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Toxicity of Two Potential Bio-insecticides Against Moveable Stages of *Tetranychus urticae* Koch

[1]Gamal A.El Kady, [1]H.M. El Sharabasy, [1]M.F. Mahmoud and [2]I.M. Bahgat

[1]Plant Protection Department, Faculty of Agriculture, Ismailia and Faculty of Education.
[2]Port Said, Suez Canal University, Egypt.

Abstract: Two Potential bio-insecticides (Spinetoram 12% and Vertimec 1.8%) were tested against lab strain of the moveable stages of *Tetranychus urticae* Koch. Laboratory bioassays were conducted to evaluate the toxicity of bio-insecticides after 24, 48 and 72 h of treatment. Results showed a significant correlation between insecticides concentrations and mortality over 3 days period ($P \leq 0.05$). On the other hand, the LC value of vertimec was less than spinetoram at different stages of mites after 24 h and 48 h and reversed after 7h. The toxicity index values show such superior efficiency of spinetoram at LC_{50} (100%) for immature, male and female, followed by vertimec 78%, 74% and 76% after 72 h of treatment. As for slope values, spinetoram had the steepest toxicity line whereas vertimec had the flattest one at the three times of exposure. Field bioassays were conducted to evaluate the efficacy of spinetoram and vertimec on eggplant till 19 days of sprays against different stages of *T. urticae* (egg, immature and adult stage). The experiment carried out in greenhouse in farm of faculty of Agriculture, Suez Canal University. All doses of spinetoram under field conditions caused reduction of infestations of different stages of *T. urticae* but the best reduction was (100% after 13 and 19 days of egg treatment, 96.7% after 5 days for adult and 87.5 after 11 days for immature stages) at dose 1 ml solution /liter water.

Keywords: *Tetranychus urticae*, spinetoram, vertimec, toxicity

INTRODUCTION

Tetranychus urticae Koch (Acri: Tetranychidae) is one of the most important mites owing to its wide distribution all over the world on different plant hosts. It is also one of the most destructive pests of many crops. Their economic importance due to their feeding on leaves, buds and fruits, causing direct injury to plants, while others phytophagous mites may transmit plant diseases, causing great damage to agricultural crops. Mites population increase in different parts of Egypt and with wide range of infesting most agriculture plant. Chemical control of mites has been extensively practiced in Egypt for control mites population. The two-spotted mite, *T. urticae* Koch is an important one in a global distribution. Its phytophagous nature, high reproductive potential and short life cycle rapid resistance development to many acaricides often after a few applications[6,7,24]. Failure in the chemical control of *T. urticae* resistance have been reported in several countries for compounds, such as organophosphates[20], dicofol[12] organotins[8,14] hexythiazox[15], clofentezine[16]; fenpyroximate[24,21] and abamectin[3].

On the other hand, the great reliance on chemical pesticides had its serious drawbacks, manifested in resistance problems and high residue levels in food products (fruits, vegetables, grains and seeds) that may hinder its marketing. Such undesirable consequences have lead to alienating effects on the irrational use of chemical agents; hence foster other approaches that capitalize on safe natural products.

The present study demonstrate the efficiency of two products of bio-insecticides against the lab. strain of moveable stage of *Tetranychus urticae* Koch. The products were Spinetoram 12% and Vertimec 1.8 EC. Moreover evaluate the effect of Spinetoram under field condition against different stage of *T. urticae* infested eggplant cultivated under greenhouse at farm of faculty of Agriculture, Suez Canal Univarsity.

MATERIALS AND METHODS

Mites Maintenance: Two spotted spider mite *Tetranychus urticae* Koch were reared on mulberry *Morus alba* leaves in an incubator maintained at 27±1∘C and 60±5% R.H. with 16:8 (L:D) photoperiod.

Bio-insecticide Used: Spinetoram is a new member of the spinosyn class of insect management tools developed by Dow Agro Sciences. It is derived from fermentation of *Saccharopolyspora spinosa* as are other

Corresponding Author: M.F. Mahmoud, Plant Protection Department, Faculty of Agriculture, Ismailia[1] and Faculty of Education, Port Said[2], Suez Canal University, Egypt.
E-mail: mfmfmousa@hotmail.com

spinosyns, but fermentation is followed by chemical modification to create the unique active ingredient in spinetoram.

