



Egyptian Journal of Plant
Protection Research Institute

www.ejppri.eg.net



Host plant-induced susceptibility of *Tetranychus urticae* (Acari: Tetranychidae) to three acaricides with different mode of action

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ARTICLE INFO

Article History

Received: 5/10 /2023

Accepted: 3/12/2023

Keywords

Performance,
preference,
Tetranychus urticae,
abamectin,
fenpyroximate and
clofentezine.

Abstract

The relationship between host plant adaptation and acaricide resistance of *Tetranychus urticae* Koch (Acari: Tetranychidae) were studied through three laboratory experiments, a non-choice test to evaluate the performance, a choice test to measure the preference of *T. urticae* females to different host plants (Squash, cucumber and kidney bean); and a bioassay of three reduced risk acaricides with different mode of action (Abamectin, fenpyroximate and clofentezine) against *T. urticae* females. Mean fecundity in the non-choice test was higher on kidney bean than on the other host plants. Survival of 5-day-old females was independent of the host plant. The percentage of females that laid at least one egg did not depend on the host plant. In the choice test, host-plant preference was independent of the host plant. The oviposition was dependent on the host plant, and a significant difference was found. Abamectin showed the highest toxicity against *T. urticae*, LC₅₀ values were 0.02, 0.031 and 0.06 mg /l on squash, cucumber, and kidney bean after 72 h. respectively, which was followed by fenpyroximate, LC₅₀ values recorded 1.69, 1.75 and 3.35 mg /l on cucumber and kidney bean and squash, respectively. While clofentezine the lowest toxicity, LC₅₀ values were 6.19, 8.34 and 14.26 mg /l on kidney bean, cucumber, and squash, respectively. Results proved the necessity of considering the host plant when studying the efficiency of the pesticides.

Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a common polyphagous arthropod herbivore that is a significant pest of greenhouse crops, particularly in the Solanaceae and Cucurbitaceae; annual field crops and flowering plants (Cazaux *et al.*, 2014). *T. urticae* is an excellent choice to

systematically elucidate the relationship between host plant adaptation and pesticide resistance. *T. urticae* is one of the most polyphagous herbivores known; it can consume over 1,100 different plants from over 140 different plant families, all of which produce a variety of chemical defenses (Grbić *et al.*, 2011). The quality of a host plant is determined by its primary and secondary metabolisms,

which may also have an impact on a phytophagous insect's ability to feed and its life history traits. Numerous studies indicate that defense chemicals at high levels with toxic, antifeedant, or repellent properties can significantly lower insect performance. These substances include alkaloids, glucosinolates, terpenoids, and phenolics (Yan *et al.*, 2018 and Fuchs *et al.*, 2020), although the development of insects benefits from relatively high nitrogen levels (Awmack and Leather, 2002). Due to mechanisms that work to detoxify plant allelochemicals in herbivores' diets, its physiological responses to host plants may promote the metabolism of pesticides. When a phytophagous pest feeds on various host plants, the secondary plant metabolites stimulate the insect's detoxification enzymes, which in turn affect the pesticides' rapid metabolism. This can have a significant impact on the pest's susceptibility to any pesticide (Abro *et al.*, 2013). The majority of contemporary acaricides work by interfering with respiratory functions or by having an impact on growth and development (Dekeyser, 2005 and Kraemer and Schirmer, 2007).

Abamectin is a neuroactive acaricide (Chloride channel activator) with reduced risk, because of their short environmental persistence, quick uptake by treated plants, and quick degradation of surface residues, it is thought to be safe for beneficial arthropods in field conditions. It is isolated from the fermentation of a soil bacterium *Streptomyces avermitilis* (Kramer and Schirmer, 2007). Fenpyroximate (Pyrazole), is reported to show high efficacy with a quick knockdown effect against both tetranychid and eriophyid mites, by

inhibition of mitochondrial electron transport (MET) at complex I (Marčić *et al.*, 2011). Clofentezine and abamectin, good selective acaricides, are more toxic to tetranychids than to phytoseiids (Yoo and Kim, 2000 and Hardman *et al.*, 2003). Clofentezine is a novel structure acaricide belonging to tetrazine group. It is registered for the protection of food and nonfood crops, ornamentals, and orchards. It conflicts with cell growth and early larval development and the final stages of embryonic differentiation [Food and Agriculture Organization (FAO, 2010) Specification]. Clofentezine is an integrated pest management (IPM) compatible pesticide. It has fast dissipation after the field application (Urbaneja *et al.*, 2008).

