

# Greenhouse and Field Evaluation of Two Biopesticides Against *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae)

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## SUMMARY

The mycopesticide Naturalis (based on *Beauveria bassiana* strain ATCC 74040) and botanical pesticide Kingbo (based on oxymatrine, an alkaloid from *Sophora flavescens*, a traditional Chinese herb) were tested against the two-spotted spider mite (*Tetranychus urticae*) on greenhouse vegetables and the European red mite (*Panonychus ulmi*) on apples. These biopesticide products were applied twice at 5-day interval and concentrations of 0.1% and 0.2%, and their effectiveness was compared to abamectin-based products and the synthetic acaricides acrinathrin and spirodiclofen, applied once at their recommended rates. The mycopesticide Naturalis, applied at 0.1% concentration against *T. urticae* on cucumber, reduced mite population density by 85-86%, achieving 91-93% efficacy. In a trial on tomato, efficacy reached some 96%, while population density was reduced by 93%. In a field trial on apple, Naturalis demonstrated an increasing and long-lasting effectiveness against the summer population of *P. ulmi* of nearly 100%, and population reduction was achieved in assessments 30 days after the first treatment. Naturalis applied at a double rate achieved a somewhat better effect but only in the first trial. The botanical pesticide Kingbo, applied at 0.1% concentration, demonstrated very high control efficacy ( $\geq 98\%$ ) and population density reduction ( $\geq 96\%$ ) of *T. urticae* in both trials. A high and long-lasting effectiveness of this bioacaricide was also achieved in a trial on *P. ulmi*. Its concentration of 0.2% achieved similar effect. The results in these trials indicate that applications of the mycopesticide Naturalis and the botanical pesticide Kingbo can provide effective control of *T. urticae* on cucumber and tomato grown in greenhouses, as well as *P. ulmi* on apple.

**Keywords:** Biopesticides; Spider mites; *Beauveria bassiana*; Oxymatrine

## INTRODUCTION

Spider mites (Acari: Tetranychidae) are the most important phytophagous mite pests of agricultural crops worldwide, whose population outbreaks can cause serious damage and yield losses. Among them, the two-spotted spider mite (*Tetranychus urticae* Koch) is especially significant as the most polyphagous species of spider mites, and probably the most important mite pest, common in greenhouses throughout the world. Another important pest is the European red mite, (*Panonychus ulmi* Koch), which is found on a range of deciduous trees, especially of the family Rosaceae (Zhang, 2003; Hoy, 2011; Migeon and Dorkeld, 2012). The pest status of these two species is primarily due to their remarkable intrinsic potential for rapid evolution of resistance (Cranham and Helle, 1985; Knowles, 1997; van Leeuwen et al., 2009). On a list of „top 20” resistant arthropod pests in the world, which is based on the number of compounds to which resistance has been reported, *T. urticae* and *P. ulmi* are ranked first and sixth with 93 and 45 compounds, respectively (Whalon et al., 2008, 2012). Therefore, there is a constant need for developing new acaricides with novel modes of action as a way of dealing with such scope of resistance (Dekeyser, 2005; Marčić, 2012).

Growing demands for environmentally-friendly and safe plant pest management have reactualized the potential of biopesticides (i.e. commercial plant protection agents manufactured from living organisms and natural products) as an alternative to synthetic chemical pesticides, especially broad-spectrum compounds. The advantages of biopesticides are their low mammalian toxicity, short environmental persistence, safety to beneficial and non-target organisms, lack of harvest and re-entry restrictions, as well as minimum risk for resistance development. Microorganisms and/or their products, and plant-derived products, are the most important sources for developing biopesticides. (Copping and Menn, 2000; Bailey et al., 2010).

Entomopathogenic fungi can be formulated as mycopesticides with conidia, blastospores and other live propagules as active ingredients intended for use in the control of insect and mite pests (de Faria and Wraight, 2007). Among them, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) has proved to be a promising biocontrol agent against spider mites (Chandler et al., 2005; Maniania et al., 2008; Gatarayihia et al., 2010, 2011). Testing of the strain ATCC 74040 has confirmed its pathogenicity to *T. urticae* in

the laboratory (Sáenz-de-Cabezón Irigaray et al., 2003; Chandler et al., 2005; Duso et al., 2008) and a high efficacy in reducing the species’ populations on tomato in greenhouses (Chandler et al., 2005). However, data on the effects of this entomopathogenic fungus on *P. ulmi* are scarce.

