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Biochemical responses of cucumber to *Tetranychus urticae* Koch (Acari: Tetranychidae) mediated biotic stress

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Abstract : The effect of two spotted spider mite (*Tetranychus urticae* Koch) feeding on leaf level physiological characteristics of cucumber (*Cucumis sativus* Linnaeus) was investigated. Young cucumber plants were artificially infested with different densities of *T. urticae* (5, 10, 15 and 20 mites/ grown up leaf) while uninfested plants acted as control. Post infestation, the plants differed in their support to mite density in accordance with initial infestation density and observation period. Highly significant negative correlation of -0.92, -0.93, -0.95 and -0.92 for total chlorophyll, chlorophyll a, chlorophyll b and carotenoids, respectively, at the highest infestation level) was recorded between mite density and photosynthetic pigments in infested leaves as compared to uninfested ones. There was a significant decrease (P= 0.05) in the level of (a progressive decline from 2.82, 0.36 and 2.17% dry weight in control to the maximum of 2.09, 0.26 and 1.87% dry weight for N, P and K, respectively, at highest infestation level) in the infested leaves in response to mite infestation. Interaction between initial infestation level and observation period also suggested a significant impact of *T. urticae* infestation on the leaf phytochemicals of cucumber (P= 0.05).

Keywords: *Cucumis sativus*, Feeding, Infestation density, Phytochemicals**,** *Tetranychus urticae*

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is amongst the most damaging agricultural pests worldwide belonging to an assemblage of web-spinning mites. *T. urticae* represents one of the most polyphagous arthropod herbivores, feeding on more than 1,100 plant species belonging to more than 140 different plant families including species known to produce toxic compounds (Van Leeuwen *et al*., 2010). It is the most notorious pest responsible for significant yield losses in many economic crops, vegetables and fruit trees (Salman, 2007) and also ornamental and agronomic crops worldwide (James and Price, 2002). The rapid developmental rate, high reproductive potential, and arrhenotokous parthenogenesis in *T. urticae* allows them to achieve damaging population levels very quickly when growth conditions are good, resulting in an equally rapid decline of host plant quality. This mite is particularly dominant in intensive, high yield cropping systems and affects crops by direct feeding; thereby reducing the area of photosynthetic activity and causing leaf abscission in severe infestations (Gorman *et al.*, 2001). Puncturing of cells by mite stylets and injection of saliva causes mechanical damage, changes in cell cytology, physiological and biochemical processes of punctured as well as non-punctured adjacent cells (Tomczyk and Kropczynska, 1985). Heavy damage may cause leaves to dry and drop and the plants may be covered with webbing (Abdel-Wali *et al*., 2012). The webbings produced by protonymphs, deutonymphs and adults and hampering of photosynthetic activity may result in a reduction in crop yield as well as aesthetic injuries (Dutta *et al*., 2012). When the plant begins to decline, resulting in a reduced food supply, the mites enter a dispersal phase from sedentary phase and aggregate on the uppermost parts of the plants. Dispersal includes both intraplant and interplant movement. Aerial dispersal begins with the mites aggregating on the uppermost portions of the plants. The mites produce threads of silk, which they use to "balloon" into the wind, which sometimes carry them great distances (Kennedy and Smitley, 1985). *T. urticae* infestation represents potential biotic stress to its host plant and adversely affects many physiological and biochemical processes (Sivritepe *et al.*, 2009). Computer modelling suggests that with intensifying global warming, the detrimental effects of spider mites in agriculture will markedly increase due to accelerated development at higher temperatures. A thorough understanding of pest ecology and host plant physiology is necessary to understand host-plant relationships and host resistance mechanisms. This is especially important for elaborating integrated pest management strategies in agriculture and reducing yield loss. There is only scarce information on the impact of two spotted spider mites

on cucumber physiology (Park and Lee, 2002). The objective of the present study was to investigate the effect of *T. urticae* infestation on leaf-level physiological characteristics of cucumbers.

MATERIALS AND METHODS

Mite culture: The base population was originally collected from okra plants from University Research Farm Area and since then maintained on potted okra (var.Varsha uphar) and cucumber (var. Damini hybrid) plants in the screenhouse of Department of Zoology, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Raising of crop and *T. urticae* inoculation: The field experiment was conducted in the Research Farm Area, Department of Entomology, CCSHAU, Hisar during 2012. Cucumber (*Cucumis sativus* L.) var. Damini hybrid seeds were sown in early April, 2012 and the crop was raised following standard agronomical practices. No insecticide, miticide or any other chemicals were applied to control the pest. The plants were divided into five treatment groups within a complete randomized block design consisting of ten replicates per treatment. The adult females of *T. urticae*, from stock culture in screenhouse, were artificially inoculated on 20 days old plants by placing them onto the fully expanded grown up leaves with the help of bird feather pick. Inoculation was done as 5, 10, 15 and 20 mites released per grown up leaf under different treatments (T_2, T_3, T_4 and T_5, respectively). Uninfested plants served as control (T_1).

