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Scientific paper

The Effects of Kingbo Biopesticide on *Tetranychus urticae* Koch Female Adults

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SUMMARY

Toxic and sublethal effects of the biopesticide Kingbo (oxymatrine 0.2% + psoralen 0.4%) on female adults of two-spotted spider mite (*Tetranychus urticae* Koch) were investigated in two laboratory bioassays. The assays were set up in four replicates on bean leaf discs (30 mm in diameter) placed on moistened cotton wads in Petri dishes. Preovipositional females were then released on them and treated directly with the biopesticide at a concentration series using a Potter device (2 ml liquid, 100 kPa air pressure, 2.7 ± 0.2 mg/cm^2 aqueous deposit). Each replicate included 4-7 Petri dishes containing a total of 20-35 females. In the first assay, females were exposed to continuous acaricidal activity on treated discs over a period of 96 h; in the second assay, they were exposed for 24 h and then transferred to untreated discs and kept there for the next 72 h. Kingbo toxicity to females, expressed as LC_{50}, was significantly higher in the first bioassay (14.83 µl/l) than in the second one (26.39 µl/l). Total gross fecundity of females in the first assay was reduced by 37-95% and net fecundity by 48-97%, depending on concentration; in the second assay, the respective fecundity reductions were 15-87% and 23-91%. We found that a 24 h exposure to the biopesticide Kingbo was sufficient for sustaining significant toxic and sublethal effects. Further research should provide additional data on the recovery potential of *T. urticae* populations.

Keywords: Sublethal effects; Oxymatrine; *Tetranychus urticae*; Biopesticides

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and highly polyphagous species, and probably the most important mite pest of greenhouse crops and ornamentals around the world (Zhang, 2003; Hoy, 2011). Its pest status is the result of its polyphagous character but considerably also of its remarkable intrinsic potential for rapid evolution of resistance to acaricides. Considering the number of compounds to which resistance has been reported, *T. urticae* is ranked first among arthropod pest species (Whalon et al., 2008; van Leeuwen et al., 2010). Developing new acaricides with novel modes of action has been a strategy of dealing with such scope of resistance. Nowadays,

newly developed pesticides are required to meet the increasingly rigorous toxicological and ecotoxicological criteria. It has reactualized the role of biopesticides (i.e. commercial plant protection agents manufactured from living organisms and/or their products) as an alternative to synthetic compounds (Dekeyser, 2005; Marčić, 2012). The advantages of biopesticides include their low risk to human health and the environment, compatibility with other biological control agents, lack of harvest and re-entry restrictions and a minimal or no risk for resistance development. Plant oils, extracts and other plant-derived products are the most important sources for development of biopesticides (Copping and Menn, 2000; Chandler et al. 2011).

Oxymatrine is a major alkaloid found in *Sophora flavescens* Aiton (Fabaceae), an ancient Chinese herb whose dry roots („Ku Shen") have long been used in traditional medicine. Ku Shen extracts have been formulated lately as pesticides and recommended to be used in commercial products to manage populations of various plant pests and diseases (Zheng et al., 2000; Fu et al., 2005). In greenhouse and field trials conducted in Serbia, the oxymatrine-based product Kingbo, which also contains furanocoumarin psoralen (= prosuler) as a natural regulator, has demonstrated high efficacy in controlling *T. urticae* on greenhouse vegetables when applied twice at 5-day interval and at 0.1% and 0.2% application rates (Marčić et al., 2012a, 2012b). The objective of the present study was to evaluate toxic and sublethal effects of Kingbo on *T. urticae* female adults in order to obtain baseline data that can be used in further research aimed to improve management of this important mite pest.

MATERIAL AND METHODS

Spider mite population

A population of *T. urticae,* originating from individuals collected from a ruderal weed habitat on the outskirts of Belgrade, has been reared on bean plants in a climate-controlled room under a long day conditions (16/8 h of light/dark photoperiod) since March 2004.

Acaricide

The commercial product Kingbo, aqueous solution; active ingredients: 0.2% oxymatrine + 0.4% psoralen (or prosuler; natural regulator); manufacturer Beijing Kingbo Biotech Co. Ltd., Beijing, China.