Therefore, the present study was planned to discuss the performance of *T. urticae* on three different host plants namely kidney bean (*Phaseolus vulgaris* L.) (Family: Leguminosae), squash (*Cucurbita pepo* L.) and cucumber (*Cucumis sativus* L.) (Family: Cucurbitaceae). Then, find out the toxicity of three reduced risk acaricides viz. abamectin, fenpyroximate and clofentezine on the *T. urticae* population reared on these host plants.

Materials and methods

1. The performance of *Tetranychus urticae* on three different susceptible host plants:

1.1. The first experiment:

Consisted of non-choice test to evaluate the performance of the females on different host plants, we registered the fecundity (number of eggs per female per 5 days), the survival of 5-day-old females, and the percentage of females that oviposited at least one egg. Twenty individual newly mated females were assigned to discs of each host plant: Squash (New Iskandar v.),

Cucumber (El Prince v.) and kidney bean (Nebraska v.).

1.2. The second experiment:

Consisted of a choice test to measure the preference of females for different host plants. Ten females were put in the center of the Petri dish, and three discs, one of each plant was put in the angles of a triangle. Six replicates were performed, with a total number of 60 mites. The fate of each female, the number and position of the eggs were computed after 24 hrs.

2. Tested reduced risk acaricides:

Three reduced risk acaricides with different modes of action according to IRAC (2019), Insecticide Resistance Action Committee (IRAC) were selected for the bioassay.

2.1. Abamectin (Abazin 1.8% EC):

gp.6, a risk reduced neuroactive acaricide, allosterically activates glutamate-gated chloride channels (GluCl_s), causing paralysis. Glutamate is an important inhibitory neurotransmitter in insects. It was purchased from North China pharmaceutical group aino co. ltd. China.

2.2. Fenpyroximate (Volitan extra 5% SC):

21A Mitochondrial complex I electron transport inhibitors. Inhibit electron transport complex I, preventing the utilization of energy by the cells, manufactured by *Shandong United Pesticide Industry Co., Ltd.* China.

2.3. Clofentezine (Azocip 50% W/V):

10A (tetrazine acaricides group), Mite growth inhibitors affecting CHS1 Inhibit the enzyme that catalyzes the polymerization of Chitin, was obtained from *Nantong Baoye Chemical Co., Ltd.* China. Application rate, 30 cm/100 L water.

3. Bioassay procedures:

The toxicity of three reduced-risk pesticides against *T. urticae* was evaluated by the spray leaf disc bioassay method on three different host plant leaves under

laboratory conditions. In brief, leaf discs (2.5 cm in diameter) using leaves of kidney bean (Nebraska v.), squash (New Iskandar) and cucumber (El prince) were prepared and placed on wet cotton pads in Petri dishes. The acaricide solutions were prepared by diluting in water and five concentrations were used for each acaricide to determine the LC₅₀ values. The control group was treated with distilled water only. There were four replicates for each treatment and 10 adult female mites were used in each replicate. After treatments leaf discs were kept at 25±2°C temperature, 80±5% RH. and 12 L:12 D photoperiod. Mortality was recorded in 24, 48 and 72 hrs.

4. Statistical analyses:

The mortality percentage of spider mites was corrected using Abbott's (1925) formula. The median lethal values (LC₅₀) were determined by probit analysis using 'Ldp line' software (<http://www.ehabsoft.com/ldpline/>), Bakr (2000). By using IBM SPSS statistics 20, fecundity was analysed using Anova and Tukey's test, while survival of females and percentage of ovipositing females by a chi-square test. The fate of each female, the number and position of eggs in a choice test were analysed by Anova and Tukey test.

