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Sanjeet Kumar Singh
Department of Entomology &
Agricultural Zoology, Institute
of Agricultural Sciences, Banaras
Hindu University, Varanasi,
Uttar Pradesh, India

Shafia Zainab
Department of Entomology &
Agricultural Zoology, Institute
of Agricultural Sciences, Banaras
Hindu University, Varanasi,
Uttar Pradesh, India

SK Singh
Department of Soil Science &
Agricultural Chemistry, Institute
of Agricultural Sciences, Banaras
Hindu University, Varanasi,
Uttar Pradesh, India

RN Singh
Department of Entomology &
Agricultural Zoology, Institute
of Agricultural Sciences, Banaras
Hindu University, Varanasi,
Uttar Pradesh, India

Correspondence
RN Singh
Department of Entomology &
Agricultural Zoology, Institute
of Agricultural Sciences, Banaras
Hindu University, Varanasi,
Uttar Pradesh, India

Two-Spotted spider mite (*Tetranychus Urticae* Koch) induced biochemical alterations across different okra varieties

Sanjeet Kumar Singh, Shafia Zainab, SK Singh and RN Singh

Abstract

Tetranychus urticae is a prolific feeder of vegetable crops. The present study aimed at studying the biochemical changes and qualitative loss due to the mite feeding in the form of major (N, P and K) and micro-nutrients (Fe, Zn, Cu and Mn) from okra leaves. Sixteen varieties of okra were randomly raised in experimental plots at Horticulture farm of Institute of Agricultural Sciences, Banaras Hindu University. The healthy and mite infested leaves were plucked from the field and their biochemical analysis was conducted at laboratory. It was observed that there was considerable reduction in nutrients across all varieties due to mite feeding. The nutrient of healthy and infested leaves was correlated against mite population. Among infested leaves, it was found that the N, P, K and Cu concentration were non-significantly positively correlated to mite population whereas a significant negative correlation was noticed with Fe, Zn and Mn to mite infestation. It was observed that mite infestation besides degrading the nutritional value of crop also reduces the yield.

Keywords: Biochemical response, Okra, *Tetranychus urticae*

Introduction

Okra (*Abelmoschus esculentus*) is one of the most important vegetable crops in India during summer and rainy season. India ranks first in the world with a production of 5.78 mt of okra (75.4% of the total world production) from over 0.518 million ha land. The global area under okra cultivation has been estimated at 1.08 million hectares with total production of about 8.3 million metric tons (Anonymous, 2013) [1]. The fully ripened fruits and stem contain carbohydrate (7.7%), protein (2.2%), fat (0.02%), fibers (1.2%), minerals (0.7%), calcium (0.9%) and are also a good source of iron, iodine and vitamins (Chauhan, 1965) [4]. Okra plants are also used for treating diseases like stones in kidney, leucorrhoea, backache and goiter in human beings (Nadkarni, 1972) [13]. Mucilage extract of stem and root are used for clarifying sugarcane juice for making jaggery (molasses) (Chauhan, 1972) [5].

The crop is attacked by about 72 insect pests and they infest the crop from seedling to harvest stage (Srinivasa and Rajendran, 2003) [19]. *Tetranychus urticae* Koch (Arachnida: Tetranychidae) is voracious feeder on okra plant. It causes considerable damage in the eastern regions of Uttar Pradesh particularly during summer when elevated temperature prevails during the month of April–June. In Varanasi region, the crop is attacked by mite pests throughout summer season from young stage till senescence.

The attack of spider mite is severe and persistent on its host plants (Roy *et al.*, 2018) [17]. It induces various types of damage to the host plant ranging from complex physiological to simple mechanical injury. The stylets of mite puncture the cells; inject saliva causing mechanical damage, change the cell cytology, as well as the physiological and biochemical processes of punctured and non-punctured cells nearby (Tomczyk and Kropczynska, 1985) [21]. The physiological damage includes decrease in photosynthesis and transpiration rates leading to decreased vegetative growth and production (Reddall *et al.*, 2004) [16]. It sucks the cell sap from the leaves and produce white spots which later get covered by thick web. The photosynthetic activity is retarded, affected leaves lose green colour, appear stippled, dry up and drop pre-maturely, finally resulting in poor fruit setting (Rahman and Sapra, 1945; Khot and Patel, 1956; Bharodia and Talati, 1976; Puttaswamy and Reddy, 1980; Dutta *et al.*, 2012) [15, 12, 2, 14, 7]. In addition to mechanical and physiological damage it also brings out changes in biochemical composition of host plant. Considering all the damage symptoms which appear, the present study was undertaken to study the biochemical response of plants by estimating change in nitrogen, phosphorus, potassium and different micro nutrient (Fe, Zn, Cu, Mn) concentration in infested and healthy leaves of okra since these macro and micro nutrients

are essential for proper growth and development of plant.

