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EFFECT OF THE HOST PLANT ON SUSCEPTIBILITY OF THE TWO-SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH, (ACARI: TETRANYCHIDAE) TO SOME ACARICIDES

Ibrahim, M. M. S.
Plant Protection Research Institute, ARC, Egypt

ABSTRACT

The two-spotted spider mites, *Tetranychus urticae* Koch, reared on kidney bean plants were moved to eggplant, cucumber and new kidney bean plants (the latter being as control) and estimated after one month for changes in susceptibility to some acaricides and in levels of some related detoxification enzymes.

The largest consistent changes were observed in mites feeding on eggplant and cucumber. Susceptibility of mites on kidney bean were significantly more than susceptibility of mites on eggplant and cucumber after feeding on the respective hosts to the all tested acaricides. The LC_{50} values of mites that feeding on kidney bean plants were 12.3, 35.9, 76.1, 56.6, and 6.7 mg/L. for the tested acaricides; abamectin, dicofol, dinobutone, ethion, and fenbropathrin, respectively. Whereas, it were 32.5, 190.8, 247.3,192.1, and 21.5 mg/L. to mites that feeding on eggplant and 21.7, 279.4, 297.5, 215.3, and 10.5 mg/L. to mites that feeding on cucumber plans for the same above tested acaricides, respectively.

Susceptibility was inversely related to activities of both general esterase and glutathione-*S*-transferase (GST) in mites on eggplant and cucumber; general esterase and GST activities were (1.9 to 3.1-fold) and (2.7 to 3.5-fold), respectively, than their activities of mites feeding on kidney bean. Both of the enzyme groups examined, general esterases and GST appeared to be involved in the changes in susceptibility and play a role in the detoxification of the two-spotted spider mite to the all tested acaricides.

Thus, plant-induced changes in general esterase activity in combination with GST activity; in the two-spotted spider mite appear to be possibly responsible for changes in susceptibility of the two-spotted spider mite to several acaricides. In addition to probably allelochemicals associated with host plant may induce changes in the general detoxification enzymes of spider mites.

These changes may have a significant impact on the ability to control spider mites with some acaricides. Better understanding of the biochemical interactions between the two-spotted spider mite and the host plants will need to develop better IPM programs on different hosts.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is a phytophagous mite with worldwide distribution and a large number of host plants. The species has been heavily exposed to insecticides and acaricides, and organophosphate-resistant populations have been reported in both greenhouses and open field crops.

Host plants can modify the susceptibility of herbivorous arthropods to pesticides (Yu *et al.* 1979, Yu 1982, Yu 1983, Brattsten 1988). For example, within several aphid species, populations from different host plants showed different susceptibilities to pirimicarb (Furk *et al.* 1980) and organophosphate (OP) insecticides (Saito 1989, Ambrose and Regupathy 1992).

Many enzymes involved in detoxification pathways act on a broad array of substrates, including both naturally occurring plant allelochemicals and artificial pesticides (Gordon 1961). Therefore, physiological response of herbivores to host plants may lead to enhance metabolism of pesticides because mechanisms that function in detoxification of plant allelochemicals in their diets may also be effective at detoxifying pesticides.

General esterases, glutathione-*S*-transferase (GST), and cytochrome P450-dependent monooxygenase are common detoxification enzymes that metabolize pesticides in arthropods. General esterases, which are capable of degrading or sequestrating pesticides, can play a significant role in the detoxification of OP and pyrethroid pesticides (Anspaugh *et al.* 1995, Argentine *et al*. 1995, Valles 1998).

Mullin and Croft (1983) reported 0.4 to 2.4-fold changes in activities of general esterases in the two-spotted spider mite, *Tetranychus urticae* Koch feeding on plants other than beans. Mites may escape some plant allelochemicals during exposure. Furthermore, digestion in mites occurs largely in phagocytes, which eventually are egested. Sequestration of toxic compounds in these cells may be an alternative defense strategy to metabolic detoxification in mites.