Phytochemical screening: Mite population was allowed to increase naturally. Each treatment group was further subdivided into three sets of different durations *viz.*, 20, 40 and 60 days. Estimation of respective phytochemicals on 0 day acted as corresponding control. Leaf samples were collected per plant and the number of *T. urticae* per sq. cm leaf counted. Visual symptoms of chlorosis appeared on the leaves. Collected leaves were then weighed on electronic balance, washed with distilled water in order to avoid contamination and air dried for 15 minutes. The samples were then allowed to dry in an oven at 60^{o}C till they attained a constant dry weight and ground in a Micro-Willey grinding mill (0.2 mm sieve) for analysis of various phytochemicals (nitrogen, potassium, phosphorus) except photosynthetic pigments for which fresh leaf samples were used. The photosynthetic pigments were estimated as per the method of Hiscox and Israelstam (1979). To estimate minerals, digestion of sample was carried out. Powdered material (100 mg) was taken in 100 ml conical flask and 10 ml diacid mixture (Sulphuric acid + Perchloric acid; 4:1) was added to each flask (Fiske and Subba Row, 1925). These flasks were covered with watch glass and allowed to stand overnight. Heating was done until solid particles had nearly disappeared and clear colourless solution was obtained. Then one ml of HCl concentrate was added to each sample and again heated until colourless solution was obtained. Solutions were allowed to cool and the volume was made to 25 ml with 1 per cent HCl in distilled water. Blank was also run simultaneously but without sample. These solutions were mixed thoroughly and used for analysis mineral estimation. Leaf-nitrogen content was estimated using the method of Lindner (1944). Phosphorus was estimated by the method of Jackson (1973). Potassium in the digested sample was estimated in the above acid digest with a Micro Flame Photometer (Elico CL 361, India) by direct reading (Richards, 1954).

Statistical analysis: The statistical significance of data was assessed through two factorial analysis of variance (ANOVA) using OPSTAT software and means were then compared using Duncan's multiple range test (P = 0.05). Correlation coefficient 'r' was calculated to see the effect of mite incidence on various parameters evaluated.

RESULTS AND DISCUSSION

The number of two spotted spider mite mobile forms elevated in the inoculated plants during the 60 days experimental period. Heavy patching on tender, grown up and older leaves of cucumber coincided with initial inoculum of *T. urticae*. Data pertaining to changes in the photosynthetic pigments (total chlorophyll, chlorophyll a, chlorophyll b, carotenoids) due to mite infestation on cucumber leaves are presented in Tables 1-4. Results showed a significant progressive decline in all the photosynthetic pigments with increase in duration and density of mite infestation (P= 0.05). Total chlorophyll content was found to be at par for the treatments T_2 and T_3; T_4 and T_5, respectively (Table 1; P= 0.05). The chlorophyll a content in T_3 showed no significant difference with the treatments T_4 and T_5 (Table 2; P= 0.05). Likewise, the chlorophyll b content in T_2 and T_3; T_4 and T_5 was found to show statistically insignificant difference between respective treatments (Table 3; P= 0.05). The chlorophyll c content in T_4 was found to be at par with chlorophyll c content recorded for T_3 and T_5 (Table 4; P= 0.05). Reduction in chlorophyll content is a primary response to spider mite infestation as it feeds on photosyntetically active cells. When the two spotted spider mites start to feed on the under surface of leaves, the mesophyll tissue collapses and a small chlorotic patch is formed at each feeding site. The amount and rate of change of the chlorophyll has been reported to depend on *T. urticae* density and duration of feeding (Alatawi *et al*., 2007) and is supported in the current work. Decrease in chlorophyll-a was more pronounced than chlorophyll-b at different stages of *T. urticae* infestation as per the findings of Farouk and Osman (2011). The major principles behind yield losses due to spider mite infestation in various crops have been established as biomass reduction, disturbance of water conduction, dry matter partitioning, CO_2 gas exchange, chlorophyll