Materials and Methods

The experiment on biochemical response of okra varieties against *T. urticae* infestation was conducted during the year 2013-14 and 2014-15. The field was laid and okra plants were cultivated as per standard horticultural practices. Sixteen varieties were screened for their biochemical response to *T. urticae* infestation and analysis was carried out at Laboratory, Department of Soil Science & Agricultural Chemistry, I. Ag. Sc, B.H.U, Varanasi. The protocol for biochemical analysis of leaf samples was as follows:

The fresh, healthy leaf as well as mite damaged leaf of okra were plucked, washed sequentially with 0.2% detergent solution, 0.1 N HCl and finally with double distilled water. The plant material was dried at 60 °C for 48 hrs in a hot air oven. Dry leaf was finely grounded.

Estimation of nitrogen in okra plant leaves

Nitrogen concentration in plant leaves of okra was determined by Modified Kjeldahl Method as per procedure outlined by Tandon (2001) [20]. In a digestion tube 0.5 g of powdered plant leaf was taken and 10 ml of di-acid solution (9:1, H₂SO₄:HClO₄) was added and kept for overnight. Then 10g of sulphate mixture [(20 parts K₂SO₄) + 1 part catalyst mixture (20 parts CuSO₄ + 1 part selenium powder)] was added and heating was done in a digestion chamber till a clear colourless solution appeared, then cooled and filtered through Whatman No. 42 filter paper in a 50 ml volumetric flask and made up to the volume with distilled water.

10 ml of 4% boric acid solution containing bromocresol green and methyl red indicator was taken in a conical flask, outlet of distillation apparatus was dipped into boric acid solution. 5 ml of the aliquot was taken and transferred to the distillation tube of Kjeltex Semi-Auto Nitrogen Analyser and 10 ml of 40% NaOH was sucked and added to the distillation tube. Then the instrument was put on distillation for 9 min. After completion of distillation, the boric acid was titrated against 0.02 N H₂SO₄. Blank was also run and N concentration was calculated by formula:

$$\text{Total N \% in Plant material} = \frac{0.02 \times T \times 0.014 \times 50 \times 50}{5 \times 0.5}$$

Where, T= Sample reading - Blank reading

Digestion for P, K, Fe, Mn, Zn, and Cu, in okra plant leaves

One gram dried and powdered plant sample (20 mesh) was taken in a 50 ml digestion tube and 10 ml di-acid mixture (4:1 v/v HNO₃: HClO₄) was added to it and was kept overnight. It was then digested on a block digester till a colourless solution was obtained. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 25 ml of distilled water was added. The solution was filtered into a 50 ml volumetric flask and diluted up to mark.

Colour development of Phosphorus

Two ml of digest was taken in a 25 ml volumetric flask and 2 drops of 2, 4 di-nitrophenol indicator was added. Ammonium solution was added till yellow colour appeared and then 6 N HCl was added (drop wise) till it become colourless. Then 5 ml of Vanadate molybdate solution was added and diluted to 25 ml with distilled water, mixed well and the intensity of

yellow colour was read on spectrophotometer by using blue filter at 440 nm wave lengths. A blank was also run without P solution simultaneously. First standard reading and then sample reading was taken. Phosphorus concentration in leaf was calculated using standard curve and expressed as total P (%).

Calculation

$$\text{Total P \% in plant material} = \frac{\text{Abs.} \times \text{dilution factor}}{\text{Slope of standard curve} \times 10000}$$

For Potassium concentration in okra leaf

Potassium concentration in okra leaves was determined by Flame Photometer method (Jackson, 1973). In case of potassium, digested extract was used directly for flame photometer determination of potassium. Potassium concentration of digested leaf was determined by Flame Photometer. K concentration was calculated using the standard curve and expressed as total K.

Calculation

$$\text{Total K \% in plant material} = \frac{R \times \text{dilution factor}}{10000}$$

Where, R = Flame photometer reading

Estimation of Fe, Zn, Mn, and Cu,

The concentration of Fe, Zn, Mn, and Cu in the okra leaves was determined using atomic absorption spectrophotometer (Agilent FS 240) as per procedure outlined by Tandon (2001) [20].