Neiswander *et al.* (1950) reported that populations of the two-spotted spider mite responded differently to the same acaricide after feeding on different host plants. Gould *et al.* (1982) reported that tolerance of the two-spotted spider mite to several OP pesticides was influenced by feeding on mite-resistant and susceptible cucumber varieties.

The objective of this study was to determine differences in susceptibility of the two-spotted spider mite on different plants to same acaricide used in vegetable crops, and if these differences could be related to plant induced changes in certain detoxification enzymes.

This information could be useful in the two-spotted spider mite management on different hosts and in understanding changes in acaricides susceptibility and the development of resistance to acaricides in the two-spotted spider mite.

MATERIALS AND METHODS

Maintenance of the colonies:

The strain of the two-spotted spider mite, *T. urticae* Koch were collected in April 2007, and reared at El- Hosainia Agricultural Research Station on Kidney bean, *Phaseolus vulgaris* (L.) plants according to the method of Nassar (1974).

The strain was kept in cheese cloth cage (60x 60 x 100 cm.) away from pesticides contamination under laboratory conditions (25 ± 2°C and 65±5 % (RH) relative humidity and 12 hours daily illuminations by using fluorescent tubes of 40 watt). Leaves from a laboratory culture infested with the two-spotted spider mite were distributed over the new foliage of the Kidney bean.These mites were subsequently maintained on without exposure to pesticides for several months under the greenhouse conditions.

General methods and host plants used:

Kidney bean, *Phaseolus vulgaris* (L.), eggplant, *Solanum melongena* (L.) and cucumber, *Cucumis sativus* (L.) were used as testing host plants. All plants were grown in pots under greenhouse conditions. Two weeks after planting, all plants were received usual agricultural practices and no pesticides were used.

Leaves contains adult female of mites (1500- 2000 individuals) from the two-spotted spider mite colony were placed on the test host plants; Kidney bean, eggplant and cucumber under greenhouse conditions. Acaricides bioassay and enzyme assays were performed after mites had been fed on these hosts for one month.

The tested acaricides:

1. Vertimec (1.8% EC) abamectin

A mixture containing: of ≥80% avermectin BLa (i): 5-0-demethyl avermectin BLa and ≤ 20% avermectin BLb (ii): 5-0-demethyl 1-25-de (1-methylpropyl)-25-(1-methyl) avermectin BLb.

2. Dicomite (18.5 % EC) dicofol
   2, 2, 2-trichloro-1, 1-bis (4-chlorophenyl)ethanol
3. Acarelate (40% EC) dinobutone
   isopropyl-2 (1-methyl-n-propyl)-4, 6- dinitrophenyl carbonate
4. Endo (50 % EC) ethion
   O,O,O,O- tetraethyl *S, S*- methylenediphosphorodithioate
5. Vetafol (40% EC) fenbropathrin
   α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl cyclopropanecarboxylate

Acaricides Bioassay:

Kidney bean leaf disc technique was used to estimate the toxicity of the tested acaricides against the adult females of the two-spotted spider mite, *T. urticae* that had been fed on each above host plant.

Each acaricide was diluted with distilled water to generate five serial dilutions, and each concentration was repeated four times. The leaf discs (diameter 3.5 cm) were immersed in the dilutions for five seconds. After drying, twenty five adult females of mites were placed on each treated leaf disc that placed on wet cotton in a Petri dish. The treatments were kept under constant conditions (25 ± 2°C and 65±5 % (RH) relative humidity) at a photoperiod of 12:12 (L: D) h for 24 hrs.

The mortality counts were estimated after 24 hrs of each treatment and the percentage mortality was corrected using formula of Abbott (1925). The criterion for mortality was the failure of mite to respond positively by leg movement following light prodding with a fine brush. The LC_{50} values and slopes of the regression lines were estimated with probit analysis according the method described by finney (1971).

Enzyme Assays:

The chemicals used to assay enzyme activities were analytical quality α-naphthol, α-naphthyl acetate (α-NA), tetrazotized *O*-dianisidine (fast blue B), reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), sodium dodecylsulfate (SDS), 3,4-dichloronitrobenzene (DCNB) dithithreitol (DTT), tetra-acetic ethylenediamine acid (EDTA) and phenylmethylsulfonyl fluoride (PMSF).