Table 1. Effect of initial infestation density of *T. urticae* on the total chlorophyll content (mg/ g fresh weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	Total chlorophyll	Mite no.	Total chlorophyll	Mite no.	Total chlorophyll	Mite no.	Total chlorophyll	
T_1 (No release-control)	0.00	2.58	0.00	2.57	0.00	2.57	0.00	2.57	2.57
T_2 (5 mites released)	0.00	2.57	3.43	1.17	11.97	1.15	13.13	0.93	1.45[a]
T_3 (10 mites released)	0.00	2.56	3.98	1.15	14.98	1.12	16.48	0.88	1.43[a]
T_4 (15 mites released)	0.00	2.58	4.95	1.13	16.92	1.01	19.79	0.86	1.39[b]
T_5 (20 mites released)	0.00	2.56	8.13	1.13	21.48	0.98	23.36	0.80	1.37[b]
Mean		2.57		1.43		1.36		1.21	
Correlation with mite population				0.79		0.94		-0.92	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

Table 2. Effect of initial infestation density of *T. urticae* on the chlorophyll a content (mg/ g fresh weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	Chlorophyll a	Mite no.	Chlorophyll a	Mite no.	Chlorphyll a	Mite no.	Chlorophyll a	
T_1 (No release - control)	0.00	2.02	0.00	2.02	0.00	2.01	0.00	2.02	2.02
T_2 (5 mites released)	0.00	2.02	3.43	0.93	11.97	0.89	13.13	0.70	1.13[a]
T_3 (10 mites released)	0.00	2.01	3.98	0.86	14.98	0.85	16.48	0.65	1.09[a,b]
T_4 (15 mites released)	0.00	2.03	4.95	0.84	16.92	0.76	19.79	0.64	1.06[b]
T_5 (20 mites released)	0.00	2.02	8.13	0.84	21.48	0.73	23.36	0.61	1.04
Mean		2.02		1.10		1.05		0.92	
Correlation with mite population				0.81		0.95		-0.93	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

reduction and shedding of immature flowers (Park and Lee, 2002). *T. urticae* feeding causes the destruction of chloroplasts by puncturing photosynthetically active cells (Sivritepe *et al*., 2009) which then leads to basic physiological changes in the plant. In gut content studies of two-spotted mites, Walsh (2001) observed the presence of only thylakoid granules, the key photosynthetic engines in plant cells, following feeding.

Data pertaining to changes in mineral content (Nitrogen, Phosphorus and Potassium) of cucumber leaves as a result of *T. urticae* feeding at different initial infestation levels are presented in Tables 5-7. Significant negative correlation between mite number and nitrogen content clearly depicted decrease in nitrogen content with increase in mite number at different durations of treatment (r= -0.98, -0.98 and 0.99 at 20, 40 and 60 days). Significant difference was recorded for nitrogen content of cucumber leaves in all the treatments (Table 6; P= 0.05). Similar results were obtained for phosphorus (Table 6) and potassium (Table 7) content in cucumber leaves, showing a significant decrease with increase in initial infestation density of *T. urticae* as well as duration of infestation. For the treatment T_3, the Phosphorus content was found to exhibit insignificant difference with the treatments T_2 and T_3 (Table 7; P= 0.05). However, Potassium content was found to be at par for the treatments T_2 and T_3 (Table 7; P= 0.05). Sivritepe *et al.* (2009) reported a fall in Calcium, Potassium and Magnesium contents of sultana cultivar of grapevine in response to *T. urticae* infestation. Farouk and Osman (2011) have recently suggested that infestation with *T. urticae* results in increased production of reactive oxygen species (ROS) which destroys membrane permeability thus leading to decreased content of minerals

Table 3. Effect of initial infestation density of *T. urticae* on the chlorophyll b content (mg/ g fresh weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	Chlorophyll b	Mite no.	Chlorophyll b	Mite no.	Chlorophyll b	Mite no.	Chlorophyll b	
T_1 (No release control)	0.00	0.56	0.00	0.56	0.00	0.56	0.00	0.56	0.56
T_2 (5 mites released)	0.00	0.55	3.43	0.32	11.97	0.29	13.13	0.24	0.35[a]
T_3 (10 mites released)	0.00	0.57	3.98	0.28	14.98	0.27	16.48	0.23	0.34[a]
T_4 (15 mites released)	0.00	0.56	4.95	0.28	16.92	0.25	19.79	0.21	0.33[b]
T_5 (20 mites released)	0.00	0.56	8.13	0.28	21.48	0.25	23.36	0.19	0.32[b]
Mean		0.56		0.35		0.32		0.29	
Correlation with mite population				0.82		0.94		0.95	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