Results and discussion

1. The performance of *Tetranychus urticae* on three different susceptible host plants:

1.1. In the non-choice test, mean fecundity (Eggs per female per 5 days), was higher on kidney bean than on the other plants showing a highly significant difference (Anova: F = 20.514; d.f. = 2; P = 0.0001). There was no significant difference between squash and kidney bean. Where the mean fecundity of cucumber was very low and highly significantly different (Table 1). Survival of 5-day-old females was independent of the host plant and no differences were found between them ($X^2 = 0.786$; d.f. = 2; P =

0.675). The percentage of females that laid at least one egg did not depend on the host plant either ($X^2 = 1.745$; d.f. = 2; $P = 0.418$) (Table 1).

1.2. In the choice test, host-plant preference was measured as the acceptance of the females settled on different host plants after 24 hrs., it was independent of the host plant, and no difference was found between them. While more females settled on squash and less on Kidney bean and cucumber (Anova: $F=0.929$, d.f.= 2, $P=0.417$) (Table 2). The oviposition (number of eggs laid after 24 hrs.) was dependent on the host plant, and a significant difference was found between them, where it was higher on squash followed by kidney bean and very poor on cucumber (Anova: $F=5.118$, d.f.= 2, $P=0.02$), as a result of more females settled on squash and less on kidney bean and cucumber. The fecundity (Number of eggs laid/ *T. urticae* female), in the choice and non-choice tests, indicated that cucumber (El Prince v.) was more resistant than other tested plants [Squash (New Iskandar v.) and kidney bean (Nebraska v.).

As anticipated, this experimental manipulation showed a high preference and a better performance of *T. urticae* on Kidney bean v. leaves followed by Squash v. than on cucumber v. In the choice test for oviposition, *T. urticae* laid more eggs on kidney bean and squash than on cucumber v., which seem to be unsuitable for the development of the two-spotted spider mite, where they had a poor performance: low fecundity and low survival rate. In addition, when choice tests were done, females selected cucumber but laid the lowest no. of eggs/female which suggests the presence of some chemical that reduce subsequent spider-mite performance. The results of this study provide a better understanding of the host range of *T. urticae* and the patterns of movements of spider mites between host plants, which may suggest ways of

modifying the habitat or agroecosystem to reduce spider mite populations on specific crops.

The results of this research are in line with the results presented by (Agrawal *et al.*, 1999), who indicated that, when spider mites injure *Cucumis sativus*, cucurbitacin are released, which have a detrimental effect on the subsequent performance of spider-mite. It has been demonstrated that mite feeding can trigger the production of cucurbitacin in the cucumber, which can reduce the mite population by up to 40%. Najafabadi (2019) stated that in terms of the number of deposited eggs/female/day and the damaging rates, there are notable variations among cucumber cultivars, interactions between cultivars, and mite species.

The quantity of eggs laid by a female on a specific cucumber accession may be a good indicator of both the host's willingness to accept oviposition and the amount of nutrients or secondary metabolites it offers. Different genotypes of a plant have physiological and biochemical differences that can affect their nutritional value (primary metabolites) for herbivores, depending on whether they have experienced environmental stress or are genetically predisposed to it (Goncalves-Alvim *et al.*, 2004 and Zakir *et al.*, 2006).

Variations in the levels of secondary plant metabolites, particularly cucurbitacin-C, may also be to blame for the observed differences between cucumber cultivars in terms of damaging rates and the number of deposited eggs/female/days. The bitter-tasting dihaploid lines were demonstrated to be resistant, while all non-bitter dihaploid lines were highly susceptible (Balkema-Boomstra *et al.*, 2003). A female insect may either lay a small number of eggs of high quality or many eggs of low quality on a host plant of poor quality (Rossiter *et al.*, 1993).