Vertimec or Abamectin is an Acaricide/ Insecticide that is used for the control of mites and insects on number of crops. Molecular formula: C_{48} H_{72} O_{14} (avermectin B_{1a} + C_{47} H_{70} O_{14} (avermectin B_{1b})

Laboratory Bioassay: Serial concentration of Spinetoram 12% EC (2.5, 5, 10, 15, 20 and 25 ppm) and Vertimec 1.8 EC (5, 7.5, 10, 12.5, 15 and 20 ppm) were prepared. Leaf disks (22 mm diam.) were cut and dipped in the different concentrations for 30 second and left to dry. The leaf disk was kept fresh by placing it on a piece of wet cotton in a Petri dish. Approximately 30 moveable stage of *T. urticae* (immature, male and female) were transferred by means of a fine soft brush to the each disk. All Petri dishes were incubated at 27±1∘C and 60±5% R.H and three dishes were used for each treatment.

The cumulative mortality data were corrected by Abbott's formula[1] and subjected to probit analysis according to Finney[13]. The values of LC_{10}, LC_{20}, LC_{50}, LC_{90} and slope of the two bio-insecticides at different stages of mites were calculated.

Field Bioassays: Three doses of spinetoram (1, 0.83 and 0.67 ml of solution added to 1 liter of water i.e., 120, 100 and 80 ppm) were used against *T. urticae* in eggplant. Water with spinetoram dose recovered 16 m^2 of eggplant cultivated under greenhouse in the farm of Faculty of Agriculture, Suez Canal University from 26 of March till 17 of April 2007. The leaf samples had been taken regularly in the next day of spray and each two days till 19 days. A sample of 10 leaves was picked up from each replicate; the leaves were examined by using a stereomicroscope. The live moving stages were counted on each leaf. The average number of mites in treated and untreated leaves was calculated and the reduction of infestation was calculated by the equation of Henerson and Tilton[17].

Statistical Analysis: The results under laboratory condition were subjected to statistical analysis to evaluate the relative efficiency of the tested pesticides against immature, male and female stage. Abbot's formula[1] was used to get corrections for natural mortality. The toxicity lines were statistically analyzed according to the Finney[13]. Toxicity index = LC_{50} of Spinetoram / LC_{50} of Vertimec Henderson and Tilton equation[17], Reduction of infestation = (1- AxB / CxD X100)

Where the average number in untreated plot; A: before - C: after treatment, where the average number in treated plot; B: after - D: before treatment.

RESULTS AND DISCUSSIONS

Data presented in Table 1, 2 and 3 show that the effectiveness of Spinetoram and Vertimec against moveable stages of the two–spotted spider mites *T. urticae* Koch after 24, 48 and 72h of treatment at (LC_{10}, LC_{20}, LC_{50}, LC_{90} and slope).

Results indicated that the female stage of *T. urticae* was more susceptible than male and immature stages at different rates of LC and the both bio-insecticides. LC_{50} value of Vertimec in female and male was low compare with LC_{50} in Spinetoram after 24h and 48h but after 72h of treatment LC_{50} of Vertimec was higher than Spinetoram (and 5.26, 5.09 and 3.94, 3.89) for male and female respectively. The LC_{90} showed high value in Spinetoram compare with Vertimec in all time of test and decreased with time of the test.

As for slope values, spinetoram had the steepest toxicity line whereas vertimec had the flattest one at the three times of exposure.

The comparative toxicity after 72h of treatment indicated that the Spinetoram was more sensitive than Vertimec; that is differed after 24h and 48h of treatment.

Ali[11] reported that the concentration of spinosad produced 50% of mortality (LC_{50}) for egg, larva, nymph, female and male adults was 11.681, 6.850, 3.447, 12.404 and 3.944 ppm respectively. It's well Known that spinosad is highly toxic to mites compared with other aquatic organisms such as rainbow trout fish (30 ppm), carp fish (5 ppm), Daphnia magna (9.7 ppm) and grass shrimp (9.8 ppm) as reported by smith and Grothe[23], Weinberg *et al.*[25] and Bert *et al.*[4]. Spinosad still represents a reduced acute risk to fish species. Also spinosad is 1.000 to 10.000 time less toxic to fish than many synthetic insecticides such as pyrethroids. Moreover, spinosad is only slightly to moderately toxic to most aquatic invertebrates and is at least 2 to 5 times less toxic than most synthetic alternatives[5,18,19,26]. Spinosad demonstrates low mammalian and environmental toxicity with reduced risk to humans and other forms of wildlife comparable to traditional biological insecticides[4]. The low toxicity of spinosad toward beneficial allows it to be incorporated into most integrated pest management (IPM) programs that heavily rely on predators and parasitoids.

