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Assessment of Acute Toxicity of Abamectin, Spinosad and Chlorpyrifos to *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) on Sweet Pepper by using Two Bioassay Techniques

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Abstract: The onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae), is one of the major greenhouse pest on sweet pepper in Iran. Lethal effects of abamectin, spinosad and chlorpyrifos were evaluated on three life stages (1st, 2nd larval instars and adult) by using two bioassay techniques, leaf dipping method and Thrips Insecticides Bioassay System (TIBS). All insecticides were significantly more toxic to first instar than 2nd instar larvae and adult but there were not significant difference between 2nd instar of larvae and adult. Comparison between two methods showed that abamectin was the most toxic insecticide in TIBS method and its lethal concentration values were significantly less than leaf-dipping method. Chlorpyrifos has the least toxicity in both methods and its toxicity in TIBS was higher than leaf dipping method. Spinosad was the most toxic among the tested insecticides in leaf dipping method and its toxicity was not significantly different in two methods. Results indicated that TIBS is an appropriate method for estimating spinosad toxicity. But this method can not be used for abamectin and chlorpyrifos toxicity evaluation.

Key words: Sweet pepper, *Thrips tabaci,* abamectin, spinosad, chlorpyrifos, leaf dipping method and TIBS

INTRODUCTION

Thrips sp. (Thysanoptera: Thripidae) are serious pests of ornamental, vegetable and fruit crops both in fields and greenhouses throughout the world (Tommasini and Maini, 1995). Onion thrips, *Thrips tabaci* Lindeman, is a serious insect pest of onion, other *Allium* sp. and green houses plants in many parts of the world (Lewis, 1997), including Iran (Yousefi and Abbasi, 2004) that can colonize crops from sea level up to an altitude of 2000 m above sea level (Rueda and Shelton, 1995; Stoll, 2000; Jean-Simon and Victor, 2005). Economic damage by onion thrips infestation is caused by both nymph and adult feeding on green leaf parts. They feed by piercing surface tissues and suck up the exuded cellular contents. The empty cells show silvery-white spots on the leaf surface, which are referred to as silver damage. Onion thrips can reduce total yields from 4 to 27%, depending on variety, but may reduce yields of colossal sized bulbs from 28 to 73% (Jensen and Simko,

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2001). In fact, in addition to the direct damage, thrips are known to be a vector of the Tomato Spotted Wilt Virus (TSWV) (Boonham *et al*., 2002), which is an economically important plant disease. This worldwide tospovirus infects several cultivated and wild plants including sweet pepper (Whitfield *et al*., 2005). The first assessed vector of TSWV was *T. tabaci* (Tedeschi *et al*., 2001; Whitfield *et al*., 2005).

The activity of a pesticide may be affected by the substrate upon which it is deposited (Cogburn, 1972; Studebaker and Kring, 2003). Jain and Yadav (1989) found that some insecticides persisted much longer when applied to a plastic substrate as compared with glass or painted wood. Potentially more realistic testing methods use treated excised leaves (Elzen and Elzen, 1999; Studebaker and Kring, 2003). These methods should provide a more realistic picture of actual toxicity from contact with residues on a natural substrate. Some factors which may affect actual toxicity are not addressed. Thus, assaying these factor affects is important in precise results. We still have limited information on bioassay methods that evaluate insecticide efficacy on thrips in Iran.

This test was conducted to evaluate the efficacy of three insecticides against on *T. tabaci* and to compare two bioassay methods: Thrips Insecticides Bioassay System (TIBS) and leaf-dipping method.

MATERIALS AND METHODS

This project was conducted from October 2007 to April 2009 in Entomological Laboratory of Department of Plant Protection, Shahid Chamran University, Ahvaz, Khuzestan Province, Iran.