Bioassays

Two bioassays involving *T. urticae* adult females were conducted. The assays were carried out in four replicates at 27 ± 2°C, 40-60% RH and under 16/8 L/D photoperiod, on bean leaf discs (30 mm diameter) placed on moistened cotton wads in Petri dishes (90 mm diameter). From a synchronous mite culture, 5 older pre-ovipositional females were transferred to a single leaf disc in each Petri dish. In Bioassay 1, three other discs were added to each Petri dish prior to treatment, so that all four discs were treated simultaneously while the females were released onto one of them alone. In Bioassay 2, only the disc with the females was treated, while the other three were inserted in Petri dishes after treatment. Between 4 and 7 Petri dishes with a total of 20-35 females, were treated per replicate, i.e. a total of 155 females. The pesticide Kingbo, suspended in distilled water, was applied to discs by a Potter spray tower (2 ml of spray liquid, 100 kPa air pressure, 2.7 ± 0.2 mg/cm^2 aqueous deposit) at the following series of concentrations: 34.3, 24, 16.8, 11.8 and 8.2 µl/l. Distilled water was applied in control treatments. The surviving females were transferred daily to new leaf discs, and both their number and the number of laid eggs were counted. In Bioassay 1, the females were continuously exposed to the acaricide for 96 h, while the exposure period was only 24 h in Bioassay 2, and the females were then kept on untreated surface for the next 72 h.

Assessment of acute toxicity

The number of dead females was counted 96 h after the beginning of the assay and concentration-mortality data were subjected to Probit analysis using POLO Plus software, LeOra Sotfware, Berkeley, CA. The lethal concentrations ratio (LCR) test was the criterion of significant differences between the calculated lethal concentrations (LC): if the 95% confidence limits for LC ratios included 1 the LCs were not significantly different (Robertson et al., 2007).

Assessment of sublethal effects

Gross fecundity (*FCg*; the number of eggs hatched daily per female alive at the midpoint od 24 h) and net

fecundity (*FCn*; gross fecundity weighted by female survival rates) were defined and calculated according to Carey (1993). Female survival rates were calculated as $(Sa/N + Sb/N)\times 0.5$, where N is the number of treated females, Sa is the number of live females at the beginning, and Sb the number of live females at the end of each day. Female longevity (L) was defined as the mean number of days that females lived in bioaassay after treatment, an assumption being that females died at the midpoint of 24 h. Longevity data and fecundity data were transformed by $\sqrt{x}$ and $\sqrt{x}+0.01$, respectively, and analyzed by one-way ANOVA with the means separated by Fisher's LSD test ($\alpha=0.05$). Untransformed means are presented in this paper.

RESULTS AND DISCUSSION

Parameters of acute toxicity of the botanical pesticide Kingbo for *T. urticae* females are presented in Table 1. Females in Bioassay 1, which were continually exposed to acaricidal activity, were expectedly found more susceptible than females in Bioassay 2, which were exposed only during the initial 24 h. At the LC_{50} level, which is the most reliable parameter in bioassays of the present design (Robertson et al., 2007), the results of LCR test indicated significant differences between the acquired LC values (Table 1). However, 24 h exposure was still sufficient to achieve relatively high toxicity: the applied biopesticide concentrations caused a corrected mortality that ranged from 23% to 90% in the first assay, while the range was 12-60% in the second assay. The values of lethal concentration in both assays were considerably below 0.2% (2000 µl/l), which is the concentration recommended for application of Kingbo.

Pesticide concentrations that were acutely toxic caused a significant reduction in fecundity of the treated *T. urticae* females. Figures 1 and 2 show gross and net fecundity values per day of oviposition. In Bioassay 1, a rising fecundity trend was observed, which is a feature of the adaptive strategy that *T. urticae* has as a colonizing species (Sabelis, 1985). However, continued exposure to the acaricide concentrations of 8.2 µl/l and 11.8 µl/l stopped the trend after three days, while all other concentrations stopped it after two days. Both gross fecundity and net fecundity decreased as concentration increased (Figure 1). Compared to the control, the percentage of fecundity reduction increased with exposure duration: gross fecundity reduction increased from 34-89% to 43-99.5%, while net fecundity reduction rose from 44-94% to 55-99.9% (depending on concentration) from the second to fourth day of oviposition. In Bioassay 2, fecundity was also found to decrease in a concentration-dependent manner. Only Kingbo concentrations of 24 µl/l and 34.3 µl/l stopped the rising fecundity trend in the initial days of oviposition. Fecundity reduction was 20-86% and 25-88% on the second day of oviposition, and 14-91% and 25-96% on the fourth day (gross fecundity and net fecundity, respectively). The reduction percentage increased only for females exposed to 24 µl/l and 34.3 µl/l.