Field Application: Three rats of Spinetoram (1, 0.83 and 0.67 ml of solution added to 1 liter of water i.e., 120, 100 and 80 ppm) were used against *T. urticae* on eggplant cultivated under greenhouse in farm of Faculty of Agriculture. Data presented in Fig. 1 indicated that the bio-insecticide Spinetoram under field condition caused reduction of infestation toward all stages tested of *T. urticae*. The highest reduction of infestation was

Table 1: Toxicity of two potential bio-insecticides against moveable stages of *Tetranychus urticae* after 24 hours of treatement

Toxicity parameter	Spinetoram 12%			Vertimec 1.8%		
	Immature	Male	Female	Immature	Male	Female
LC_{10}	1.460 (0.61-3.53)	0.937 (0.24-3.65)	1.674 (0.85-3.29)	2.513 (1.01-6.27)	2.560 (1.35-4.85)	1.739 (0.69-4.40)
LC_{20}	3.026 (1.65-5.56)	2.460 (1.02-5.94)	3.023 (1.84-4.96)	4.635 (2.61-8.23)	3.970 (2.51-6.29)	2.925 (1.49-5.75)
LC_{50}	12.317 (8.9-17.05)	15.789 (9.86-25.27)	9.44 (7.25-12.3)	15.065 (10.87-20.88)	9.238 (7.58-11.26)	7.966 (6.14-10.34)
LC_{90}	103.911 (38.03-283.9)	265.989 (44.7-1581.5)	53.252 (28.42-99.8)	90.314 (24.92-327.3)	33.333 (19.47-57.67)	36.496 (18.55-71.82)
Slope	5.231	8.948	3.828	4.013	2.706	3.258
Alpha (0.05) CHI2 (X^2)	2.73<9.5(4,0.05)	1.79<9.5(4,0.05)	1.62<9.5(4,0.05)	0.18<9.5(4,0.05)	0.44<9.5(4,0.05)	0.17<9.5(4,0.05)

Table 2: Toxicity of two potential bio-insecticides against moveable stages of *Tetranychus urticae* after 48 hours of treatment

Toxicity parameter	Spinetoram 12%			Vertimec 1.8%		
	Immature	Male	Female	Immature	Male	Female
LC_{10}	0.858 (0.3-2.48)	0.457 (0.08-2.57)	0.457 (0.08-2.57)	2.530 (1.41-4.54)	2.142 (1.1-4.17)	1.505 (0.6-3.8)
LC_{20}	1.813 (0.84-3.92)	1.251 (0.38-4.17)	1.251 (0.38-4.17)	3.760 (2.43-5.81)	3.224 (1.95-5.34)	2.409 (1.19-4.89)
LC_{50}	7.662 (5.45-10.78)	8.682 (5.61-13.43)	8.682 (5.61-13.43)	8.065 (6.62-9.83)	7.09 (5.62-8.94)	5.957 (4.32-37.62)
LC_{90}	68.450 (28.5-164.2)	164.808 (33.7-805.4)	164.808 (33.7-805.4)	25.711 (17.01-38.87)	23.473 (15.7-35.1)	23.581 (14.78-37.62)
Slope	5.469	9.815	9.815	2.459	2.532	2.908
Alpha (0.05) CHI2 (X^2)	3.78<9.5(4,0.05)	2.19<9.5(4,0.05)	2.19<9.5(4,0.05)	2.43<9.5(4,0.05)	1.03<9.5(4,0.05)	0.52<9.5(4,0.05)

Table 3: Toxicity of two Potential bio-insecticides against moveable stages of *Tetranychus urticae* after 72 hours of treatment

Toxicity parameter	Spinetoram 12%			Vertimec 1.8%		
	Immature	Male	Female	Immature	Male	Female
LC_{10}	0.613 (0.2-1.87)	0.249 (0.04-1.7)	0.833 (0.38-1.82)	1.885 (0.92-3.87)	1.671 (0.77-3.61)	1.876 (0.97-3.62)
LC_{20}	1.240 (0.53-2.90)	0.640 (0.15-2.7)	1.410 (0.76-2.62)	2.825 (1.62-4.92)	2.473 (1.35-4.54)	2.640 (1.56-4.46)
LC_{50}	4.825 (3.23-7.21)	3.944 (2.16-7.21)	3.89 (2.76-5.49)	6.156 (4.71-8.05)	5.263 (3.85-7.2)	5.094 (3.82-6.79)
LC_{90}	37.999 (19.6-73.7)	62.545 (21.2-184.7)	18.144 (12.34-26.7)	20.104 (14.01-28.86)	16.576 (12.14-22.6)	13.829 (10.8-17.7)
Slope	4 .959	8.536	3.305	2.505	2.436	2.170
Alpha (0.05) CHI2 (X^2)	6.0<9.5(4,0.05)	3.35<9.5(4,0.05)	4.76<9.5(4,0.05)	2.0<9.5(4,0.05)	2.03<9.5(4,0.05)	3.28<9.5(4,0.05)

100% after 13 and 19 days of egg treatment, 96.7% after 5 days for adult and 87.5 after 11 days for immature stages at dose 1 ml solution /liter water.