Activities of general esterase and glutathione *S*-transferase (GST) were measured following the method of Habig *et al*. (1974).

Extracts of total proteins were prepared by homogenizing 2500 adult females of *T. urticae* from each host plant separately in 2.5 ml 0.05 M ice-cold phosphate buffer, pH 7.5, containing 0.1 mM dithithreitol, 1 mM tetra-acetic ethylenediamine acid (EDTA) and 0.4 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 15,000 rpm for 15 min at 4°C. For each test, after centrifugation the supernatant was collected and kept on ice for no more than 4 hours as an enzyme source. The supernatant was used as a source of soluble proteins to determine both of general esterase and GST activity.

For general esterase activity assays, 15 μl of enzyme preparation was incubated in a final reaction volume of 150 μl containing 0.27 mM of α-NA as a substrate at 37°C for 30 min. The reaction was stopped by addition of 50 μl of freshly prepared fast blue B-SDS solution (30 mg fast blue B in 10 ml 5% SDS solution) in a glass cuvette. The absorbance was determined 15 min later at 600 nm using Phoenix-2000 UV/VIS spectrophotometer. Quantification of enzymatic product was based on a standard curve prepared using α-naphthol according the method of van Asperen (1962) as modified by Zhu and Gao (1998).

For the GST activity assay, 50 μl of enzyme preparation was mixed with 690 μl of 10 mM glutathione (in 0.1 M ice-cold phosphate buffer, pH 7.5) and 10 μl of either 150 mM CDNB or DCNB (in acetone) in a glass cuvette. The reaction mixture (final volume: 1 ml) consisted of a volume of homogenate equivalent to 50 μg soluble proteins in substrate solution at varying concentrations and 0.01 M ice-cold phosphate buffer pH 7.5 in a glass cuvette.

The change in absorbance was recorded at 340 nm for CDNB-conjugating reaction or at 344 nm for DCNB-conjugating reaction for two minuets using Phoenix-2000 UV/VIS spectrophotometer. Each assay was replicated four times, each with three replicated readings.

All assays were corrected for non-enzymatic conjugation that occurred in a sample that contained substrate and 10 mM glutathione in 0.1M phosphate buffer, pH 7.5. The amount of glutathione conjugate formed was calculated using the extinction coefficients of 9.6 $mM^{-1}cm^{-1}$ for CDNB and 10.0 $mM^{-1}cm^{-1}$ for DCNB.

Activities of general esterase and glutathione-*S*-transferase (GST) were measured concurrently with the above-described bioassays. The influence of host plant on the enzyme activity of the two-spotted spider mite was tested at a significance level of 5% by analysis of variance.

RESULTS AND DISCUSSION

Susceptibility to acaricides:

Kidney bean plants are suitable hosts for the two-spotted spider mite and are often used for laboratory rearing. Susceptibility of the moving stages of mites on eggplant or cucumber decreased compared with that on kidney

beans to same tested acaricides, although the magnitude of the decrease varied among acaricides and host plants.

The obtained data in table (1) and fig. (1) showed the susceptibility of mites on kidney beans plants were significantly more than the susceptibility of mites on eggplant and cucumber after feeding on the respective hosts to the all tested acaricides.

The LC_{50} values of mites that feeding on kidney bean plants were 12.3, 35.9, 76.1, 56.6, and 6.7 mg/L. for the tested acaricides; abamectin, dicofol, dinobutone, ethion, and fenbropathrin, respectively. Whereas, it were 32.5, 190.8, 247.3,192.1, and 21.5 mg/L. to mites that feeding on eggplant and 21.7, 279.4, 297.5, 215.3, and 10.5 mg/L. to mites that feeding on cucumber plants for the same above tested acaricides, respectively.

From aforementioned results it is clear that acaricides abamectin and fenbropathrin were more effective on mites infested cucumber and eggplant than other tested acaricides. While, acaricide dicofol was less effective one on these host plants.