Awmack and Leather (2002) concluded that both at the individual and population level, host plant quality has an impact on the fecundity of herbivorous insects.

The same host plant frequently affects the fecundity of sap-feeding and chewing herbivores differently, and plant quality (Especially defensive compounds) may also affect generalists and specialists differently. Puspitarini *et al.*

(2021) mentioned that mite development, oviposition period, adult lifespan and female fertility were significantly influenced by the host plant. Their results also show that protein rich in leaves significantly increases mite fertility. Long mite development time and low fecundity may be due to nutritional deficiencies and/or imbalances of poor host plant quality.

Table (1): Performance of the two-spotted spider mite *Tetranychus urticae* on squash, cucumber, and kidney bean.

Host plants	Fecundity (eggs per female per 5 days)	Percentage of survival at fifth day	Percentage of females that laid at least one egg
Kidney bean	26.5 ± 4.19 a	75	95
Squash	20.45 ± 2.07 a	85	95
Cucumber	2.85 ± 0.48 b	75	85
Static analysis	F= 20.514	X ² _{0.95} = 0.786	X ² _{0.95} = 1.745
	P= 0.0001	P= 0.675	P= 0.418
	df= 2 X ² _{tabulated} = 5.991		

Values are mean ± SE. Different letters in columns mean statistical difference (P < 0.05).

Table (2): Mean ± SE number of eggs laid on the three plants and acceptance of *Tetranychus urticae* females in the choice test after 24hrs.

Host plants	Choice test (Number of females per disc)	Choice test (Number of eggs per disc)
Squash	3.58 ± 0.71 a	21.33 ± 4.92 a
Kidney bean	2.83 ± 0.31 a	16.5 ± 1.66 ab
Cucumber	2.58 ± 0.52 a	7.33 ± 1.63 b
F (df=2)	0.929	5.118
P	0.417	0.02

Values are mean ± SE. Different letters in columns mean statistical difference (P < 0.05).

2. Bioassay:

Abamectin showed the highest toxicity against *T. urticae*, LC₅₀ values were 0.02, 0.031 and 0.06 mg/l on squash, cucumber, and kidney bean after 72 hrs. respectively, which was followed by fenpyroximate, LC₅₀ values recorded at 1.69, 1.75 and 3.35 mg/l on cucumber and kidney bean and squash, respectively. While clofentezine had the lowest toxicity, LC₅₀ values were 6.19, 8.34 and 14.26 mg/l on kidney bean, cucumber, and Squash respectively (Table 3). Spider mites' populations were susceptible to abamectin followed by fenpyroximate; however, they showed the lowest susceptibility to the mite growth regulator clofentezine (Figures 1-3).

Similar results were reported by Kumari *et al.* (2015), who stated that abamectin was the most toxic to the adults (LC₅₀ = 0.39 ppm) followed by fenpyroximate (5.67 ppm), spiromesifen (12.53 ppm), chlorfenapyr (32.24 ppm), propargite (77.05 ppm) and dicofol (146.65 ppm). Hexythiazox was the least toxic. Lin (2019) concluded that only bifentazate has high effectiveness against *T. urticae* female mite whereas other acaricides (Clofentezine) have marginally effective control over female mite. Zhao *et al.* (1996) stated that the adult *Rhizoglyphus echinopus* bulb mite was not toxic to clofentezine, but its eggs and larvae were poisoned. The effectiveness of clofentezine in eradicating *Tetranychus*

spp. has been reported by Hardman *et al.* (2007).

Interestingly, the susceptibility of *T. urticae* to the selected pesticides was significantly influenced by the host plants. It was obvious that the spider mites treated with abamectin were the most susceptible on squash plant discs, followed by the mites fed on cucumber and kidney bean leaves respectively. While, the spider mites treated with fenpyroximate, were the most susceptible on cucumber plant discs, followed by the mites fed on kidney bean and squash leaves respectively. In the case of the spider mites treated with Clofentezine were the most susceptible on kidney bean plant discs, followed by the mites fed on cucumber and squash leaves respectively. Many factors could contribute to this variation under field conditions; nevertheless, we conducted a bioassay on the leaf discs under completely controlled conditions which indicates that the host chemistry is one of the most important contributing factors for the difference in LC₅₀ values.

