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## Toxicity of biopesticides to green apple aphid, predatory insects and mite in an apple-tree orchard

Laimutis RAUDONIS, Laisvūnė DUCHOVSKIENĖ, Alma VALIUŠKAITĖ,  
Elena SURVILIENĖ

Institute of Horticulture  
Lithuanian Research Centre for Agriculture and Forestry  
Kauno 30, Babtai, Kaunas distr., Lithuania  
E-mail: l.raudonis@lsdi.lt

### Abstract

The investigations were carried out in 2005–2006 at the Lithuanian Institute of Horticulture. A two-year trial examined the toxicity of biopesticides BioNature R2000 (a.i. *Azadirachta indica* 210 g l<sup>-1</sup>, *Pinus resinosa* 180 g l<sup>-1</sup> and *Ricinus communis*), Bioshower (a.i. 100% fatty acids) and Insecticidal Soap (a.i. 20% fatty acids) to two species of predatory insects (*Chrysopa perla* L., *Coccinella septempunctata* L.) and predatory mite (*Amblyseius andersoni* Chant) in an apple-tree orchard. All tested products showed no toxicity to larvae of *C. perla*. BioNature R2000 was slightly or moderately toxic to larvae of *C. septempunctata*, meanwhile Bioshower and Insecticidal Soap had no toxicity to this predatory insect. Bioshower and Insecticidal Soap were moderately or very toxic to *A. andersoni*, but BioNature R2000 was only slightly toxic. Biopesticides BioNature R2000, Bioshower and Insecticidal Soap are allowed for use in organic farming in many countries and could be safely applied to control aphids.

Key words: *Amblyseius andersoni*, biopesticides, *Chrysopa perla*, *Coccinella septempunctata*, toxicity.

### Introduction

In Integrated Pest Management (IPM), important key predators, for example predatory mites and insects survive; thus the use insecticides against green apple aphid (*Aphis pomi* Deg.) can be reduced or omitted. The IPM, which is based on selective toxicity of pesticides to the invertebrate pests and harmless to predatory mites and insects, has become the most relevant strategy of plant protection (Leake, 2000). For the development of successful IPM and Organic Farming (OF) strategies, information is required on the direct effect of chemicals upon the invertebrate pests and their predators of a given ecosystem. The toxicity of pesticides on pests and their predators has been widely studied in many countries, using a range of different chemical pesticides at different rates on various developmental stages (Sterk et al., 1999; Martínez-Villar et al., 2005). However, the existing active substances of pesticides are reviewed by the European Commission and many old active substances with detrimen-

tal effects will be withdrawn from the market.

The organic fruit industry has shown the lowest growth rates compared to other approaches. One major reason is the high production risk due to high pest pressure and lack of control means (Tamm et al., 2004). Only some biopesticides, like insecticidal soap, azadirachtin complex mixtures or extracts of Neem tree *Azadirachta indica* etc. are allowed for pest control in organic farming. Meanwhile, little is known concerning the toxicity of biopesticides on pests and their predators in orchards. The toxicity of biopesticides on aphids, predatory mites and insects has been studied in some experiments, using insecticidal soap or products based on complex mixtures or extracts of Neem tree *Azadirachta indica*. However, studies showed controversial toxicity of biopesticides depending on pest or its predator's life stage, species, abiotic factors or even different years (Schuster, Stansly, 2000; Ahmad et al., 2003; Bostanian et al., 2005; Kara-

gounis et al., 2006; Duchovskienė, Karklelienė, 2008; Kraiss, Cullen, 2008; Tremblay et al., 2008). Biopesticides like BioNature R2000 and preparations based on fatty acids are mainly known as control means used against different species of aphids (Hummel, Kleeberg, 1997; Karagounis et al., 2006; Kraiss, Cullen, 2008; Raudonis et al., 2009). Meanwhile in sustainable agriculture it is very important to know how preparations affect different species of beneficial insects.

Therefore the aim of this study was to investigate the toxicity of some biopesticides on predatory mites and predatory insects and if they could be safely used in Integrated Pest Management or Organic Farming, without having significant detrimental effects on predatory mites and predatory insects.

