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Spirotetramat toxicity to immatures and sublethal effects on fecundity of female adults of *Tetranychus urticae* Koch*

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Abstract

Acute toxicity of spirotetramat to immatures and its sublethal effects on adult females of two-spotted spider mite, *Tetranychus urticae* Koch, were investigated in laboratory bioassays, at 27 ± 2°C, 50–80% RH and 16/8 L/D photoperiod. Acute toxicity was tested by successive treatments of eggs, larvae, protonymphs and deutonymphs on bean leaf discs, and mortality was evaluated based on the number of mites reaching the adult stage. Concentration-mortality data were subjected to probit analysis and the following LC_{50} data were computed: 0.10 mg/l (larvae), 0.17 mg/L (protonymphs) and 0.15 mg/L (deutonymphs). The acaricidal effect after the treatment of eggs was not the result of spirotetramat toxicity to this developmental stage, but rather of its residual activity on hatched larvae (LC_{50}=0.62 mg/L). Sublethal effects were evaluated after the treatment of pre-ovipositing adult females on leaf discs using the concentrations of 2 mg/L, 20 mg/L and 200 mg/L. After 18–20 hours of exposure on treated discs, females showing no visible symptoms of poisoning were transferred to untreated leaf discs and re-transferred to new discs at 48 h intervals over ten days. Based on the number of eggs laid and survival rate of females, gross fecundity and net fecundity were calculated. At the end of the tenth day, the survival rate was 0.72 in untreated and 0.40, 0.27 and 0.05 in females treated at the concentrations mentioned above. Compared with the control mites, gross fecundity was reduced by 9% (2 mg/L), 29% (20 mg/L) and 93% (200 mg/L), while net fecundity decreased by 40% (2 mg/L), 67% (20 mg/L) and 98% (200 mg/L). The results indicate that spirotetramat exerts similar effects on spider mites as do the acaricides spirodiclofen and spiromesifen.

Key words: Spirotetramat, *Tetranychus urticae*, toxicity, sublethal effects.

Introduction

Spirotetramat, a tetramic acid derivative, has been recently introduced as a new product intended for controlling whiteflies, aphids and other sucking insect pests of agricultural crops. The insecticide has high acute toxicity to immature insects and significantly reduces the fecundity and fertility of adult females (Bretschneider *et al*., 2007; Cantoni *et al*., 2008; Brück *et al*., 2009). Even though some suppressive side effects of spirotetramat have also been detected on spider mite populations in field trials targeted against insects (Brück *et al*., 2009), there are still no adequate studies on possible acaricidal properties of this compound.

Similarly to spirodiclofen and spiromesifen, derivatives of tetronic acid, spirotetramat inhibits lipid biosynthesis. Spirodiclofen and spiromesifen are highly toxic to eggs and other immature stages of spider mites, while their activity against adult females is through reduction of fecundity and fertility (Wachendorff *et al*., 2002; Nauen *et al*., 2005; Marcic, 2007; van Pottelberge *et al*., 2009; Marcic *et al*., 2010). The objective of this paper is to provide an evaluation of spirotetramat toxicity against immature stages of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), as well as its sublethal effects on fecundity.

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Materials and Methods

Spider mite population

The mites used in this work were taken from a population of *T. urticae* initiated with mites collected from a ruderal weed habitat in Belgrade environs. The colony had been reared on bean plants in a climate chamber (16/8 h of light/dark photoperiod, 25–30°C) since March 2004.

Chemical pesticide

Spirotetramat, commercial formulation Movento (suspension concentrate, 100 g a.i./L, Bayer CropScience, Germany), was obtained from Bayer d.o.o. (Belgrade, Serbia).