Statistical analysis and interpretation of data

The raw data obtained during the whole experiment was analysed following the Factorial Randomized Block Design and the correlation coefficient 'r' was calculated to observe impact of mite infestation on various biochemical parameters. SPSS software version 16 was used to draw the valid differences among the treatments.

Results and Discussion

The results of this study indicate that the nitrogen concentration (Table 1) in healthy leaves varied significantly among okra varieties, ranged highest in Varsha Uphar (2.48%) followed by Panjab-7 (2.46%) to lowest in No-315 (2.27%). Among infested leaves, the maximum nitrogen concentration was 1.76% in Panjab-7 and lowest in Azad Bhindi-1 (1.52%). The maximum percent decrease in nitrogen concentration was observed in Azad Bhindi-1 (36.93%) followed by Hisar Naveen (38.78%) and Varsha Uphar (34.27%). The least percent decrease in nitrogen concentration was observed in Panjab-7 (28.46%) followed by Pant-A-4 (29.00%). Early workers have also reported biochemical changes in various plant parts due to mite infestation resulting in their physiological and morphological changes (Golek, 1975, Shree and Nataraja, 1993). Chatterjee and Gupta (1997) reported percent reduction of nitrate and nitrite ranging between 51.1% and 3.12% in case of *Luffa acutangula* by feeding of *Tetranychus ludeni* while in our experiment we found percent nitrogen reduction ranging between 36.93% and 28.46%.

The phosphorus concentration (Table 1) in healthy leaves was the highest (0.57%) in Panjab Padmini and the lowest in Azad Bhindi-1 (0.44%). While in infested leaves; it was 0.40% in Azad Bhindi-3 and 0.26% in Azad Bhindi-1. The maximum decrease in phosphorus concentration across the varieties was found in No-315 (46.43%) followed by HRB-231 (41.67%) and Azad Bhindi-2 (41.07%). The minimum decrease in phosphorus concentration was observed in Azad Bhindi-3 (28.57%) while the decrease in other varieties was statistically at par to each other.

The potassium concentration (Table 1) varied significantly in healthy leaves across all the tested varieties. It was maximum in Panjab-7 (2.44%) and minimum in Azad Bhindi-1 (1.94%). Moreover, in case of infected leaves the potassium concentration was least in Pule Utkarsh (1.34%) and showed the maximum in both, Pusa Sawani and Panjab-7 (1.61%). The overall maximum percent decrease in potassium concentration was observed in Azad Bhindi-2 (34.45%) followed by Panjab-7 (34.02%) and least in Kashi Lila (22.56%).

The plant analysis for iron concentration of healthy leaves (Table 2) resulted the maximum concentration in Varsha Uphar (188.43 mg kg⁻¹) followed by Pant-A-4 (186.05 mg kg⁻¹). While the least amount of iron concentration in healthy leaves was found in variety Pusa Sawani (156.72 mg kg⁻¹). The percent decrease in iron concentration in mite infested leaves was highest in HRB-231 (18.60%) followed by Pant-A-4 (16.35%) and the minimum in Pusa Sawani (2.74%).

The maximum zinc concentration (Table 2) among healthy leaves was found in VRO-6 (35.07 mg kg⁻¹) followed by Hisar Naveen (34.01 mg kg⁻¹). The zinc concentration in healthy leaves of remaining varieties remained on par with each other and no significant variation was observed among them. The maximum percent decrease in zinc post infestation was observed in Azad Bhindi-1 (39.51%) followed by Hisar Naveen (38.81%) whereas the least was found in Kashi Lalima (33.53%).

The leaf analysis for copper concentration (Table 2) in healthy leaves revealed the highest copper concentration in Panjab-7 (19.78 mg kg⁻¹) followed by Azad Bhindi-1 (19.00 mg kg⁻¹). The maximum percent decrease in copper post infestation was recorded in Panjab-7 (37.82%) followed by Varsha Uphar (37.28%). Although Pant-A-4 (19.33%) showed least percent decrease in Cu concentration post mite infestation.