Source of Test Insects

Onion thrips were collected from several commercial onion (*Allium cepa* L., cultivar, Azarshahr) fields in the Ahvaz area. Samples were sent to Safiabad International Agriculture Research Center for determination and were confirmed to be *Thrips tabaci* Lindeman. The insects were reared on potted plants of sweet pepper without exposure to insecticides under laboratory conditions at 26±1°C, 60 RH and 16 L: 8 D photoperiod. Five to ten adult thrips were introduced on potted plants after sowing and allowed to multiply. The potted plants were transferred into insectarium in order to prevent the egg laying by other insects and natural enemies. First and 2nd instar nymphs and adults were used for bioassay. The adults of per generation were transferred to fresh plants for egg laying and development of subsequent generations.

Insecticides

Insecticides tested were abamectin (Agrimec®1.8 EC) (Partonar, Tehran, Iran, http://www.partonaragro.com); chlorpyrifos (Dursban®, 40.8EC) (Ghazal chemistry, Babol, Iran, http://www.ghazalshimi.com) and spinosad (SpinTor 2®, 22.8 SC) (both from Dow AgroSciences, Indianapolis, U. S. A., http://dowagro.com/uk/products).

Thrips Insecticide Bioassay System (TIBS)

TIBS, a method developed by Rueda and Shelton (2003) was used. Thrips were collected from plants and put into a plastic 0.5 mL microcentrifuge tube previously treated with an insecticide. Thrips mortality was assessed after 24 h with the help of a dissecting stereomicroscope. Mortality was corrected using Abbotts' formula (1925).

The tube has a flexible plastic cap and on the inside of the cap is a small well into which 0.08 mL of a 10% sugar-water solution with food colorant is deposited. The solution was sealed into the well with a small piece of stretched parafilm through which the thrips can feed on the solution. The 10% sugar solution was used as the food source for thrips and it greatly prolongs thrips survival, whereas the food colorant was added to the solution to facilitate determining whether the parafilm membrane became broken and the solution contaminated the tube when the assay was read. The flexible cap only serves as a container for the solution and to seal the tube. The tube, but not the cap, is treated with an insecticide by filling the tube to its top with 0.75 mL of the insecticide solution (although the microcentrifuge tube is listed as a 0.50 mL tube, it can be hold 0.75 mL if filling to the top). After 4 h, the insecticide was poured out and the tube was allowed to dry overnight. For tests with TIBS, we selected five to six concentrations that we calculated, based on preliminary studies, would encompass a mortality range of 10-90%, plus an untreated control. We used five replicates of each concentration with 10 thrips per replicate (tube).

Leaf-Dipping Bioassay Method

Excised sweet pepper leaf discs of the same size were dipped for 30 sec in five concentrations of each insecticide and allowed to dry for an hour. The bases of small ventilated polythene petri dishes (50 mm diameter) were filled with agar gel (12 g L^{-1}, 5 mL) to maintain the turgidity of leaf. The leaf discs were placed on the agar with their adaxial surface downwards. Insects were collected from the pot culture pepper plant by using aspirator and placed on a black cloth. Using a fine hairbrush, twenty thrips were then transferred onto the treated leaf disc and set up was covered with a lid. Leaf discs were immersed in water alone served as control. The test was replicated thrice. Mortality was recorded 24 h after exposure to the insecticides. Mortality was corrected using Abbotts' formula (1925).

Data Analysis

The SAS program (Version 9.1) (SAS Institute, 2003) was used for probit analysis of dose-response data. To compare toxicity of the same insecticide in different bioassay methods, different life stages, as well as the toxicity of different chemicals with each other, the ratios of the LC_{50} values and their related 95% confidence limits were calculated (Robertson *et al*., 2007).

RESULTS

Lethal concentrations of the three insecticides for first instar, 2nd instar of larvae and adults of *T. tabaci* in the two bioassay methods were shown in Table 1, 2 and 3, respectively. In all cases, there were nonsignificant χ^2 values, which indicate a good fit of the data to the probit model. For first instar, all insecticides were significantly more toxic than 2nd instar of larvae and adults but there were not significant difference between 2nd instar of larvae and adults.