## Materials and methods

The field trials were carried out on apple-trees under organic growing conditions in 2005–2006 at the Lithuanian Institute of Horticulture. Biopesticides BioNature R2000 3.0 l ha l<sup>-1</sup> (a.i. *Azadirachta indica* 210 g l<sup>-1</sup>, *Pinus resinosa* 180 g l<sup>-1</sup> and *Ricinus communis*), Bioshower 5.0 l ha l<sup>-1</sup> (a.i. 100% fatty acids) and Insecticidal Soap 20.0 l ha<sup>-1</sup> (a.i. 20% fatty acids) were tested. Apple trees were directly sprayed with a spray mixture equivalent to 500 l ha<sup>-1</sup> at growth stage 73 (during the second fruit

fall) according to BBCH scale (Meier, 1997). 5 trees were sprayed for each replicate, predatory mites and insects were assessed on 3 trees, meanwhile the first and the fifth tree was as protective separating the treatments from each other. The plots were randomly distributed and replicated four times.

Tests on predatory mite (*Amblyseius andersoni* Chant) and two predatory insect (sevenspotted lady beetle *Coccinella septempunctata* L., common green lacewing *Chrysopa perla* L.) species were carried out according to the following standard characteristics (Hassan et al., 1985; EPPO standards, 1999; Candolfi et al., 2000).

The number of living larvae of lacewing and lady beetle were counted or estimated on 10 previously marked extension shoots per plot. 25 leaves were collected from each replication and living predatory mites (adults and large nymphs) were counted under stereomicroscope. Predatory mites were identified according to keys of species (Chant, Hansell, 1971; Chant, Yoshida-Shaul, 1987; Chant, McMurtry, 1994). The first assessment was made shortly before application at growth stage 73, the second 3 days after application (growth stage 73) and the last 14 days after application (growth stage 75).

Meteorological data (air temperature and amount of precipitation) were recorded using scab warning equipment Metos D (Table 1).

**Table 1.** Meteorological conditions in 2005–2006

Month	Air temperature °C			Precipitation mm		
	2005	2006	average of 1924–2000	2005	2006	average of 1924–2000
June	14.8	16.3	16.6	66.6	13.8	50.4
July	19.4	19.3	17.6	3.8	30.2	71.8
August	14.7	17.5	16.3	109.4	173.4	75.8

The mortality of predators, was calculated by the formula  $x = 100 (1 - Ab/Ba)$ : x – mortality (%), A – number of predatory mites or insects before spraying in untreated plot, B – number of predatory mites or insects before spraying in treated plot, a – number of predatory mites or insects after spraying in untreated plot, b – number of predatory mites or insects after spraying in treated plot (Žemės ūkio augalų..., 2002). The mortality is explained as quantitative toxicity categories which were employed by the International Organization for Biological Control for assessment of pesticide toxicity to predatory and phytophagous mites in field trials: non-toxic (<25% mortality), slightly toxic (25–50%), moderately toxic (51–75%), very toxic (>75%) (Hassan et al., 1985).

The number of predators was compared among treatments with a single factor analysis of variance (*Anova*). Specific differences were identified with Fisher's criterion.

## Results and discussion

In both years of study predatory insects and predatory mites seemed to develop very similarly on untreated plots (Table 2, 3, 4). Meteorological conditions for predatory mite and predatory insect development were favourable, because it was warm in 2005–2006 (Table 1). Lower temperature was recorded in June and August of 2005, but it did not negatively affect insect and mite development, because the temperature was higher than the long-

term average for July, during the main insect and mite development period. Table 2 shows that on the trees treated with BioNature R2000, Bioshower and Insecticidal Soap, larvae of predatory insects *C. perla* survived and slightly increased 14 days after treatment. All biopesticides had no toxic effects

on *C. perla*, which increased to 7–11 per 10 shoots 14 days after treatment. Statistical reduction of the number of larvae was not found between these treatments and untreated plots. The mortality of biopesticides to *C. perla* was only 2.0–22.8%.

**Table 2.** Effects of biopesticides on survival of larvae of common green lacewing (*C. perla*) in apple-tree orchard

Treatment	Rate l ha <sup>-1</sup>	Mean number of larvae per 10 shoots			Mortality %	
		*before treatment	days after treatment		3	14
			3	14		
2005						
Control	–	6a	7b	9a	–	–
BioNature R2000	3.0	5a	5a	7a	14.3	6.7
Bioshower	5.0	7b	8b	9a	2.0	14.3
Insecticidal Soap	20.0	6a	6a	8a	14.3	11.1
LSD <sub>05</sub>		1.36	1.73	2.10		
2006						
Control	–	6a	6a	10a	–	–
BioNature R2000	3.0	7a	6a	9a	14.3	22.8
Bioshower	5.0	8b	7a	11a	12.5	17.5
Insecticidal Soap	20.0	6a	5a	8a	16.7	20.0
LSD <sub>05</sub>		1.64	2.06	2.18		

Note. \* – means in columns followed with different letters indicate significant differences between treatment at 95% significance level by Fisher's criterion.