The pooled data analysis for manganese (Table 2) concentration in healthy leaves across the varieties ranged between 84.93 to 69.74 mg kg⁻¹. It was found that HRB-231 and Punjab Padmini had the highest amount with 84.93 mg kg⁻¹ and 80.90 mg kg⁻¹ in healthy and infested leaves, respectively. The least concentration of manganese in healthy leaves was found in Hisar Naveen (69.74 mg kg⁻¹) and Azad Bhindi-3 (69.86 mg kg⁻¹). The maximum percent decrease in

Mn concentration post infestation was found in HRB-231 (32.24%) followed by Kashi Lalima (27.94%) whereas the least decrease was found in Hisar Naveen (14.10%).

It was observed that there was reduction in overall nutrient concentration in *T. urticae* infested leaf and this reduction was found across all the varieties.

Vishnupriya *et al.*, (2016) [22] studied the biochemical aspects of feeding of Jasmine blister mite (*Aceria jasmini*) on jasmine and reported a non-significant decrease in calcium and magnesium, whereas significant decrease in iron, manganese, copper and zinc. Ghoshal (2013) [9] also found a considerable decrease in concentration of Mg, Zn, Cu and Fe by 21.32, 26.43, 25.22, and 21.02% respectively in *Ocimum sanctum* leaves when infested by *Tetranychus neocalidonicus*. These results are in line with our investigation.

Ghoshal *et al.*, (2006) [9] have also reported depletion of magnesium, zinc, copper and iron as 8.33%, 22.22%, 13.88% and 8.66%, respectively in case of jute (*Corchorus capsularis* Linn.) infested by *Polyphagotarsonemus latus* (Banks). Iron and zinc depletions have been reported by Chatterjee and Gupta (1997) [3] up to 66.4% and 70% on *Luffa acutangula* due to feeding of *T. ludeni* which were much higher as compared to observation found in our study. Das (1987) [6] reported reduction in Iron and Zinc concentrations by 42.9% and 31.11% respectively in case of *Dolichotetranychus floridanus* on pineapple and those results are also on much higher side as compared to those obtained in the present study.

Correlation between *T. urticae* infestation and biochemical changes in okra varieties (Table 3)

We found that there was reduction in Nitrogen, Phosphorus, Potassium, Iron, Zinc, Copper and Manganese concentration and this decrease was observed across all the varieties (Table 3). Infestation of *T. urticae* was correlated with biochemical changes in all the varieties of okra. The correlation observations made during 2013 and 2014 have been pooled for more comprehensive understanding. In healthy leaves, the Nitrogen ($r = 0.453$), Phosphorus ($r = 0.397$), Iron ($r = 0.447$) and Zinc ($r = 0.391$) were found to be significantly and positively correlated whereas Potassium ($r = 0.385$), Copper ($r = 0.251$) and Manganese ($r = 0.326$) were found to be non-significantly and positively correlated with mite population.

In case of infested leaves, Nitrogen ($r = 0.239$), Phosphorus ($r = 0.221$), Potassium ($r = 0.123$) and Copper ($r = 0.321$) were found to be non-significantly and positively correlated whereas Iron ($r = -0.438$), Zinc ($r = -0.475$) and Manganese ($r = -0.360$) were significantly and negatively correlated with mite infestation. The study helps in deducing the overall loss of nutrients across all the varieties due to reduced photosynthetic activity thus indicating indirect losses incurred due to mites besides yield.

Table 1: Pooled data (2013-14 and 2014-15) of percent Nitrogen, Phosphorus and Potassium concentration in healthy and *T. urticae* infested leaves of Okra

Varieties	% Nitrogen concentration			% Phosphorus concentration			% Potassium concentration		
	Healthy	Infested	%Decrease	Healthy	Infested	%Decrease	Healthy	Infested	%Decrease
Azad Bhindi-1	2.41	1.52	36.93	0.44	0.26	40.91	1.94	1.40	27.84
Azad Bhindi-2	2.32	1.61	30.60	0.56	0.33	41.07	2.09	1.37	34.45
Azad Bhindi-3	2.37	1.55	34.60	0.56	0.40	28.57	2.07	1.49	28.02
Pule Utkarsh	2.33	1.63	30.04	0.52	0.34	34.62	2.02	1.34	33.66
No-315	2.27	1.56	31.28	0.56	0.30	46.43	1.98	1.51	23.74
HRB-231	2.32	1.57	32.33	0.48	0.28	41.67	2.00	1.44	28.00
Varsha Uphar	2.48	1.63	34.27	0.53	0.37	30.19	1.96	1.47	25.00