Plant-derived products that have been used commercially as crop protection agents against insect and mite pests include neem-based products, pyrethrum, essential oils and their constituents, other plant extracts and oils (Isman, 2006; Miresmailli et al., 2006; Marcic et al., 2009; Bailey et al., 2010). Oxymatrine is one of the most significant secondary metabolites of the plant species *Sophora flavescens* Aiton (Fabaceae), whose dry root is commonly known as Ku Shen in traditional Chinese medicine. This alkaloid is also a pesticide that has been recommended lately to be used in commercial products for controlling insects and mites (Zheng et al., 2000; Fu et al., 2005). However, data on its effects on spider mite populations are currently insufficient.

This study focused on examining the effectiveness of two commercial biopesticide products: the mycopesticide Naturalis (based on *B. bassiana* strain ATCC 74040) and the botanical pesticide Kingbo (based on oxymatrine) in controlling *T. urticae* and *P. ulmi* populations in greenhouse and field trials. The objective was to evaluate their potential for management of these important mite pests.

## MATERIAL AND METHODS

During 2010-2011, the bioacaricides Naturalis and Kingbo were tested against spider mites in two greenhouse and one field trials conducted in Serbia and arranged in a randomized complete block design with four replications. These products were applied twice at an interval of 5 days, and their efficacy was compared to the standard biopesticide products based on abamectin. Synthetic acaricides were also included in the trials (Table 1). The abamectine-based products and synthetic acaricides were applied once at their recommended rates. All pesticides were sprayed to run-off by the portable mister Solo 423 Port.

The trials against *T. urticae* were conducted on cucumber and tomato grown in commercial greenhouses (Table 2), on plots containing 10 plants. Motile forms of *T. urticae* were counted on 4-5 leaves per plot *in situ*, once immediately before spraying and twice after spraying.

**Table 1.** Bioacaricides and synthetic acaricides applied in greenhouse (GH) and field (F) trials

Products (rates, %)	Active ingredients	Formulations	Manufacturers	Trials
Naturalis (0.1; 0.2)	<i>B. bassiana</i> ATCC 74040 (2,3×10 <sup>7</sup> conidia/ml)	Oil dispersion	Intrachem, Italy	GH1-2, F
Kingbo (0.1; 0.2)	Oxymatrine (2 g/l)	Aqueous solution	Beijing Kingbo Biotech, China	GH1-2, F
Kraft 1.8 EW (0.05; 0.1)	Abamectin (18 g/l)	Emulsion oil in water	Cheminova A/S, Denmark	GH1, F
Abastate ME (0.05)	Abamectin (18 g/l)	Microemulsion	Galenika-Fitofarmacija, Serbia	GH2
Rufast E FLO (0.1)	Acinathrin (75 g/l)	Emulsion oil in water	Cheminova, Denmark	GH1
Envidor (0.06)	Spirodiclofen (240 g/l)	Suspension concentrate	Bayer CropScience, Germany	GH2
Masai (0.05)	Tebufenpirad (200 g/kg)	Wettable powder	BASE, Germany	F

**Table 2.** Greenhouse (GH) and field (F) trials against spider mites

Species	Trial	Date	Locality	GPS	Crop	Cultivar
<i>T. urticae</i>	GH1	2011, 6/29-7/15	Padinska Skela	N44°56.958'; E020°25.679'	Cucumber	Caman
	GH2	2011, 10/6-10/24	Padinska Skela	N44°57.008'; E020°25.726'	Tomato	Lorely
<i>P. ulmi</i>	F	2011, 7/11-8/10	Bela Crkva	N44°53.623'; E021°23.142'	Apple	Idared

The trial against the summer population of *P. ulmi* was conducted in a commercial apple orchard (Table 2), on plots of five trees. Motile forms of *P. ulmi* were counted on 25 leaves per plot *in situ*, once immediately before spraying and another three times after spraying.

For each treatment, the number of motile forms per plot was subjected to ANOVA and the means were separated by Duncan test. The data were transformed by  $\sqrt{x+0.1}$  before analysis. Treatment effectiveness was estimated in two ways: as a percentage change in population density and efficacy percentage relative to the unsprayed (control) plots.

The change in population density was calculated as follows

$$CPD\% = [(X_i - X_0)/X_0] \times 100$$

where  $X_0$  is the mean number of motile forms before spraying, and  $X_i$  is the mean number of motile

forms at  $i^{\text{th}}$  assessment after spraying. Positive values imply an increase.