Table 1. Toxicity of the botanical acaricide Kingbo to *T. urticae* adult females

Treatments	N	LC_{10} (µl/l) (95% CLs)	LC_{50} (µl/l) (95% CLs)	LC_{90} (µl/l) (95% CLs)	b (± SE)	χ^2	df
Bioassay 1 (E 96)	620	6.43 (3.44 - 8.89)	14.83 (11.51 - 17.65)	34.19 (27.88 - 48.34)	3.53 (± 0.36)	3.28	3
Bioassay 2 (E 24 + NT 72)	620	8.42 (5.27 - 11.06)	26.39 (22.85 - 30.82)	82.70 (61.42 - 138.47)	2.58 (± 0.38)	1.74	3
LCR (95% CLs)		0.76 (0.50 - 1.18)	0,56 * (0,47 - 0,68)	0,41 * (0,27 - 0,62)			

E 96 = continuous exposure to the acaricide for 96 h
E 24 + NT 72 = exposure to the acaricide in the first 24 h, then a recovery for 72 h on untreated surface

N = total number of females tested;
CLs = confidence limits;
b = slope of regression line (± standard error)
df = degrees of freedom
LCR = lethal concentrations ratio

* The LC values are significantly different, based on LCR test (Robertson et al., 2007)

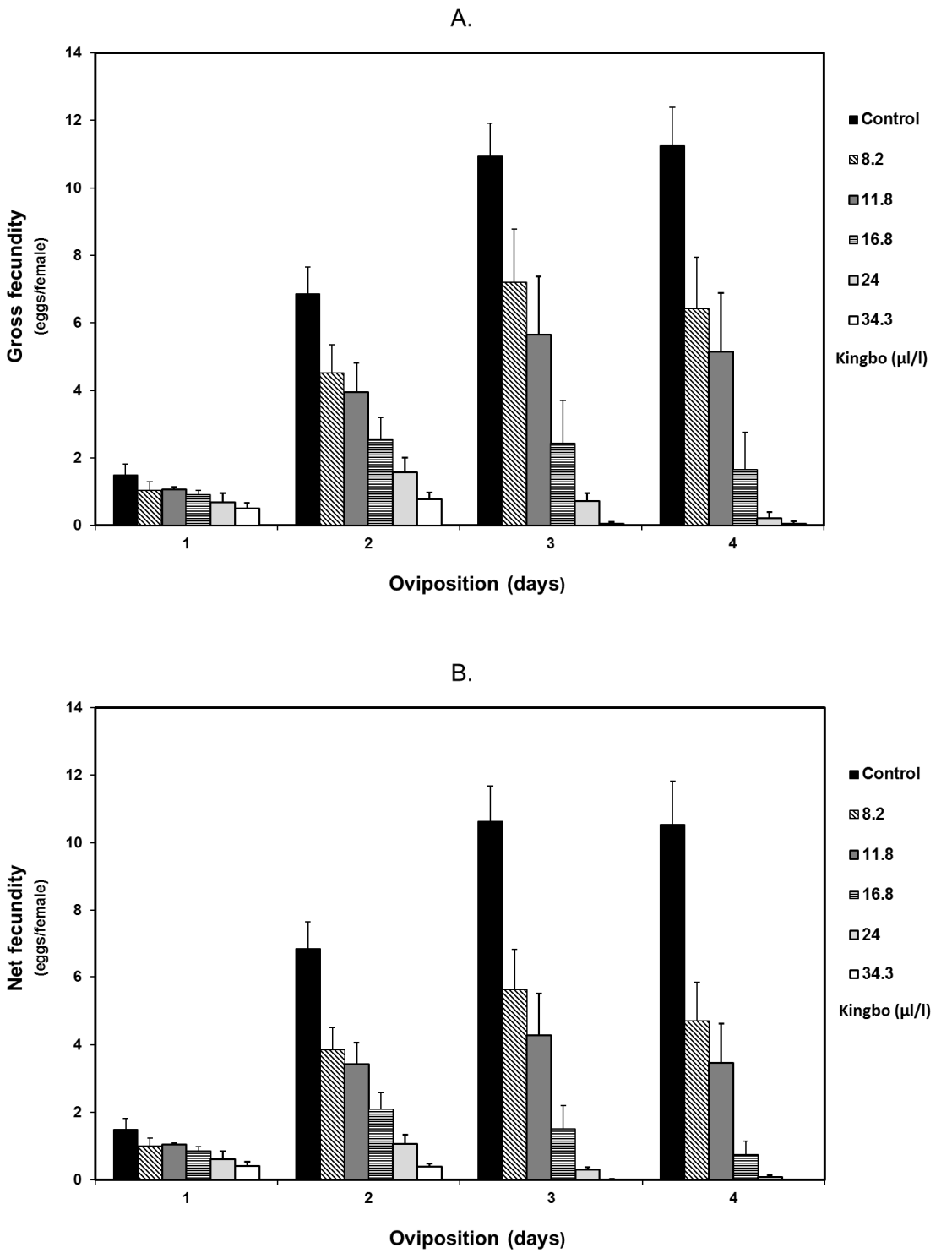

Figure 1. Gross fecundity (A) and net fecundity (B) of *T. urticae* females continuously exposed to the bioacaricide Kingbo (µl/l)