Punjab Padmini	2.36	1.58	33.05	0.57	0.34	40.35	2.04	1.46	28.43
Kashi Lila	2.34	1.56	33.33	0.50	0.33	34.00	1.95	1.51	22.56
Kashi Sathdhari	2.33	1.63	30.04	0.56	0.35	37.50	2.01	1.45	27.86
Kashi Lalima	2.35	1.58	32.77	0.50	0.30	40.00	2.11	1.51	28.44
Pant-A-4	2.31	1.64	29.00	0.50	0.30	40.00	2.12	1.51	28.77
Pusa Sawani	2.37	1.68	29.11	0.53	0.35	33.96	2.27	1.61	29.07
Hisar Naveen	2.41	1.57	34.85	0.49	0.30	38.78	2.26	1.56	30.97
Panjab-7	2.46	1.76	28.46	0.48	0.30	37.50	2.44	1.61	34.02
VRO-6	2.39	1.62	32.22	0.49	0.32	34.69	2.14	1.47	31.31
S.Em.±	0.0249	0.016		0.027	0.012		0.024	0.024	
CD at 5%	0.0720	0.047		0.079	0.035		0.069	0.071	

Table 2: Iron, Zinc, Copper and Manganese concentration (mg/kg⁻¹) in healthy and *T. urticae* infested leaves of Okra (Pooled data 2013-14 and 2014-15).

Varieties	Iron (Fe) concentration			Zinc (Zn) concentration			Copper (Cu) Concentration			Manganese (Mn) concentration		
	Healthy	Infested	%Decrease	Healthy	Infested	%Decrease	Healthy	Infested	%Decrease	Healthy	Infested	%Decrease
Azad Bhindi-1	182.46	158.63	13.06	33.31	20.15	39.51	19.00	13.13	30.89	76.74	57.83	24.64
Azad Bhindi-2	171.72	154.2	10.20	33.70	21.89	35.04	18.44	11.82	35.90	76.78	60.11	21.71
Azad Bhindi-3	180.76	152.86	15.43	33.02	21.20	35.80	16.79	12.61	24.90	69.86	58.97	15.59
Pule Utkarsh	182.28	155.83	14.51	32.76	20.70	36.81	18.17	13.02	28.34	75.09	59.57	20.67
No-315	170.04	159.28	6.33	33.58	21.98	34.54	17.22	11.99	30.37	74.35	59.06	20.56
HRB-231	185.74	151.2	18.60	32.95	21.33	35.27	18.82	12.80	31.99	84.93	57.55	32.24
Varsha Uphar	188.43	158.37	15.95	33.79	21.54	36.25	17.97	11.27	37.28	72.99	59.82	18.04
Punjab Padmini	182.03	154.77	14.98	33.51	21.61	35.51	18.20	11.75	35.44	80.90	59.12	26.92
Kashi Lila	176.07	149.79	14.93	32.57	21.36	34.42	17.46	11.47	34.31	77.11	60.95	20.96
Kashi Sathdhari	168.71	145.63	13.68	33.78	21.84	35.35	16.97	12.37	27.11	73.61	59.77	18.80
Kashi Lalima	166.96	157.32	5.77	33.22	22.08	33.53	18.16	13.42	26.10	80.78	58.21	27.94
Pant-A-4	186.05	155.64	16.35	33.74	21.37	36.66	17.43	14.06	19.33	74.97	59.18	21.06
Pusa Sawani	156.72	152.54	2.74	33.71	21.70	35.63	18.04	11.35	37.08	73.40	58.84	19.84
Hisar Naveen	176.82	155.53	12.04	34.01	20.81	38.81	17.42	12.57	27.84	69.74	59.91	14.10
Panjab-7	170.58	155.63	8.76	33.52	21.18	36.81	19.78	12.30	37.82	71.83	59.10	17.72
VRO-6	173.75	158.97	8.51	35.07	21.78	37.90	17.54	12.43	29.13	75.22	58.57	22.14
S.Em.±	2.933	2.597		0.417	0.335		0.224	0.551		1.692	0.455	
CD at 5%	8.473	7.501		1.205	0.967		0.648	1.592		4.888	1.314	

Table 3: Correlation(r) of *T. urticae* (Koch) infestation with biochemical changes in different varieties of okra (Pooled data 2013-14 and 2014-15).

Pooled Data (2013-14 and 2014-15)	Parameters	N	P	K	Fe	Zn	Cu	Mn
	Healthy	0.453*	0.397*	0.385	0.447*	0.391*	0.251	0.326
	Infested	0.239	0.221	0.123	-0.438*	-0.475**	0.321	-0.360*

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level

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