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Effects of Abamectin on Two Spotted Spider Mite and *Trichogramma brassicae* and Efficacy of Its Residual Effects Applied on the Bean Plants

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For evaluating lethal effects of abamectin on *T. urticae* leaf dipping method was used. Then *T. brassicae* adults were treated with LC_{10} and LC_{50} values obtained from probit analysis of the mite bioassay results using toxicity bioassay with fresh residue method. For evaluating residual effects of abamectin, bean plants were sprayed with twentieth of recommended field dose of abamectin (equal to 25 ppm of trade product). Leaf pieces were prepared from treated plants in different time intervals and the adult mites were transferred on them. Mortality was recorded after 24 h. Probit analysis of lethal experiment against *T. urticae* revealed 0.05, 0.417 and 3.26 ppm from trade product as LC_{10}, LC_{50} and LC_{90} values, respectively. Results of residual effects experiment showed that mite mortality was 100% in 1 day after plant spraying which decreased to 55.62% in 21 days after spraying. LC_{10} and LC_{50} values of abamectin tended to 53.87 and 72.57% mortality of *T. brassicae,* respectively.

Keywords: Probit analysis, recommended field dose, *Tetranychus urticae*.

Two-spotted spider mite, *Tetranychus urticae* Koch, 1836 is one of the important pests on many crop, greenhouse and garden products (Jeppson et al., 1975; Hoy, 2011). Two-spotted spider mite is the most important species from the family Tetranychidae with 1200 described species. *T. urticae* is able to create multiple generations (12 to 25 generations) and adapt to new climates quickly. It also has a broad host range, where more than 960 species host plants has been reported (Bolland et al., 1998). Its short life cycle, haploid-diploid sex-determination and high fecundity leading to the rapid development of resistance to the pesticides (Van Leeuwen et al., 2010).

Abamectin is the major component of avermectins derived from *Streptomyces avermitilis*. Abamectin is used as an insecticide and acaricide component in different parts of the world, including America, Europe and Asia (Clark et al., 1995; Sato et al., 2005) however, resistance of *T. urticae* against abamectin has been reported in some crops (Khajehali et al., 2011; Monteiro et al., 2015).

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Moghan region (39° 41' N 47° 32' E, with 40-50 m above from sea level) is one of the most important agricultural regions in Iran. Abamectin is just recently used for *T. urticae* control for most crops including maize and soybean in the region; therefore apparently there was not increased selective pressure on Moghan *T. urticae* population. On the other hand, *Trichogramma* sp. (Hymenoptera; Trichogrammatidae) is one of the most important and valuable biocontrol agents against lepidopteran pests in different crops in the world (Sumer et al., 2009). *Trichogramma brassicae* Bezdenco is the most important species in Iran and this species is active and efficient species in Moghan region which release as Tricho cards annually (Ebrahimi et al., 1998). Since abamectin has some contact toxicity on insects, awareness of abamectin used against *T. urticae* effects on *T. brassicae* is necessary for improved integrated pest management programs. The main goal of this study is evaluating of the susceptibility of the mite population against abamectin, and then assessing the obtained LC_{50} and LC_{10} values of abamectin on mortality of *T. brassicae*. Also, persistence of abamectin lethal effects against the mite on the bean plants was evaluated.

Materials and Methods

Two-spotted spider mite colony

The initial population of two-spotted spider mites includes different life stages was collected from soybean fields in Ardabil Agricultural Research Station in Moghan region and transferred to the laboratory of Plant Protection Research Department. The mites were reared on bean plants (*Phaseolus vulgaris* Linnaeus, 1753) under laboratory conditions at 25±1 °C and relative humidity of 70 ± 10% and photoperiod 16:8 (L:D). Species identification based on male and female mites was carried out and *T. urticae* was identified.

Mite synchronization

To synchronize the adult stage for bioassay experiments 10–12 adult *T. urticae* were transferred on a bean leaf piece (2 cm × 2 cm) on water soaked cotton in a plastic Petri dish (6 cm diameter) with ventilated lid. The adult mites were removed after 24 h. The Petri dishes were incubated at 25 ± 1 °C and relative humidity of 70 ± 10% and photoperiod 16:8 (L:D) to allow the eggs to hatch and develop into adults. The water soaked cotton was wetted before drying.

Bioassays
Chemicals

Abamectin (EC 1.8%) was purchased from Golsam Gorgan Chemicals (Gorgan, Iran).