Table 4. Effect of initial infestation density of *T. urticae* on the carotenoid content (mg/ g fresh weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	Carotenoid	Mite no.	Carotenoid	Mite no.	Carotenoid	Mite no.	Carotenoid	
T_1 (No release control)	0.00	0.56	0.00	0.54	0.00	0.55	0.00	0.55	0.55
T_2 (5 mites released)	0.00	0.56	3.43	0.29	11.97	0.26	13.13	0.19	0.33
T_3 (10 mites released)	0.00	0.55	3.98	0.26	14.98	0.23	16.48	0.18	0.31[a]
T_4 (15 mites released)	0.00	0.55	4.95	0.26	16.92	0.22	19.79	0.17	0.30[a,b]
T_5 (20 mites released)	0.00	0.56	8.13	0.25	21.48	0.21	23.36	0.17	0.29[b]
Mean		0.55		0.32		0.29		0.25	
Correlation with mite population				0.83		-0.94		-0.92	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

due to its effect on ion uptake. This reduction may also be due to less drain of phloem sap by *T. urticae* population. However, accumulation of some minerals (Sodium, Copper and Zinc) and inorganic ions has also been reported in muskule cultivar of grapevine which may contribute to osmotic adjustment in the leaves experiencing *T. urticae* induced water stress (Sivritepe *et al*., 2009). To date, the data on the relationship between mite feeding and changes in the mineral composition of leaf tissue are scarce and more studies are needed to shed light on this arena.

Conclusion

Highly significant negative correlation was recorded between mite density and various phytochemicals (photosynthetic pigments and mineral content) in infested leaves as compared to uninfested ones. Interaction between initial infestation level and observation period suggested a significant impact of *T. urticae* infestation on the leaf phytochemicals of cucumber. The findings indicated that substantial physiological impact on cucumber is possible even at low *T. urticae* densities and timely management of the pest is suggested to allow potential higher benefit to the growers.

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Table 5. Effect of initial infestation density of *T. urticae* on the Nitrogen content (% dry weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	N	Mite no.	N	Mite no.	N	Mite no.	N	
T_1 (No release control)	0.00	2.84	0.00	2.83	0.00	2.82	0.00	2.82	2.83
T_2 (5 mites released)	0.00	2.84	3.43	2.71	11.97	2.44	13.13	2.39	2.59
T_3 (10 mites released)	0.00	2.84	3.98	2.68	14.98	2.37	16.48	2.28	2.54
T_4 (15 mites released)	0.00	2.84	4.95	2.65	16.92	2.29	19.79	2.19	2.49
T_5 (20 mites released)	0.00	2.85	8.13	2.58	21.48	2.22	23.36	2.09	2.43
Mean		2.84		2.69		2.42		2.35	
Correlation with mite population				-0.98		-0.98		-0.99	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15

Table 6. Effect of initial infestation density of *T. urticae* on the Phosphorus content (% dry weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	P	Mite no.	P	Mite no.	P	Mite no.	P	
T_1 (No release -control)	0.00	0.36	0.00	0.36	0.00	0.36	0.00	0.36	0.36
T_2 (5 mites released)	0.00	0.36	3.43	0.35	11.97	0.33	13.13	0.31	0.34^{a}
T_3 (10 mites released)	0.00	0.36	3.98	0.34	14.98	0.32	16.48	0.29	$0.33^{a,b}$
T_4 (15 mites released)	0.00	0.36	4.95	0.33	16.92	0.31	19.79	0.27	0.32^{b}
T_5 (20 mites released)	0.00	0.36	8.13	0.31	21.48	0.29	23.36	0.26	0.30
Mean		0.36		0.34		0.32		0.30	
Correlation with mite population				-0.96		-0.98		-0.99	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

Table 7. Effect of initial infestation density of *T. urticae* on the Potassium content (% dry weight) of cucumber leaves.

Treatment	Days of infestation								Mean
	0		20		40		60		
	Mite no.	K	Mite no.	K	Mite no.	K	Mite no.	K	
T_1 (No release -control)	0.00	2.17	0.00	2.17	0.00	2.17	0.00	2.17	2.17
T_2 (5 mites released)	0.00	2.17	3.43	2.08	11.97	2.03	13.13	1.96	2.05^{a}
T_3 (10 mites released)	0.00	2.18	3.98	2.06	14.98	1.99	16.48	1.92	2.04^{a}
T_4 (15 mites released)	0.00	2.18	4.95	2.05	16.92	1.97	19.79	1.89	2.02
T_5 (20 mites released)	0.00	2.17	8.13	2.03	21.48	1.93	23.36	1.87	2.00
Mean		2.17		2.08		2.02		1.96	
Correlation with mite population				-0.93		-0.99		-0.96	

Mite no. expressed as no. of mites/sq. cm leaf; CD (P=0.05) for Treatment (T) = 0.06; Duration (D) = 0.06; T×D = 0.15; Values with the same superscript do not differ significantly

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