Table (1): Effects of host plant on the susceptibility of the two-spotted spider mite, *T. urticae* to the tested acaricides.

Acaricides	Host plant	LC_{50} (mg/L.)	Slope	Ratio of susceptibility*
abamectin	Kidney bean	12.3	0.43	100
	Eggplant	32.5	0.31	37.8
	Cucumber	21.7	0.33	56.7
dicofol	Kidney bean	35.9	0.43	100
	Eggplant	190.8	0.32	18.8
	Cucumber	279.4	0.31	12.8
dinobutone	Kidney bean	76.1	0.42	100
	Eggplant	247.3	0.31	30.8
	Cucumber	297.5	0.31	25.6
ethion	Kidney bean	56.6	0.42	100
	Eggplant	192.1	0.31	29.5
	Cucumber	215.3	0.32	26.3
fenbropathrin	Kidney bean	6.7	0.42	100
	Eggplant	21.5	0.32	31.2
	Cucumber	10.5	0.31	54.3

*** Ratio of susceptibility was obtained by dividing LC_{50} from mites on kidney bean (Control) by that of mites on eggplant or cucumber to the same acaricide.**

From the previous results it is clear that the significant differences in susceptibility of the two-spotted spider mite on different host plants to the same tested acaricide.

These results are in agreement with the known differences in dosages of acaricide that are required to manage spider mite populations on different host plants. Many factors appear to contribute to these differences, but it is useful to know that the host plant itself can be responsible for some of these differences (Yang *et al.* 2001).

Enzymes Activity:

Data presented in table (2) and fig. (2) showed that mites on eggplant and cucumber had significantly higher general esterase activity than that on kidney bean.

The general esterase activity of mites on kidney bean remained stable compared with control, but for mites on eggplant and cucumber showed an increase in activity (1.9 to 3.1-fold). GST activity showed a slightly different pattern than general esterase activity. Mites from eggplant and cucumber had significantly higher increasing in GST activity (2.7 to 3.5-fold) than did mites on kidney bean plants.

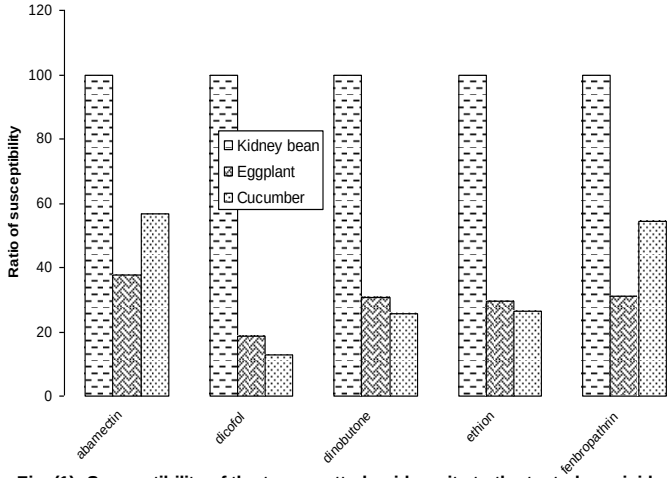

Fig. (1): Susceptibility of the two-spotted spider mite to the tested acaricides on different host plants.

Both of the enzyme groups examined, general esterases and GST, appeared to be involved in the changes in susceptibility of the two-spotted spider mite to the tested acaricides. An inverse relationship occurred between detoxification enzyme activity in mites and susceptibility to tested acaricides. Activities of general esterases and, GST (in mites on eggplant and cucumber) appeared to be associated with lower susceptibilities of the two-spotted spider mite to the tested acaricides.

Table (2): General esterase and GST activities in the two-spotted spider, *T. urticae* mite feeding on different host plants.