The efficacy of acaricides was calculated by Henderon-Tilton's formula:

$$Ef\% = [1 - (X_{iT}/X_{iC})(X_{0C}/X_{0T})] \times 100$$

where  $X_{0C}$  and  $X_{0T}$  are respectively the mean numbers of motile forms in unsprayed and treated plots before treatment, and  $X_{iC}$  and  $X_{iT}$  are respectively the mean numbers of motile forms in unsprayed and treated plots at  $i^{\text{th}}$  assessment after treatment.

## RESULTS

The mycopesticide Naturalis applied at the concentration of 0.1% significantly reduced the mean number of motile forms and showed high efficacy in controlling *T. urticae* and *P. ulmi*. In the trial on cucumber

(Table 3), population density showed an increasing trend in untreated plots: at the end of the trial, 16 DAT1 (=days after the first treatment), the mean number of *T. urticae* motile forms more than tripled, compared to the initial number. The mycopesticide reduced population density by 85-86%, and the efficacy was 91-93%. In the tomato trial (Table 4), population density in untreated plots was more than double 11 DAT1, while it was 77% higher than the initial population size 18 DAT1. The mycopesticide achieved around 96% efficacy and reduced the population density by 93%. In the field trial on apple (Table 5), Naturalis showed an increasing and long-lasting effectiveness against the summer population of *P. ulmi*: in an assessment 11 DAT1, efficacy was 92.5% and population density reduction 81%, while nearly 100% efficacy and equal population reduction were achieved 30 DAT1. Compared to the situation before treatment, population density in untreated plots was two and a half times higher in the first assessment before falling below the initial population size in the third assessment. Applying Naturalis at a double rate achieved better effect only in the first trial.

The botanical pesticide Kingbo, applied at the rate of 0.1%, showed a very high control efficacy ( $\geq 98\%$ ) and reduction of population density ( $\geq 96\%$ ) of *T. urticae* in both trials (Tables 3 and 4). In the trial on *P. ulmi*, this bioacaricide demonstrated a high and long-lasting effectiveness (Table 5). Similar effects were achieved by applying 0.2% concentration.

The products based on abamectin, as well as the synthetic acaricides acrinathrin and spiroticlofen, also achieved very high efficacy in controlling and reducing population density of *T. urticae* in the greenhouse trials. In the field trial, the abamectin-based products were less effective than other treatments, while the synthetic acaricide tebufenpyrad was highly effective against *P. ulmi*.

Naturalis and Kingbo were found to be effective bioacaricides when applied at the concentration of 0.1% twice at five-day interval. Improvements in effectiveness should be sought in combinations with adjuvants that would prolong their activity and reduce the number of treatments, rather than in increasing concentration.

**Table 3.** Population densities of *T. urticae* (N)<sup>†</sup> on cucumber and effectiveness of acaricides (Trial GH1)

Products	Rates (%)	BT		9 DAT1		16 DAT1		
		N	N	CPD (%)	EF (%)	N	CPD (%)	EF (%)
Naturalis	0.1	35.88 a	4.94 b	-86.2	90.8	5.38 b	-85.0	95.3
Naturalis	0.2	47.06 a	1.56 bc	-96.7	97.8	2.31 c	-95.1	98.5
Kingbo	0.1	90.06 a	1.44 bc	-98.4	98.9	1.13 cd	-98.4	99.6
Kingbo	0.2	48.00 a	0.25 c	-99.5	99.6	0.19 d	-99.6	99.9
Kraft 1.8 EW	0.05	81.38 a	0.81 c	-99.0	99.3	0.19 d	-99.8	99.9
Rufast E FLO	0.1	68.31 a	0.63 c	-99.1	99.4	0.38 d	-99.4	99.8
Untreated	-	38.44 a	57.50 a	49.6	-	122.94 a	219.8	-
	F	0.54	50.58			234.36		
	p	0.7740	< 0.0001			< 0.0001		

<sup>†</sup> The mean number of motile forms/leaf (4 leaves per plot, 50 cm<sup>2</sup> per leaf)

BT = before treatment; DAT1 = days after 1<sup>st</sup> treatment of Naturalis and Kingbo

CPD (%) = change in population density; EF (%) = efficacy according to Henderson-Tilton formula

Within a column, the means followed by the same letter are not significantly different (Duncan-test,  $\alpha = 0.05$ )