The trend of toxicity of abamectin and chlorpyrifos were similar in both methods. Abamectin was the most toxic insecticide in TIBS method and its lethal concentration values were significantly more than leaf-dipping method. Abamectin was 2.5 times more toxic to 1st instar onion thrips than spinosad and 161.5 times than chlorpyrifos in TIBS (Table 1). For 2nd instar of larvae, abamectin was 1.7 and 29 times more toxic than spinosad and chlorpyrifos, respectively (Table 2). Abamectin was 1.6 times more toxic to adults than spinosad and 27.6 times than chlorpyrifos (Table 3).

Table 1: Toxicity of the insecticides tested on first instar *T. tabaci* in dip-leaf method and TIBS

		TIBS				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	198	0.63±0.09	0.0039 (0.0015-0.008)	0.40 (0.15-2.03)	9.87
Spinosad	Spinosyns	220	0.92±0.20	0.0097 (0.0026-0.018)	0.24 (0.12-1.01)	9.11
Chlorpyrifos	Organophosphates	174	1.6±0.210	0.6300 (0.45-0.87)	3.98 (2.49-8-29)	9.85
		Dip-leaf				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	128	0.67±0.15	0.0053 (0.00061-0.016)	0.43 (0.15-3.11)	3.54
Spinosad	Spinosyns	109	0.61±0.22	0.0046 (0.00000012-0.32)	0.56 (0.16-10.9)	6.77
Chlorpyrifos	Organophosphates	112	3.33±0.71	5.5000 (4.14-7.49)	13.30 (9.3-28.6)	6.79

Table 2: Toxicity of the insecticides tested on second instar *T. tabaci* in dip-leaf method and TIBS

		TIBS				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	224	0.71±0.09	0.037 (0.02-0.07)	2.28 (0.83-10.8)	14.49
Spinosad	Spinosyns	257	1.32±0.17	0.064 (0.047-0.087)	0.59 (0.35-1.4)	19.93
Chlorpyrifos	Organophosphates	197	1.93±0.22	1.072 (0.82-1.4)	4.95 (3.38-8.65)	9.63
		Dip-leaf				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	140	1.02±0.14	0.990 (0.53-1.83)	17.70 (8.88-63)	13.48
Spinosad	Spinosyns	147	1.19±0.24	0.056 (0.019-0.1)	0.67 (0.37-1.9)	8.80
Chlorpyrifos	Organophosphates	137	2.39±0.50	9.80 (7.28-16.8)	33.70 (7.28-16.8)	4.60

Table 3: Toxicity of the insecticides tested on adult of *T. tabaci* in dip-leaf method and TIBS

		TIBS				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	217	0.78±0.09	0.030 (0.017-0.053)	1.30 (0.55-4.69)	16.60
Spinosad	Spinosyns	239	1.52±0.20	0.049 (0.037-0.065)	0.34 (0.22-0.72)	25.90
Chlorpyrifos	Organophosphates	185	1.98±0.23	0.830 (0.63-1.09)	3.67 (2.52-6.36)	10.07
		Dip-leaf				
Insecticide	Category	n	Slope±SE	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	χ^2-values
Abamectin	Avermectins	135	1.57±0.16	0.70 (0.39-1.25)	8.99 (4.27-28.8)	16.06
Spinosad	Spinosyns	146	1.01±0.20	0.059 (0.018-0.12)	1.09 (0.56-3.62)	6.26
Chlorpyrifos	Organophosphates	133	2.04±0.40	8.470 (6.15-14.4)	36.00 (19.3-137.8)	2.80

Chlorpyrifos was the least toxic in both methods and its toxicity in TIBS was higher than leaf-dipping method. The LC_{50} values in leaf-dipping method were 8.7, 9.1 and 10.2 times more than the TIBS for first instar, 2nd instar of larvae and adults of *T. tabaci*, respectively (Table 1-3). These differences in toxicity of chlorpyrifos were significant in the two bioassay methods.