Host plant	Means of general esterase activity* (nmol/ min/ mg protein)	Change of General esterase activity**	Means of GST Activity* (nmol/ min/ mg protein)	Change of GST Activity**
Kidney bean (Control)	(0.13 – 0.35) 0.24^{c}	Normal	(0.89 – 1.13) 1.01^{c}	Normal
Eggplant	(0.35 – 0.57) 0.46^{b}	1.9-fold	(2.62 – 2.90) 2.76^{b}	2.7-fold
Cucumber	(0.65 – 0.85) 0.75^{a}	3.1-fold	(3.34 – 3.67) 3.51^{a}	3.5-fold
LSD 0.05	0.14		0.31	

*** Means of enzyme activity followed by the same letter are not significantly different.**

**** The changes of activity were obtained by dividing the activity from mites on eggplant or cucumber by that of mites on kidney bean (Control).**

The changes in enzyme levels of the two-spotted spider mite introduced to eggplant and cucumber occurred relatively higher increased may be related to feeding on unusual host or to inherently poorer nutritional

quality of eggplant and cucumber compared with kidney bean. Mites faced with a new host may not feed, or not feed as much, as mites on a more familiar host. This could cause initially changes in general esterase activity and related changes in susceptibility to acaricides.

These results are in agreement with those reported by Mullin and Croft (1983), in which mites on maize and cucumber showed increased levels of general esterase activity compared with mites on beans. However, their study employed mites reared for several generations on the new host plants (maize and cucumber) before they conducted enzyme assays. Many generations of selection may result in adaptation to a new host (Gould 1979, Fry 1989), including changes in general esterase activity.

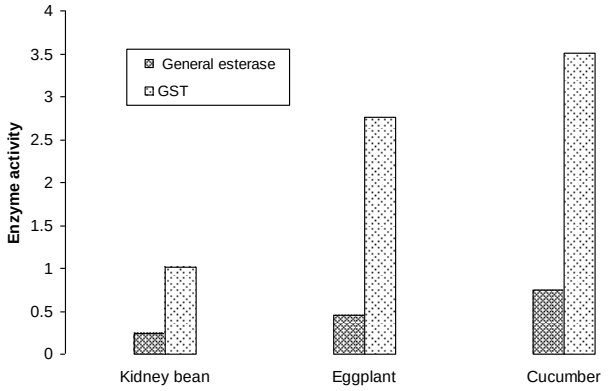

Fig. (2): General esterase and glutathione-*S*-transferase (GST) in the two-spotted spider mite feeding on different host plants.

Also Mullin and Croft (1983) noted that physiological response of herbivores to host plants may lead to enhanced metabolism of pesticides because mechanisms that function in detoxification of plant allelochemicals in their diets may also be effective at detoxifying pesticides.

Plant-induced changes in general esterase activity, perhaps in combination with GST activity, in the two-spotted spider mite appear to be inversely related to, and possibly responsible for, changes in susceptibility of the two-spotted spider mite to several pesticides. Furthermore, host plant resistance to the two-spotted spider mite has become an important component of integrated pest management (IPM) (Yang *et al.* 2001).

The population dynamics and the host plant characteristics with differential suitability to the two-spotted spider mite is an important factor to consider when exploring integrated pest management (IPM) solution for the two-spotted spider mite (Ibrahim *et al*. 2008).

In addition to the changes in detoxifying enzymes associated with host plants, allelochemicals associated with host plant may induce changes in the general detoxification enzymes of spider mites Mullin and Croft (1983). These changes may have a significant impact on the ability to control spider mites with some acaricides. Better understanding of the biochemical

interactions between the two-spotted spider mite and the host plants will need to develop better IPM programs for the two-spotted spider mite on different hosts.

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تأثير العائل النباتى على حساسية العنكبوت الأحمر ذو البقعتين تيترانيكس يورتيكا لبعض مبيدات الأكاروسات

محمد منصور سليمان إبراهيم
معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر

تم تربية أكاروس العنكبوت الأحمر ذو البقعتين تيترانيكس يورتيكا على نباتات الفاصوليا فى المعمل ثم نقله الى ثلاث عوائل من عائلات نباتية مختلفة هى الباذنجان والخيار والفاصوليا (والأخير أستخدم للمقارنة) للتغذية عليها لمدة شهر بغرض دراسة تأثير هذه العوائل النباتية على حساسية العنكبوت الأحمر ذو البقعتين لبعض مبيدات الأكاروسات وهى:
فيرتيمك (أباميكتين)- ديكوميت (ديكوفول)- أكاريليت (دينوبيتون) - إندو (إثيون)- فيتافول (فينبروباثرين). وكذلك دراسة تأثير تغذية أكاروس العنكبوت الأحمر ذو البقعتين على هذه العوائل على نشاط الإنزيمات العامة ومجموعة إنزيمات الجلوتاثيون- إس- ترانسفيريز والتى تلعب دورا هاما فى تقليل سمية العديد من مبيدات الآفات.

وقد تبين من هذه الدراسة أن حساسية أكاروس العنكبوت الأحمر ذو البقعتين تختلف لذات المركب باختلاف العائل النباتى الذى تمت التغذية عليه. فقد وجد أن أكاروس العنكبوت الأحمر ذو البقعتين الذى تغذى على نباتات الفاصوليا كان أكثر حساسية للمركبات المختبرة من الأكاروس الذى تغذى على نباتات الباذنجان والخيار. فكان التركيز القاتل ل ٥٠% من أكاروس العنكبوت الأحمر ذو البقعتين والذى تغذى نباتات الفاصوليا هو: ١٢,٣، ٣٥,٩، ٧٦,١، ٥٦,٦، ٦,٧ ملجم/اللتر، فى حين أن الأكاروس الذى تغذى على نباتات الباذنجان كان التركيز القاتل ل ٥٠% هو: ٣٢,٥، ١٩٠,٨، ٢٤٧,٣، ١٩٢,١، ٢١,٥ ملجم/اللتر، وللأكاروس الذى تغذى على نباتات الخيار كان: ٢١,٧، ٢٧٩,٤، ٢٩٧,٥، ٢١٥,٣، ١٠,٥ ملجم/اللتر للمبيدات الأكاروسية: أباميكتين، دايكوفول، دينوبيوتون، إثيون، فينبروباثرين على الترتيب.

كما وجد أيضا أن تغذية أكاروس العنكبوت الأحمر ذو البقعتين على عوائل نباتية مختلفة أدى الى حدوث تغير فى نشاط الإنزيمات العامة ومجموعة إنزيمات الجلوتاثيون- إس- ترانسفيريز، حيث وجد أن نشاط الإنزيمات العامة للأكاروس الذى تغذى على نباتات الباذنجان والخيار إزداد (١,٩ إلى ٣,١ مرة على الترتيب) عنه فى حالة الأكاروسات التى تغذت على نباتات الفاصوليا. كما وجد أن نشاط مجموعة إنزيمات الجلوتاثيون- إس- ترانسفيريز للأكاروس الذى تغذى على نباتات الباذنجان والخيار إزداد (٢,٧ إلى ٣,٥ مرة على الترتيب) عنه فى حالة الأكاروسات التى تغذت على نباتات الفاصوليا.

يتضح من هذه الدراسة أن العائل النباتى له تاثير هام على حساسية أكاروس العنكبوت الأحمر ذو البقعتين لبعض مبيدات الأكاروسات حيث أن بعض العوائل النباتية بما تحتوى عليه من مواد كيماوية ثانوية قد تعمل كسموم بتركيزات أقل من التركيزات المميتة والتى قد تؤدى بدورها إلى تحفيز وزيادة نشاط بعض النظم الإنزيمية للآفة ومن بينها مجموعة إنزيمات التحلل ومجموعة إنزيمات الجلوتاثيون- إس- ترانسفيريز والتى تلعب دورا هاما فى تقليل سمية العديد من مبيدات الآفات. لذا يجب أن يؤخذ فى الإعتبار نوع العائل النباتى المصاب واختيار المبيد المناسب عند تطبيق عملية المكافحة المتكاملة لآفة زراعية معينة.

قام بتحكيم البحث

أ.د / عادل عبد المنعم صالح	**كلية الزراعه – جامعة المنصوره**
أ.د / سيد على احمد ابراهيم	**خارجى**