Toxicity bioassays

Toxicity of spirotetramat to developmental stages was tested by successive treatments of 25 eggs, 20 larvae, 20 protonymphs and 20 deutonymphs on bean leaf discs (30 mm in diameter). The acaricide was suspended in distilled water and applied to the discs by using a Potter spray tower (3 mL of spray fluid, 1 bar air pressure). In the control treatments, distilled water was applied. Mortality was evaluated based on the number of treated individuals reaching the adult stage. The bioassays were conducted in four replicates in a climatically controlled room at 27 ± 2°C, 50–80% RH and 16/8 L/D photoperiod. The lethal concentration (LC_{50}) values, slopes, and 95% confidence limits were calculated by probit analysis using POLO Plus software, LeOra Software, Berkeley, CA (Robertson *et al*., 2007). Non-overlapping of the 95% confidence limits was the criterion for significant differences.

Assessment of sublethal effects on fecundity

The effects of spirotetramat on fecundity were evaluated after the treatment of adult females at the pre-ovipositional period on bean leaf discs. From the synchronous mite culture, newly emerged pre-ovipositional females were transferred to 30 mm-diameter discs (15 females per disc, 3–4 discs per replicate) and treated with spirotetramat. The acaricide was suspended in distilled water and applied to the discs in the same way as in toxicity bioassay. The following concentrations of spirotetramat were used: 200 mg/L, 20 mg/L and 2 mg/L (the concentration that produced 100% mortality of eggs and immatures in preliminary studies). The assays were carried out in four replicates under the same condition as the toxicity bioassays. After 18–20 h of exposure on treated discs, females showing no visible symptoms of poisoning were transferred to untreated leaf discs (5 females/disc, 3 discs/replicate) and re-transferred to new discs at 48 h intervals during ten days, and both the number of surviving females (*Fs*) and eggs laid were counted. Female survival rates were calculated as *Fs*/15. Gross fecundity (the number of eggs laid daily per female) and net fecundity (gross fecundity weighted by female survival rates) were defined and calculated according to Carey (1993). Fecundity data for all ten days were summed, square-root transformed and analyzed by one-way ANOVA and significant differences in means identified by Tukey's test ($p < 0.05$).

Results and Discussion

Spirotetramat proved to be highly toxic to post-embryonic immatures of *T. urticae* (Table 1). At LC_{50} level, the highest susceptibility to this acaricide was observed for larvae, followed by protonymphs and deutonymphs. At LC_{90} level, however, there were no pronounced variations in susceptibility between those three developmental stages. The treated larvae, protonymphs and deutonymphs usually died up reaching the next quiescent stage, which was also observed for spirodiclofen and spiromesifen (Wachendorff *et al*., 2002; Nauen *et al*., 2005).

On the other hand, spirotetramat did not show any significant effect on eggs. The acaricidal ef-

TABLE 1. Spirotetramat toxicity to *Tetranychus urticae* after the treatment of eggs (E), larvae (L), protonymphs (PN) and deutonymphs (DN).

Developmental stages	n[a]	LC_{50} (mg/L) (95% CL)	LC_{90} (mg/L) (95% CL)	Slope (± SE)	χ^2	df[b]	H[c]
E	500	0.62 c (0.58–0.66)	1.14 b (1.03–1.30)	4.90 (± 0.42)	2.97	3	0.99
L	525	0.10 a (0.09–0.12)	0.24 a (0.20–0.34)	3.40 (± 0.29)	6.37	5	1.27
PN	375	0.17 b (0.15–0.19)	0.27 a (0.24–0.34)	6.37 (± 0.60)	3.63	3	1.22
DN	300	0.15 b (0.12–0.18)	0.23 a (0.20–0.37)	6.98 (± 0.79)	2.52	2	1.26

For each column, means followed by different letters differ significantly based on non-overlapping 95% CLs.
[a] total number of test subjects.
[b] degree of freedom.
[c] heterogeneity.

fect after the treatment of eggs was not the result of spirotetramat toxicity to this developmental stage, but rather of the toxic activity of its three day old residues on larvae that hatched from the eggs and died in the protochrysalis stage. Therefore, the LC values and other parameters calculated after the treatment of eggs (Table 1) represent residual toxicity of spirotetramat to hatched larvae. This feature makes spirotetramat different from spirodiclofen and spiromesifen which exhibit excellent ovicidal activity (Wachendorff *et al*., 2002; Nauen *et al*., 2005).