**Table 4.** Population densities of *T. urticae* (N)<sup>†</sup> on tomato and effectiveness of acaricides (Trial GH2)

Products	Rates (%)	BT	11 DAT1			18 DAT1		
		N	N	CPD (%)	EF (%)	N	CPD (%)	EF (%)
Naturalis	0.1	15.00 bc	1.10 cd	-92.7	96.5	1.05 b	-93.0	96.0
Naturalis	0.2	13.15 bc	2.75 c	-79.1	96.0	2.25 b	-82.9	90.3
Kingbo	0.1	9.15 c	0.10 d	-98.9	99.5	0.35 b	-96.2	97.8
Kingbo	0.2	30.70 abc	0.10 d	-99.7	99.8	0.55 b	-98.2	99.0
Abastate ME	0.05	31.70 ab	1.65 c	-94.8	97.6	0.95 b	-97.0	98.3
Envidor	0.06	42.40 a	8.10 b	-80.9	91.0	4.25 b	-90.0	94.3
Untreated	-	15.70 bc	33.50 a	113.4	-	27.80 a	77.1	-
	F	3.26	66.07			17.98		
	p	0.0199	< 0.0001			< 0.0001		

<sup>†</sup> The mean number of motile forms/leaf (5 leaves per plot, 100 cm<sup>2</sup> per leaf area)

BT = before treatment; DAT1 = days after 1<sup>st</sup> treatment of Naturalis and Kingbo

CPD (%) = change in population density; EF (%) = efficacy according to Henderson-Tilton formula

Within a column, the means followed by the same letter are not significantly different

(Duncan-test,  $\alpha = 0.05$ )

**Table 5.** Population densities of *P. ulmi* (N)<sup>†</sup> on apple and effectiveness of acaricides (Trial F2)

Products	Rates (%)	BT	11 DAT1			25 DAT1			30 DAT1		
		N	N	CPD (%)	EF (%)	N	CPD (%)	EF (%)	N	CPD (%)	EF (%)
Naturalis	0.1	3.13 a	0.60 b	-80.8	92.5	0.11 b	-96.5	97.6	0.01b	-99.7	99.6
Naturalis	0.2	1.18 ab	0.30 b	-74.6	90.1	0.12 b	-89.8	93.0	0.06 b	-94.9	93.3
Kingbo	0.1	0.68 b	0.06 b	-91.2	96.6	0.06 b	-91.2	93.9	0.02 b	-97.1	96.1
Kingbo	0.2	0.91 b	0.08 b	-91.2	96.6	0.08 b	-91.2	94.0	0.00 b	-100.0	100.0
Kraft 1.8 EW	0.1	0.55 b	0.18 b	-67.3	87.2	0.23 b	-58.2	71.2	0.10 b	-81.8	76.0
Masai	0.05	0.64 b	0.01 b	-98.4	99.4	0.08 b	-87.5	91.4	0.04 b	-93.8	91.8
Untreated	-	1.65 ab	4.23 a	156.4	-	2.40 a	45.4	-	1.25 a	-24.2	-
	F	2.32	3.77			8.06			2.36		
	p	0.0705	0.0106			0.0001			0.0673		

<sup>†</sup> The mean number of motile forms/leaf (25 leaves per plot)

BT = before treatment; DAT1 = days after 1<sup>st</sup> treatment of Naturalis and Kingbo

CPD (%) = change in population density; EF (%) = efficacy according to Henderson-Tilton formula

Within a column, the means followed by the same letter are not significantly different

(Duncan-test,  $\alpha = 0.05$ )

## DISCUSSION

So far, different *B. bassiana*-based products have been tested against the two-spotted spider mite on vegetables in greenhouse trials. Chandler et al. (2005) applied a commercial product based on the strain ATCC 74040 twice at 7-day interval at a rate of  $1 \times 10^5$  conidia/ml and achieved a reduction in the number of *T. urticae* motile forms on tomato by 85-97% 7 days after the second treatment. In trials on various crops (cucumber, tomato, eggplant), conidia of the *B. bassiana* isolate R444 were suspended in a silicone surfactant and applied at a rate of  $4.2 \times 10^6$  conidia/ml. The formulation was applied twice at 7-day interval and caused 60-86% mortality of adult mites 7 days after the second spray (Gatarayiha et al. 2010). Further studies showed that extending the interval between two treatments from 7 to 14 days could not improve the efficacy of this formulation significantly (Gatarayiha et al. 2011). These trials were conducted after artificial pest inoculations on host plants. In our trials conducted in commercial greenhouses under natural infestation, two applications of the mycopesticide Naturalis at a rate of  $2.3 \times 10^4$  conidia/ml and an interval of 5 days achieved >95% efficacy and reduced population density by >85%, 16-18 DAT1.