T. urticae *lethal bioassay*

Preliminary dose-response experiments were carried out for determining the concentration range making about 20 to 80 percentage mortality. Serial dilutions as ppm of the trade product were prepared using distilled water. Leaf immersion method was used for bioassay. In brief, bean leaf pieces (2 cm × 2 cm) were prepared and immersed in desired abamectin concentrations (0.15 to 2.5 ppm from trade product) for 10 s. Distilled water was used as control. Then air dried leaf pieces were transferred into the experimental units, individually. The experiment units were composed of a plastic Petri dish (6 cm diameter) with ventilated lid which lined with water soaked cotton. Margins of the leaf piece were limited with a fine layer of tissue paper to prevent the mite from leaving the leaf piece. Twenty synchronized adult mites were transferred on each leaf piece. Experimental units were incubated at 25 ± 1 °C and relative humidity of 70 ± 10% and photoperiod 16:8 (L:D). Mortality was recorded 24 h post treatment. The experiment was replicated three times. The mortality percentages were corrected for the mortality of the control (Abbott, 1925).

Residual acaricide efficacy

For evaluating the persistence of lethal effects of the abamectin on bean plants against *T. urticae*, bean plants were sprayed with concentration of twentieth of recommended field dose of abamectin (equal to 25 ppm of trade product). Leaf pieces (2 cm × 2 cm) were prepared from treated plants in different time intervals including 1, 3, 7, 10 and 21 days after treatments. The experimental unit and incubation conditions were the same described before. Twenty adult mites were transferred on each of the leaf pieces in experimental units and their mortality was recorded 24 h post transfer. The experiment was repeated three times and distilled water was used as control.

Residual acute toxicity on T. brassicae *adults*

Lethal effects of contact toxicity of the LC_{50} and LC_{10} values of abamectin calculated from *T. urticae* bioassay were evaluated against *T. brassicae* adults. Residual bioassay using glass Petri dishes (9 cm in diameter) were performed. In brief, bottom and lid of the Petri dishes were treated with 1 ml of the desired abamectin concentrations. After the Petri dishes were dried, 30–40 adult parasitoids (2–4 days old) were released in each Petri dish for 24 h then the mortality was recorded and the experiment was replicated three times for each insecticide concentration. Incubation condition was similar with mite bioassay. The mortality percentages were corrected for the mortality of the control (Abbott, 1925).

Statistics

Lethal experimental data were transformed into square root of (x + 1) before analysis. Probit analysis, analysis of variance and mean evaluation by Duncan's multiple-range test were carried out using SAS software (SAS Institute, 2004). Diagrams and SE calcu-

lations were performed using Excel software. A significance level of $\alpha = 0.05$ was used. LC_{10}, LC_{50} and LC_{90} values were computed by Probit analysis using SAS software (SAS Institute, 2004).

Results and Discussion

T. urticae *lethal bioassay*

Bioassay results indicate the effectiveness of abamectin against *T. urticae* population. LC_{10}, LC_{50} and LC_{90} values and the correlation between abamectin concentrations and the mite mortality were presented in Table 1 and Figure 1. Regression analysis of the logarithm of abamectin concentrations and probit of the mite mortality revealed a significant relationship between them ($R^2 = 0.95$) (Table 1 and Fig. 1.).

Table 1

LC_{10}, LC_{50} and LC_{90} values for abamectin against *Tetranychus urticae*

Chi-square (df, P value)	LC_{10} (95% CL)		LC_{50} (95% CL[c])		LC_{90} (95% CL)		N
	TP[a]	AI[b]	TP	AI	TP	AI	
3.50 (3, 0.32)	0.05 (0.02–0.09)	0.001 (0.000–0.002)	0.417 (0.314–0.533)	0.010 (0.007–0.012)	3.26 (2.12–6.55)	0.066 (0.044–0.128)	360

[a] LC values are based on ppm of trade product
[b] LC values are based on ppm of active ingredient
[c] Confidence limits

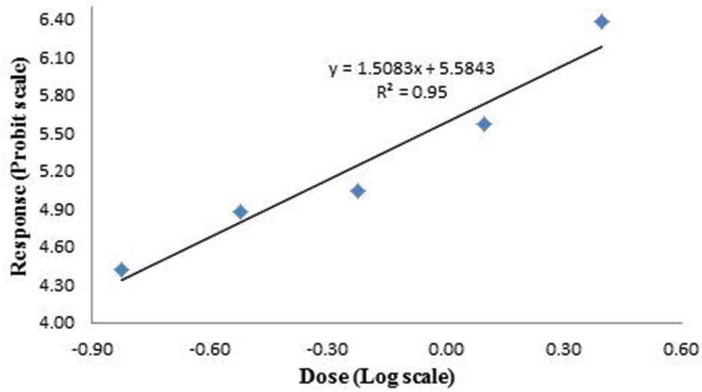

Fig. 1. Mortality (probit-transformed) of *Tetranychus urticae* adults treated with different doses of abamectin