Baranowski[2] stated that avermectin is a new product for the control of harmful spider mites (*Tetranychus urticae*) and leafminers (*Liriomyza* spp.) on ornamental crops. It is a natural product produced

Table 4: Comparative toxicity of bio-insecticide between Spinetoram and Vertimec against moveable stages of *T. urticae*

Days after treatments	Toxicity index*		
	Immature	Male	Female
24 h	0.818	1.709	1.184
48 h	1.053	1.225	1.457
72 h	0.784	0.749	0.764

Toxicity index = LC_{50} of Spinetoram / LC_{50} of Vertimec

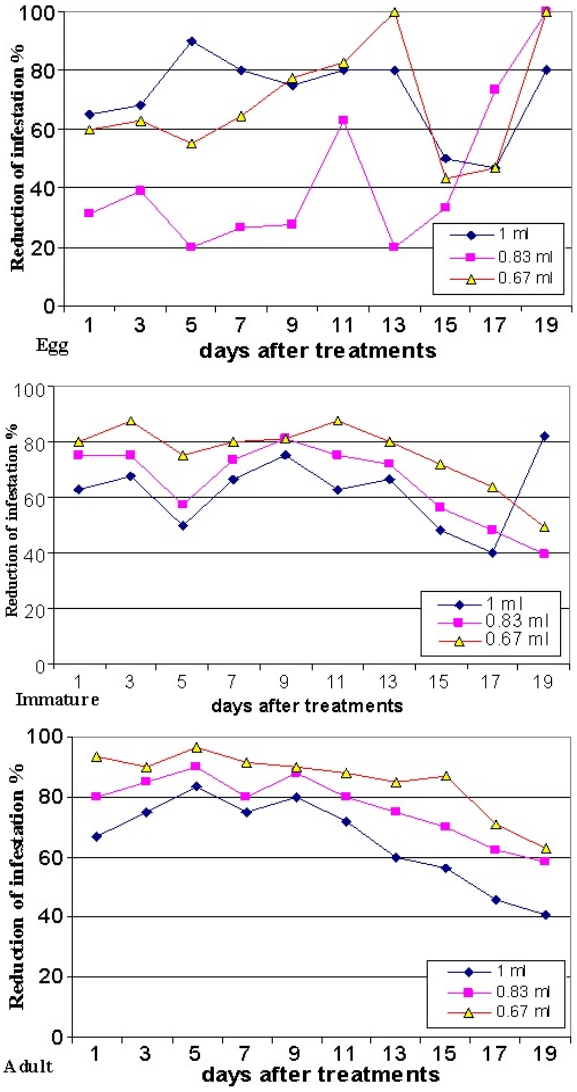

Fig. 1: Efficacy of spinetoram on reduction of infestation of T. urticae under field condition

by the soil microorganism *Streptomyces avermicis*. Avermectin has a unique mode of action. It is chemically unrelated to any other miticide or insecticide. Avermectin is non phytotoxic at the recommended dose on virtually all varieties tested. Avermectin leaves no visible residue, but provides a reservoir of long-lasting activity within the leaf. It is not considered disruptive to natural predators or beneficial insects.

EL-Adawy *et al.*[9] tested eleven acaricides from different chemical groups and the entomopathogenic fungus; *Beauveria bassiana* against the two spotted spider mite *Tetranychus urticae* infesting cucumber under plastic house conditions. Generally results, chlorfenapyr, abamactin, fenpropathrin, azocyclotin, propagate (Acargite), propagate (Comite), fenpyroximate and fenazaquim reduced the infestation to (71.25- 80.38%) whereas hexythiazox, bromopropylate and ethion reduced it to (61.25-62.87%). *Beauveria bassiana* resulted in 80.86% percentage reduction in the mite population.

Santos *et al.*[22] evaluated the practicality and efficiency of chlorfenapyr and abamectin for the control of *Polyphagotarsonemus latus* and *Tetranchus urticae* on cotton in Miguelopolis, Sao Paulo, Brazil, during Junary-February 1997. Chlorfenapyr was applied at 120, 240, 300 and 360 g a.i./ha and abamectin at 9 g a.i./ha plus 0.25% natural oil. Chlorfenapyr at 300 g.a.i./ha is recommended for the control of both mites on cotton and abamectin plus natural oil was also effective. EL-Adawy *et al*[10] mentioned that Kelthane was the most effective compound exhibited 86.93% reduction followed by bioacaricides Challenger 80.72% > Fabcomic 79.32% > Milbenkock 79.14% > Biofly 78.64% > Medmic 78.62% > Chemelite 77.38% > Biosect 75.62% redction in the two spotted spider mite population.

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