These results agree with Sakr *et al.* (2007) who found the existence of notable variations in the pesticides' effectiveness against *T. urticae* on six distinct hosts. The cucumber showed the highest abamectin percent reduction after three days (95.94%), followed by the tomato (94.44%), bean (93.24%), squash (86.48%), eggplant (85.13%), and kidney bean (75.67%). Contrarily, for fenpyroximate, a highly significant percent reduction was observed on the bean, at 97.26 percent, followed by 86.03 percent on squash, 57.53 percent on tomatoes, 53.42 percent on eggplant, 46.57 percent on cucumber, and 30.55 percent on kidney beans.

Ibrahim (2009) stated that after feeding on the respective hosts, the susceptibility of mites to all tested acaricides was significantly higher for mites on kidney bean than for mites on cucumber and eggplant. Abamectin's LC₅₀ values for mites feeding on kidney bean, cucumber, and eggplant plants were 12, 21, and 32 mg/L, respectively. On kidney bean, eggplant, and cucumber plants, respectively, dicofol showed LC₅₀ values of 35.9, 190.8, and 2790.4 mg/L. Islam (2019) concluded that the host plants play a very important role in the susceptibility of spider mites to acaricides.

Spider mites reared on country bean leaves were comparatively less susceptible to abamectin followed by mites reared on papaya and jute leaves respectively. On country bean, papaya, and jute leaf discs, the abamectin LC₅₀ values were 0.432, 0.342 and 0.324 mg/L respectively after 24 hrs. Osman (2019) stated that abamectin LC₅₀ recorded 0.003 and 0.001 ppm, while fenpyroximate LC₅₀ were 103.59 and 89.87 ppm against *T. urticae* on cotton and soybean host plant in laboratory study, respectively. EL-Kasser *et al.* (2015) reported that abamectin was more toxic against *T. urticae* than fenpyroximate recording different LC₅₀ values on three different host plants. Abamectin LC₅₀ values were 0.004, 0.006 and 0.006 ppm on castor bean, broad bean, and cucumber. While fenpyroximate LC₅₀ values showed 18.71, 16.48 and 21.25 ppm on castor bean, broad bean and cucumber respectively.

The recognized variations in acaricide dosages needed to control spider mite populations on various host plants appear to be caused by numerous factors, but it is important to note that some of these differences may also be caused by the

host plant. It is hypothesized that this variation in spider mite

susceptibility to pesticides may be related to their nutritional status. *T. urticae* may not feed or may not feed as much as spider mites on more well-known host plants, when presented with a novel host. As a result, mites on new host plants may initially exhibit decreased general esterase activity and change in pesticide susceptibility (Yang *et al.*, 2001). Dermauw *et al.* (2013) reported that the activity of spider mite detoxification enzymes may change when alternative hosts are consumed, and some acaricides may have less acute toxicity as a result.

In addition to possibly altering the general detoxification enzymes of spider mites, host plant allelochemicals may also do so. The two-spotted spider mite was introduced to eggplant and cucumber, where changes in enzyme levels occurred relatively more frequently. This may be because the two-spotted spider mite was feeding on an unusual host or because eggplant and cucumber naturally have lower nutritional quality than kidney beans. When faced with a new host, mites might not eat as much or not at all as they would on a host that is more familiar to them.

This could initially result in modifications to the overall esterase activity and associated modifications to the susceptibility to acaricides.

These modifications may significantly affect how well some acaricides work to control spider mites. To create better IPM programs on various hosts, a better understanding of the biochemical interactions between the two-spotted spider mite and the host plants is required (Ibrahim, 2009). When investigating integrated pest management (IPM) strategies for the two-spotted spider mite, it is crucial to consider the population dynamics and the host plant characteristics that differ in their suitability to the mite (Ibrahim *et al.*, 2008).