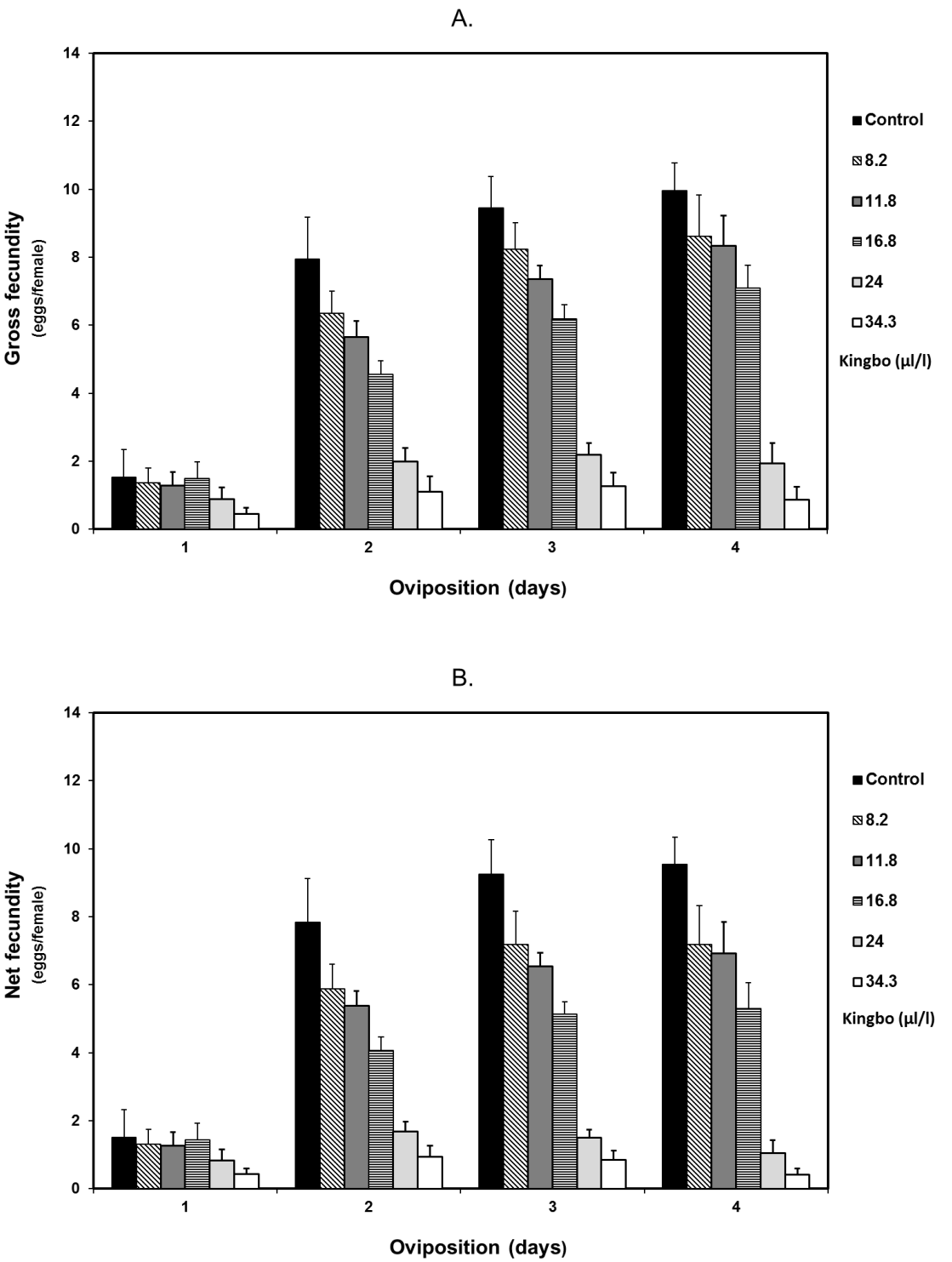

Figure 2. Gross fecundity (A) and net fecundity (B) of *T. urticae* females exposed to the bioacaricide Kingbo (µl/l) in the first 24 h, and recovered for 72 h on untreated surface

Total gross fecundity and total net fecundity over the four days of oviposition are shown in Tables 2 and 3. In Bioassay 1, fecundity of the treated females differed significantly from the control, an exception being only the gross fecundity of females treated with the lowest concentration. Treated females lived significantly shorter than untreated females. The highest concentration reduced fecundity by more than 95% and halved the average life span of treated females. A comparison of FCg and FCn values revealed that net fecundity was 21-39% lower, depending on concentration (Table 2). In Bioassay 2, gross fecundity of the females exposed to 8.2 µl/l and 11.8 µl/l, and net fecundity of females exposed to 8.2 µl/l showed no significant difference from control values. The achieved fecundity reduction was lower than the reduction in Bioassay 1. Pesticide treatment significantly shortened the life span of females in Bioassay 2 but not as much as in Bioassay 1. The highest concentration reduced fecundity by over 87% and shortened the average life span of treated females by a day. Depending on concentration, net fecundity was lower than gross fecundity by 12-28%, a smaller difference than it was in Bioassay 1 (Table 3). Greater fecundity reduction and more prominent reduction in net fecundity, compared to gross fecundity in Bioassay 1, were an expected consequence of continued exposure. The 24 h exposure, however, was still sufficient for causing significant sublethal effects, especially in females exposed to concentrations of 34.3 µl/l and 24 µl/l.