Spinosad was the most toxic of the tested insecticides in leaf-dipping method and its toxicity was not significantly different in two methods. For dip-leaf method, the toxicity of spinosad to 1st instar of larvae was 1.2 times more than abamectin and 1195.6 times more than chlorpyrifos (Table 1). For 2nd instar of larvae, spinosad was 17.6 and 175 times more toxic than abamectin and chlorpyrifos respectively (Table 2). Spinosad was 11.9 times more toxic to adults than abamectin and 143.5 times than chlorpyrifos (Table 3).

Results indicated that TIBS is an appropriate method for estimating spinosad toxicity. But this method can't be used for abamectin and chlorpyrifos toxicity evaluation.

DISCUSSION

Difference of LC_{50} values between first instar and two other stages could be due to the large difference in the body size of them. LC_{50} values of insecticides could be affected by body size of insects in bioassay experiments (Jahromi, 2008). Shelton *et al.* (2003) showed that there was no significant difference in susceptibility to spinosad, between 2nd larval instar and adults of *T. tabaci.*

Microcentrifuge tube is an inert substance; it is not likely that the pesticide deposits would be altered or somehow bound to the substrate, leaving them free for uptake by an insect therefore abamectin was the most toxic pesticide in TIBS method. Another explanation offered is that abamectin infiltrate into plant rapidly and plant maybe somehow altered or bound the pesticide deposits making them less available for uptake by the test insects. Wislocki *et al.* (1989) explained that abamectin is subject to rapid degradation when present as on treated surfaces of plants.

Chlorpyrifos is a fumigate (has higher vapor pressure) toxicant (Jahromi, 2008) that this characteristic allow it to evaporate and inundate the closed space of microcentrifuge. Hence this situation intensifies toxicity and cause higher mortality in relation to dip-leaf method which bioassay materials are ventilated.

Bioassay substrate and formulation of insecticides have high effects on toxicity. Spinosad used in this study, has been formulated in suspension, while abamectin and chlorpyrifos were formulated in emulsion. This may be due to tendency of emulsion ingredients to absorb in the waxy layers of the leaves. On the other hand, the addition of formulating materials intended to increase adhesion of particle can, above a certain limit, bind them too firmly to leaves and thus reduce toxicity. Busvine (1971) described that insecticides applied to leaves in emulsions have been found less effective than similar deposits in the form of suspensions.

Cloyd and Sadof (2000) reported that spinosad and acephate were effective at controlling thrips on greenhouse-grown plants.

Comparison between organophosphates (dicrotophos and methamidophos), spinosyn (spinosad) and neonicotinoids (thiamethoxam and imidacloprid) for *Frankliniella* sp. on cotton with two laboratory bioassay techniques (Adult vial technique and spray table bioassays) showed that spinosad is most toxic insecticide for these pests in both methods (Lopez *et al.*, 2008).

Shelton *et al.* (2003, 2006) used TIBS to evaluate the susceptibility of onion thrips, *T. tabaci* to lambda-cyhalothrin and reported that it could be instrumental for developing resistance management strategy for onion thrips. Deepa *et al.* (2005) assessed resistance of *T. tabaci* to lambda-cyhalothrin, imidacloprid, thiamethoxam and dimethoate by using dip-leaf method and TIBS. They concluded that both methods had similar results for used insecticides. Unsuitability of TIBS to abamectin and chlorpyrifos in this study may be due to different characteristics between various insecticides. Thus preliminary tests are necessary for each insecticide before TIBS application.

It is apparent that merely evaluating mortality of pesticides on pest insects by only one method does not give an accurate depiction on how those pesticides would fit into IPM programs. This study concurs with Studebaker and Kring (2003) in that multiple testing methods should be used in evaluating pesticide effects.

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