The findings demonstrated the importance of considering the plant host when evaluating and applying pesticide effectiveness. For a better understanding of the biochemical interactions between *T. urticae* and its host plants, which are affecting the toxicity of pesticides against the spider mite population. More research is necessary to develop IPM programs for the two-spotted spider mite on various hosts.

Table (3): Efficacy of abamectin, fenpyroximate and clofentezine against *Tetranychus urticae* females.

Treatment conc. mg/l	Crop	LC ₅₀	Lower limit	Upper Limit	LC ₉₀	Slope	X ² (Tabulated 7.8)	Toxicity index
Abamectin 1.8% EC	Squash	0.02	0.0033	0.046	0.585	0.871±0.169	1.25	100
	Cucumber	0.03	0.025	0.041	0.477	1.085±0.136	3.23	64.52
	Kidney bean	0.06	0.044	0.079	0.690	1.182±0.144	2.75	35.09
Fenpyroximate 5% SC	Cucumber	1.69	1.214	2.798	39.93	0.93±0.141	4.06	100
	Kidney bean	1.75	1.128	3.87	135.27	0.679±0.135	1.76	96.68
	Squash	3.35	2.366	5.701	65.73	0.991±0.133	6.61	50.51
Clofentezine 50% W/V	Kidney bean	6.19	4.431	9.768	265.499	0.785±0.135	0.81	100
	Cucumber	8.34	5.622	15.913	553.61	0.703±0.135	1.25	74.19
	Squash	14.26	10.639	21.604	261.715	1.101±0.129	1.99	43.42

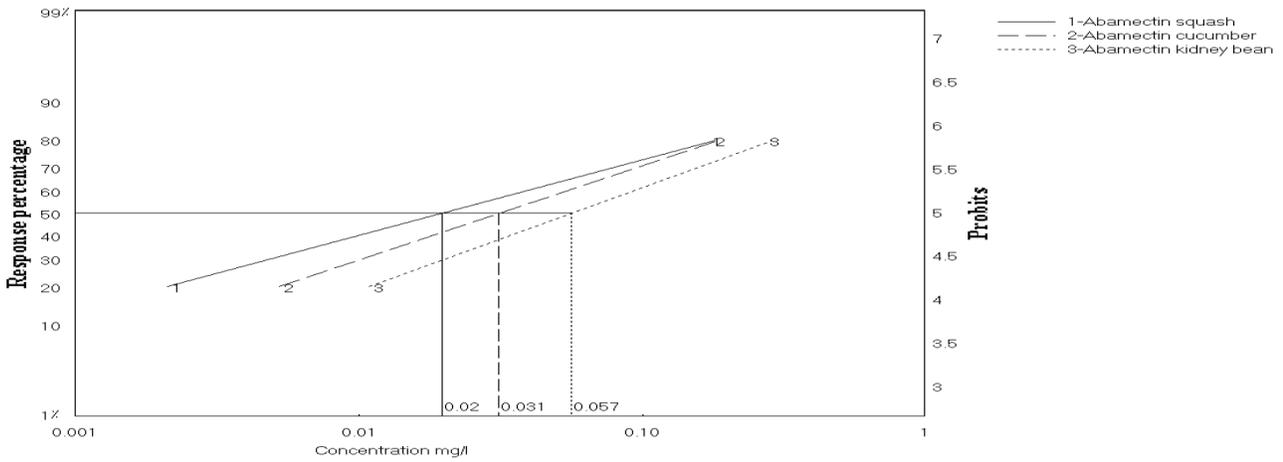


Figure (1): Toxicity lines of Abamectin 1.8% EC against *Tetranychus urticae* on three host plant squash, cucumber, and kidney bean.