Table 2. Gross fecundity (FCg) and net fecundity (FCn) of *T. urticae* adult females within 96 h of continuous exposure to the botanical acaricide Kingbo (µl/l)

µl/l	FCg (± SE)	%R	FCn (± SE)	%R	FCn/FCg	L
34.3	1.39 (0.35) d	95.4	0.85 (0.17) d	97.1	0.61	1.85 d
24.0	3.20 (0.64) cd	89.5	2.05 (0.46) cd	93.0	0.64	2.26 c
16.8	7.58 (3.00) c	75.2	5.24 (1.57) c	82.2	0.69	2.93 b
11.8	15.81 (4.37) b	48.2	12.18 (3.07) b	58.7	0.77	3.24 b
8.2	19.20 (4.10) ab	37.1	15.19 (3.14) b	48.5	0.79	3.34 b
0.0	30.53 (3.13) a	-	29.51 (3.35) a	-	0.97	3.90 a
	F = 16.62		F = 26.03			F = 28.63
	p < 0.0001		p < 0.0001			p < 0.0001

%R = percent of reduction compared to the control
L = average longevity of female adults in the bioassay (days)
Means in columns followed by different letters differ significantly (ANOVA, Fisher LSD, α=0.05)

Table 3. Gross fecundity (FCg) and net fecundity (FCn) of *T. urticae* adult females within 96 h of exposure to the botanical acaricide Kingbo (µl/l) in the first 24 h

µl/l	FCg (± SE)	%R	FCn (± SE)	%R	FCn/FCg	L
34.3	3.69 (1.35) c	87.2	2.64 (0.91) d	90.7	0.72	2.99 c
24.0	7.01 (1.58) c	75.7	5.09 (1.10) c	81.9	0.73	3.06 c
16.8	19.34 (1.21) b	33.0	15.93 (1.36) b	43.3	0.82	3.41 b
11.8	22.62 (1.60) ab	21.6	20.11 (1.64) b	28.4	0.89	3.66 ab
8.2	24.54 (2.83) ab	15.0	21.57 (2.93) ab	23.3	0.88	3.61 b
0.0	28.87 (3.18) a		28.11 (3.38) a		0.97	3.91 a
	F = 27.97		F = 35.71			F=15.45
	p < 0.0001		p < 0.0001			p < 0.0001