In preliminary trials, adult females were sprayed with spirotetramat (2 mg/L, 20 mg/L, and 200 mg/L) and left on treated discs for several days. The inspection after 96 h showed that no eggs were laid by the treated females, while they all had visible symptoms of poisoning similar to those caused by spirodiclofen and spiromesifen: sticky remains on ovipositors, swelling, and inability to lay eggs. Most of the treated females died after 5–7 days. Therefore, sublethal effects on fecundity were investigated on females that survived 18–20 h of exposure to the acaricide in the pre-ovipositional period, and showed no symptoms of poisoning. In the first two days after treatment, the females treated with 200 mg/L laid no eggs, while the females treated with 20 mg/L and 2 mg/L laid considerably reduced numbers of eggs compared to females in the control. In the following days, the females from the latter group recovered as their daily gross fecundity reached the one in the control, while the females treated with 200 mg/L began to lay eggs, but their gross fecundity remained low (Fig. 1a). If observed from the aspect of daily net fecundity, the situation is somewhat different (Fig. 1b). From the beginning to the end of the experiment, control females showed considerably higher daily net fecundity than the treated ones, and the net fecundity of treated females continually decreased with the increase of acaricide concentration rate. This was the result of varying female survival rates (Fig. 2) which steadily decreased as the concentration of spirotetramat increased. At the end of the tenth day, the survival rate was 0.72 in the control, and 0.40, 0.27 and 0.05 in females treated with 2 mg/L, 20 mg/L and 200 mg/L, respectively.

Total gross fecundity was reduced by 9% (2 mg/L), 29% (20 mg/L) and 93% (200 mg/L), while total net fecundity decreased by 40% (2 mg/L), 67% (20 mg/L) and 98% (200 mg/L), compared with the control (Table 2). However, only the net fecundity statistically decreased as the acaricide concentration rate increased.

A similar pattern of sublethal activity against two-spotted spider mite has been previously reported for spirodiclofen (Wachendorrf *et al*., 2002; Marcic, 2007; van Pottelberge *et al*., 2009) and spiromesifen (Nauen *et al*., 2005; Marcic *et al*., 2010). Lethal and sublethal effects of spirotetramat on *T. urticae* demonstrated in our laboratory trials indicate that spirotetramat could be an effective acaricide against motile stages. Additional greenhouse and field trials are needed to evaluate its acaricidal properties.

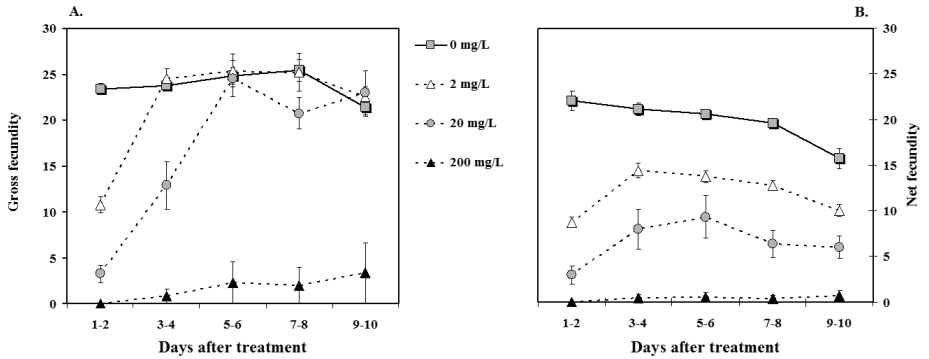

FIGURE 1. (A) Gross fecundity of *Tetranychus urticae* females that survived treatment with spirotetramat (mg/L) at pre-ovipositional period (means ± SEM); **(B)** Net fecundity of *Tetranychus urticae* females that survived treatment with spirotetramat (mg/L) at pre-ovipositional period (means ± SEM).