There is very little data on the effects of *B. bassiana* or some other fungal pathogens on European red mites. Santamarina et al. (1987) reported a high mortality and complete loss of fertility of *P. ulmi* females treated with crude extracts of *Penicillium funiculosum*. Recently, Afifi et al. (2010) tested the effect of *B. bassiana* in a laboratory trial, and the average mortality of *P. ulmi* adult females was 62.5% and 83.3% 14 days after treatment with  $2 \times 10^6$  and  $2 \times 10^8$  spores/ml, respectively. No data is available on the effects of commercial products or other formulations of *B. bassiana* on *P. ulmi* in greenhouse and field trials. One of the reasons for this situation is the fact that various environmental factors (sunlight, humidity, temperature or rain) can affect the effectiveness of fungal pathogens. Interfering effects of these factors can be significantly reduced by applying entomopathogenic fungi in oil-based formulations (Charnley and Collins, 2007; Maniania et al., 2008; Jaronski, 2010) such as Naturalis. Shi et al. (2008) showed that oil-formulated conidia of *B. bassiana* were highly viable at the regimes of 20-30°C and 51-95% RH. In our field trials, air temperature and humidity were within this range at treatment and in the first few days after it. Also, there was no rainfall in that period.

Very little data has been available on the acaricidal properties of oxymatrine, most of them in Chinese

research journals. The root extract of *S. flavescens* is known to contain matrine, oxymatrine and other substances that are also found in commercial oxymatrine-based products, and it is toxic to *T. urticae*, *T. cinnabarinus* and some other plant-feeding mites (Zheng et al., 2000; Fu et al., 2005; Han et al., 2012).

The results obtained in this study show that the mycopesticide Naturalis and botanical pesticide Kingbo can provide effective control of *T. urticae* and *P. ulmi* which is comparable to standard bioacaricides and synthetic acaricides. Considering that spider mite resistance to acaricides is a seriously increasing phenomenon (Knowles, 1997; van Leeuwen et al., 2009; Marčić, 2012), biopesticides may be a successful alternative to conventional chemical control. From the point of view of further improvement of spider mite management, additional laboratory and field research is needed, especially on compatibility of these products with predatory mites and other acaricides of natural origin, as well as their effectiveness after application with various adjuvants.

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# Evaluacija dva biopesticida u suzbijanju *Tetranychus urticae* i *Panonychus ulmi* (Acari: Tetranychidae) u plasteniku i polju

## REZIME

Ispitivana je efektivnost mikopesticida Naturalis (na bazi *Beauveria bassiana*, soj ATCC 74040) i botaničkog pesticida Kingbo (na bazi oksimatrina, alkaloida iz biljke *Sophora flavescens*, poznate u kineskoj tradicionalnoj medicini) u suzbijanju obične paučinaste grinje (*Tetranychus urticae*) na povrću u plasteniku i crvene voćne grinje (*Panonychus ulmi*) na jabuci. Ovi biopesticidi su primenjeni dva puta u razmaku od 5 dana, u koncentracijama 0,1% i 0,2%, a njihova efektivnost je upoređena sa akaricidima na bazi abamektina, akrinatrina i spirodiklofena, primenjenim jednom u preporučenim koncentracijama. Mikopesticid Naturalis, primenjen u koncentraciji 0,1% protiv *T. urticae* na krastavcu, redukovao je gustinu populacije za 85-86% i ostvario efikasnost 91-93%. U ogledu na paradajzu, efikasnost je dostigla 96%, dok je populaciona gustina redukovana za 93%. U ogledu u zasadu jabuke, Naturalis je pokazao rastuću i dugotrajnu efikasnost u suzbijanju letnje populacije *P. ulmi*: u oceni 30 dana posle prvog tretiranja zabeleženi su gotovo 100% efikasnost i redukcija populacione gustine. Efektivnost preparata Naturalis primenjenog u dvostruko većoj koncentraciji bila je nešto bolja, ali samo u prvom ogledu. Botanički pesticid Kingbo je pokazao visoku efikasnost ( $\geq 98\%$ ) i redukciju populacione gustine ( $\geq 96\%$ ) u oba oglada sa *T. urticae*. U ogledu suzbijanja *P. ulmi* postignuta je visoka i dugotrajna efektivnost ovog bioakaricida. Koncentracija 0,2% ostvarila je slične efekte. Rezultati ovih oglada pokazuju da se primenom preparata Naturalis i Kingbo obezbeđuje efikasno suzbijanje *T. urticae* na krastavcu i paradajzu u plasteniku, kao i *P. ulmi* na jabuci.

**Ključne reči:** Biopesticidi; grinje-paučinari; *Beauveria bassiana*; oksimatrin