Low values of LC_{10}, LC_{50} and LC_{90} represented the relatively high susceptibility of the studied mite population to abamectin. Lagziri and Elamrani (2009) reported 54% and 100% mortality of *T. urticae* when 2 and 9 ppm of abamectin trade product were used, respectively. In present study 50% mite mortality was achieved with using 0.417 ppm of trade product (0.010 ppm of active ingredient) of abamectin which is about 8.4 times lower than dose used by Lagziri and Elamrani (2009). Vassiliou and Kitsis (2013) reported 0.02 (0.02 to 0.03) ppm of active ingredient of abamectin as LC_{50} value for GSS population of *T. urticae* which was used as a susceptible population. This LC_{50} value was 0.010 (0.007 to 0.012) ppm for present study. It can be concluded that mite population used in present study is sensitive to abamectin. However, despite similar experimental methods, factors such as the details of the test conditions and acaricide formulation can also be involved.

Residual acaricide efficacy

Efficacy of residual abamectin on bean plant was variable for different time points. Analysis of variance revealed significant differences between mortality of *T. urticae* treated with abamectin in different time intervals post spraying ($F = 12.69$; $df = 5, 10$; $P < 0.0005$; Table 2). Abamectin showed the highest efficacy one and three days after spraying while it was lowest in 21 days after spraying. However, rate of efficacy reduc-

Table 2

Mean comparison for mortality percentage of *Tetranychus urticae* treated with abamectin in different time intervals after spraying

	Abamectin (25 ppm TP[a] equal to 0.45 ppm AI[b])					
Days after spraying	1	3	7	10	14	21
Mean of mortality	100[a]	100[a]	81.67[b]	81.67[b]	67.92[bc]	55.63[c]

[a] Trade product
[b] Active ingredient
Note: The data were analyzed using ANOVA and Duncan's multiple-range tests with a specified significance level of $P < 0.05$. No significant differences are present between the values marked with similar superscripts ($P < 0.05$)

tion was slow so that abamectin caused 55.63% mite mortality 21 days after spraying. In overall, the results were agreed with the results of other similar studies including Duchovskienė (2007) and Lagziri and Elamrani (2009). Duchovskienė (2007) reported 72.40% mortality of *T. urticae* 14 days after spraying 9 ppm of abamectin active ingredient; this amount was 54% for Lagziri and Elamrani (2009) study. However, in the present study 0.45 ppm of abamectin active ingredient caused 67.92% mite mortality 14 days after spraying. High efficacy of the relatively low concentration of abamectin revealed that the investigated mite population is highly susceptible to this pesticide.

Based on Lasota and Dybas (1991) abamectin caused 85–96% of *T. urticae* mortality on cotton plants up to 49 days after spraying. However, Cloyd et al. (2009) reported

inefficacy of abamectin residue on cotton plants. According to sensitivity of abamectin to UV degradation Wislocki et al. (1989) and influencing from plant age and species (Lasota and Dybas, 1991) such variability of the results are predictable. However, abamectin be absorbed by the plants some hours after spraying and make appropriate control of *T. urticae* (Putter et al. 1981; Lasota and Dybas, 1991).

Residual acute toxicity on T. brassicae *adults*

The mortality caused with LC_{10} and LC_{50} values used in bioassay showed significant differences in compared with control ($F = 196.35$; $df = 2, 4$; $P < 0.0001$). Analysis of variance revealed significant differences between mortality of *T. brassicae* treated with LC_{10} and LC_{50} values of abamectin (Table 3).

Table 3

Mean comparison for mortality percentage of *Trichogramma brassicae* treated with abamectin concentrations and control

Abamectin Concentrations (ppm of active ingredient)	Mean
0.001	72.570a
0.010	53.873b
control	4.180c

Note: The data were analysed using ANOVA and Duncan's multiple-range tests with a specified significance level of $P < 0.05$. No significant differences are present between the values marked with similar letters ($P < 0.05$)

High mortality of *T. brassicae* treated with approximately low concentrations of abamectin (0.001 ppm of abamectin active ingredient caused 72.57% mortality) confirms the high susceptibility of *T. brassicae*. This result is warning about using abamectin in the crop fields which *T. brassicae* is one of the major pest parasitoids. Results of the present study was similar to the other studies on abamectin effects on *Trichogramma* sp. and *Encarsia* sp. (Hassan et al., 1998; Bacci et al., 2007; Wang et al., 2012). Wang et al. (2012) reported relatively high susceptibility of *Trichogramma nubilale* adults against abamectin. Their results showed 3.57 (2.90–4.66) and 25.42 (15.77–47.38) ppm of abamectin active ingredient as LC_{50} and LC_{95} values for *T. nubilae*, respectively, while recommended field dose was 48.75 ppm of active ingredient. Due to high lethal effect of abamectin against adult *Trichogramma*, this pesticide should be applied carefully to minimizing its direct contact with the parasitoid or its feeding source.

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