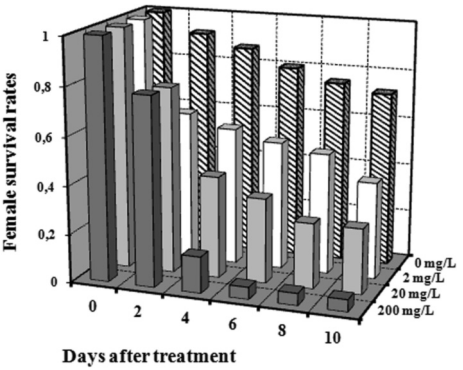

FIGURE 2. Survival rates of *Tetranychus urticae* females after treatment with spirotetramat (mg/L) at pre-ovipositional period.

TABLE 2. Gross (*G*) and net (*N*) fecundity of *Tetranychus urticae* females ten days after treatment with spirotetramat (mg/L) at pre-ovipositional period.

mg/L	*G*	*N*
0	118.78 *a*	99.17 *a*
2	108.32 *a*	69.70 *b*
20	84.29 *a*	32.65 *c*
200	8.43 *c*	2.05 *d*

Within each column, means followed by different letters differ significantly (Tukey-test; $p < 0.05$).

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References

Bretschneider, T., Fischer, R. & Nauen, R. (2007) Inhibitors of lipid synthesis. In: Krämer, W. & Schirmer, U. (eds) *Modern crop protection compounds, Vol. 3*. Wiley–VCH Verlag GmbH & Co, Weinheim, pp. 909–925.

Brück, E., Elbert, A., Fischer, R., Krueger, S., Kühnhold, J., Klueken, A.M., Nauen, R., Niebes, J.F., Reckman, U., Schnorbach, J.J., Steffens, R. & van Waetermeulen, X. (2009) Movento®, an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance. *Crop Protection*, 28, 838–844.

Cantoni, A., De Maeyer, L., Izquierdo Casas, J., Niebes, J.F., Peeters, D., Roffeni, S., Silva, J. & Villalobos, A. (2008) Development of Movento® on key pests and crops in European countries. *Bayer CropScience Journal*, 61, 349–376.

Carey, J.R. (1993) *Applied demography for biologists*. Oxford University Press, New York.

Marcic, D. (2007) Sublethal effects of spirodiclofen on life history and life-table parameters of two-spotted spider mite (*Tetranychus urticae*). *Experimental and Applied Acarology*, 42, 121–129.

Marcic, D., Ogurlic, I., Mutavdzic, S. & Peric, P. (2010) The effects of spiromesifen on life history traits and population growth of two-spotted spider mite (Acari: Tetranychidae). *Experimental and Applied Acarology*, 50, 255–267.

Nauen, R., Schnorbach, H.J. & Elbert, A. (2005) The biological profile of spiromesifen (Oberon®) – a new tetronic acid insecticide/acaricide. *Pflanzenschuz-Nachrichten Bayer*, 58, 417–440.

Robertson, J.L., Russell, R.M., Preisler, H.K. & Savin, E. (2007) *Bioassays with arthropods, 2nd edition*. CRC Press, Boca Raton.

Van Pottelberge, S., Khajehali, J., van Leeuwen, T. & Tirry, L. (2009) Effects of spirodiclofen on reproduction in a susceptible and resistant strain of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, 47, 301–309.

Wachendorff, U., Nauen, R., Schnorbach, H.J., Rauch, N. & Elbert, A. (2002) The biological profile of spirodiclofen (Envidor®) - a new selective tetronic acid acaricide. *Pflanzenschutz-Nachrichten Bayer*, 55, 149–176.