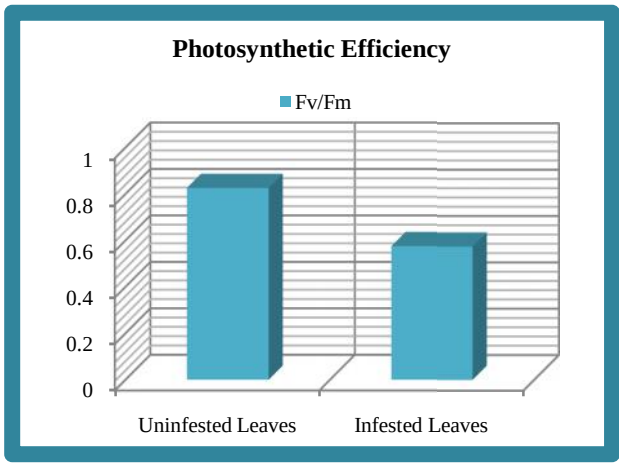

Fig. II Changes in the values of Fv/Fm in the leaves of *Mentha rotundifolia* induced by *Tetranychus neocaledonicus*

Quantitative Estimation of Total Phenol

The total phenol content of mint leaves on estimation showed an increase in level owing to mite infestation. The mean amount of total phenol in uninfested leaves was 1.901± 0.068 mg/g tissue where as that of the mite infested leaves was 2.865 ± 0.275 mg/g tissue (P<0.01). Thus, the increase in the level of phenol due to mite infestation was observed to be 0.964± 0.207 mg/g tissue, thereby leading to an increase of 50.7%. (Table 3, Fig. III).

Table 3 Alterations in Phenol contents induced by *Tetranychus neocaledonicus* in the Leaves of *Mentha rotundifolia*

Phenol in Uninfested Leaves (mg/g tissue)	Phenol in Infested Leaves (mg/g tissue)	Increase in Phenol (mg/g tissue)	% Increase in Phenol
1.901± 0.0393	**2.86475 ± 0.1589**	**0.964 ± 0.1196**	**50.7**

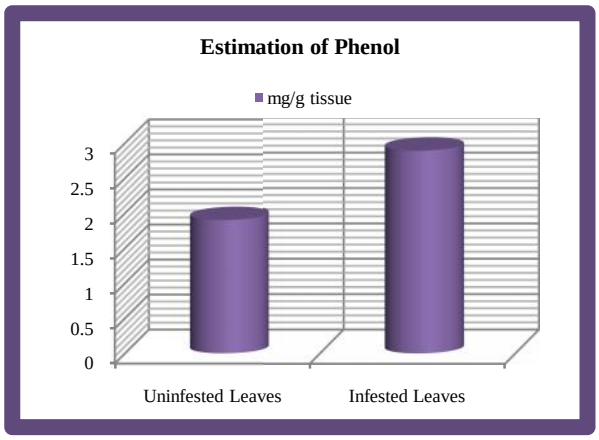

Fig.III Alterations in Phenol contents induced by *Tetranychus neocaledonicus* in the Leaves of *Mentha rotundifolia*

DISCUSSION

T. neocaledonicus has been recognized as a major pest of various economic crops like vegetables, fruit crops, ornamental plants and so on. The species exhibits a very wide host range, infesting 432 host plants (Bolland *et al.*, 1998) and most recently, the notorious pest status of the species could be observed on a very important nutritional crop like *Moringa oleifera* growing in various parts of Kerala (Sangeetha and Ramani, 2007). The present finding on the infestation by the species on a very vital medicinal cum insecticidal plant viz. *M. rotundifolia* helped to add a new host plant to the existing list thereby extending the host range of the species further.

The impact of feeding activity of *T. neocaledonicus* on *M. rotundifolia* revealed the development of visible symptoms of damage like the chlorotic spots, patches, yellowing of leaves etc. This shows the ability of the species to feed on plants like *M. rotundifolia* which contains several alkaloids with potent medicinal cum insecticidal properties (Sertkaya, *et al.* 2010; Kumar *et al.*, 2011). This is a clear indication that this notorious species has developed resistance against pesticides, probably through the production of detoxifying enzymes.

The results of biochemical analysis on the extent of damage induced by *T. neocaledonicus* on *M. rotundifolia* clearly revealed the potential of the species to induce significant quantitative reduction in photosynthetic pigments like chlorophyll a, b, total chlorophyll and also the carotenoids. The percent decrease in chlorophyll a, b, total chlorophyll and carotenoid contents were 31.22%, when estimated showed the potential of the mite as a notorious pest of medicinal plants.

Though, the population of mites are low in the monsoon, the damage potential of these mites are still noticeable. The leaf chlorophyll content was found to decrease with increase in the density and duration of feeding of spider mites (Sangeetha and Ramani, 2007) and accordingly the photosynthetic efficiency was also found to have decreased considerably as confirmed through the measurement of chlorophyll fluorescence. A decrease in the Fv/Fm value observed in the mite infested leaves is a very clear indication that the plant is under stress and chlorophyll centres and PS II system are not as active as a healthy plant (Bolhar-Nordenkampf *et al*., 1989, Iatrou *et al.* 1995, Skaloudov *et al*., 2006). Phenol serves to provide resistance to plants against pest attack and the increased amount of total phenolics in the leaves of *M. rotundifolia* as observed during the present study indicates the potential of *T. neocaledonicus* to induce stress on the plants and to overcome this, the plant produces more amount of phenols. Thus the results of the study support the earlier finding that spider mites cause increase in the production of phenolic compounds in plants (Puchalska, 2006). Therefore, the results of the present study help to confirm the potential of *T. neocaledonicus* in inducing physiological stress and significant reduction in the photosynthetic machinery of the plant, which in turn would adversely affect the vigor and economic utility of the plant, *M. rotundifolia.*

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