%R = percent of reduction compared to the control
L = average longevity of female adults in the bioassay (days)
Means in columns followed by different letters differ significantly (ANOVA, Fisher LSD, α=0.05)

In laboratory bioassays, oxymatrine and/or oxymatrine-based products have shown considerable toxic and antifeedant activity against several insect pest species (Mao and Henderson, 2007; Asghari-Tabari et al., 2009; Abd El-Mageed and Shalaby, 2011). The only available information on their acute toxicity to mites had been reported by Wang et al. (2009), who found the combination of oxymatrine/psoralen to have an LC_{50} of 150 µg/l for carmine spider mite, *T. cinnabarinus*. In our bioassay, in which different exposure methods were employed, LC_{50} after 96 h of exposure was 14.83 µl/l = 88.98 µg/l, the active ingredient content of Kingbo being 0.6% w/v (i.e. 2 g/l oxymatrine and 4 g/l psoralen). The data from our insect assays indicate that the reduction in *T. urticae* fecundity could be the result not (only) of female intoxication with oxymatrine but (also) of the antifeeding activity of this alkaloid. Since psoralen is also a feeding deterrent (Berdegué et al., 1997), it is possible that its combination with oxymatrine increased the antifeeding effect.

The data obtained in our study showed that the botanical pesticide Kingbo can achieve significant toxic and sublethal effects on females of *T. urticae* when applied at concentrations considerably lower than recommended. This finding provides a basis for further research of product application at reduced rates, as doses below recommended should be feasible in combination with predator release or other measures incorporated in integrated mite management strategies (Hoy, 2011). Pre-ovipositional adult females are most likely to exhibit dispersal behaviour, which is an important element in the biology of two-spotted spider mite as a colonizing species (Mitchell, 1973; Li and Margolies, 1993). In our second bioassay, the 24 h exposure to the biopesticide at the concentration of 34.3 µl/l caused a corrected mortality of 60% and reduceds net fertility of the surviving females by 90%, which indicates a low potential of population recovery on untreated surface. A more detailed evaluation of sublethal effects on the reproductive capacity of female dispersers would require the employment of methods of population-toxicological bioassay. On the other hand, the real potential for population recovery from eggs laid on treated surface should be evaluated, considering that preliminary results indicate a negliglible ovicidal activity of the product Kingbo (Marčić and Međo, unpublished data). Possible repellent and deterrent potentials of that botanical acaricide should also be examined more closely.

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Efekti biopesticida Kingbo na odrasle ženke *Tetranychus urticae* Koch

REZIME

Toksični i subletalni efekti biopesticida Kingbo (oksimatrin 0,2% + psoralen 0,4%) na odrasle ženke obične paučinaste grinje (*Tetranychus urticae* Koch) ispitivani su u dva laboratorijska ogleda. Ogledi su izvedeni u četiri ponavljanja na kružnim lisnim isečcima primarnih listova pasulja (prečnika 30 mm) postavljenim u petri-sudove na navlaženu vatu, na kojima su pre-ovipozicione ženke direktno tretirane serijom koncentracija biopesticida pomoću Poter-aparata (2 ml tečnosti, pritisak 100 kPa, vlažni depozit 2,7 ± 0.2 mg/cm^2). Tretirano je 4-7 petri-sudova sa 20-35 ženki po ponavljanju. U prvom ogledu ženke su bile 96 h kontinuirano izložene delovanju akaricida na tretiranim isečcima, dok su u drugom ogledu posle 24 h prebačene na netretirane isečke, gde su ostale naredna 72 h. Na nivou LC_{50}, utvrđena je značajno veća toksičnost biopesticida Kingbo za ženke u prvom ogledu (14.83 µl/l) u poređenju sa ženkama u drugom ogledu (26.39 µl/l). Ukupan bruto-fekunditet ženki u prvom ogledu redukovan je za 37-95%, a neto-fekunditet za 48-97%, u zavisnosti od koncentracije; u drugom ogledu odgovarajuća redukcija fekunditeta iznosila je 15-87% i 23-91%. Konstatovano je da je 24-časovna ekspozicija biopesticidu Kingbo dovoljna za nastajanje značajnih toksičnih i subletalnih efekata, kao i da su potrebna dalja istraživanja u cilju preciznije procene potencijala oporavka populacije *T. urticae*.

Ključne reči: Subletalni efekti; oksimatrin; *Tetranychus urticae*; biopesticidi