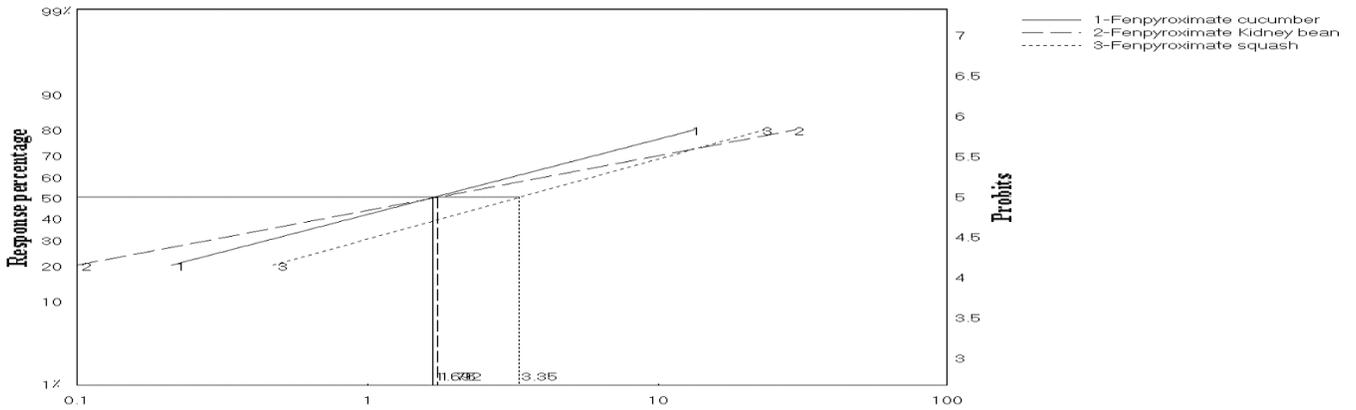


Figure (2): Toxicity lines of fenproprimate 5% SC against *Tetranychus urticae* on three host plant squash, cucumber, and kidney bean.

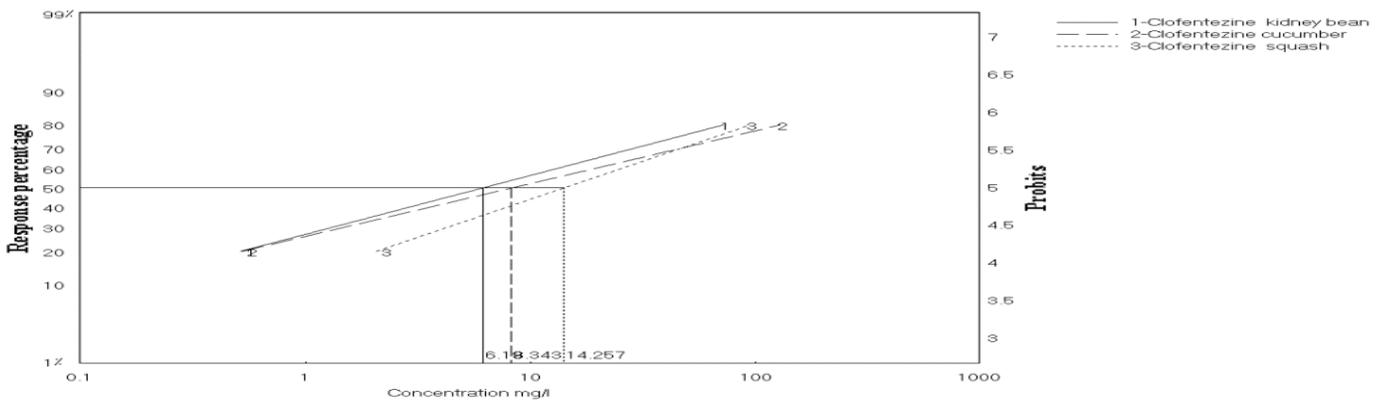


Figure (3): Toxicity lines of clofentezine 50% W/V against *Tetranychus urticae* on three host plant squash, cucumber, and kidney bean.

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