**Table 3.** Effects of biopesticides on survival of larvae of seven-spotted lady beetle (*C. septempunctata*) in apple-tree orchard

Treatment	Rate l ha <sup>-1</sup>	Mean number of larvae per 10 shoots			Mortality %	
		*before treatment	days after treatment		3	14
			3	14		
2005						
Control	–	9a	10b	12b	–	–
BioNature R2000	3.0	10a	6a	7a	46.0	47.5
Bioshower	5.0	11a	10b	12b	18.2	10.0
Insecticidal Soap	20.0	9a	8b	10b	20.0	16.7
LSD <sub>05</sub>		2.11	2.39	1.72		
2006						
Control	–	8a	8b	11b	–	–
BioNature R2000	3.0	9a	4a	7a	55.5	43.4
Bioshower	5.0	10b	9b	12b	10.0	12.7
Insecticidal Soap	20.0	9a	8b	10b	11.1	19.2
LSD <sub>05</sub>		1.62	2.54	2.13		

Note. Explanation under Table 2.

The abundance of seven-spotted lady beetle larvae reached from 9 to 11 in 2005 and from 8 to 10 per 10 shoots in 2006 respectively. BioNature R2000, compared with Bioshower, Insecticidal Soap or control statistically reduced the number of larvae of *C. septempunctata* (Table 3). With the exception,

BioNature R2000, which induced a mortality rate of 55.5% 3 days after application in 2006, showed moderate toxicity to *C. septempunctata*. Bioshower and Insecticidal Soap were rated as non toxic to larvae of this predatory insect.

The numbers of predatory mites *A. andersoni* increased from 28–36 to 36–43 per 100 leaves in untreated plots during 2005–2006. Bioshower and Insecticidal Soap significantly reduced (to 8–13 and 7–11 mites per 100 leaves, respectively) the numbers of predatory mites and were from moderately to very toxic (Table 4). The mortality caused by Bioshower and Insecticidal Soap to predatory mites ranged from 60.7% to 77.3% and from 69.4% to 77.5% 3 days after treatment, respectively. The highest toxicity was recorded in 2006, compared with 2005. The populations of *A. andersoni* started

to increase after 14 days and both biopesticides were rated only as moderately toxic. The toxicity of BioNature R2000 was statistically lower on predatory mites than that of Bioshower or Insecticidal Soap. The mortality caused by BioNature R2000 to *A. andersoni* was from 34.8% to 45.7% after 3 and from 25.4% to 29.5% 14 days after application. Although all biopesticides were rated as moderately toxic 14 days after treatment, there was significantly lower reduction of the numbers of mites after BioNature R2000 treatment, compared with Bioshower or Insecticidal Soap.

**Table 4.** Effects of biopesticides on survival of predatory mite (*A. andersoni*) in apple-tree orchard

Treatment	Rate l ha <sup>-1</sup>	Mean number of predatory mites per 100 leaves			Mortality	
		*before treatment	days after treatment		3	14
			3	14		
2005						
Control	–	36a	35c	43c	–	–
BioNature R2000	3.0	41a	26b	38b	34.8	25.4
Bioshower	5.0	34a	13a	19a	60.7	53.2
Insecticidal Soap	20.0	37a	11a	18a	69.4	59.3
LSD <sub>05</sub>		8.82	5.81	3.22		
2006						
Control	–	28a	29d	36d	–	–
BioNature R2000	3.0	32a	18bc	29bc	45.7	29.5
Bioshower	5.0	34b	8ab	20b	77.3	54.2
Insecticidal Soap	20.0	30a	7a	16a	77.5	58.5
LSD <sub>05</sub>		7.64	8.12	4.34		

Note. Explanation under Table 2.

BioNature R2000 was slightly toxic on predatory mite *A. andersoni*, slightly or moderately toxic on predatory insect *C. septempunctata*, but it had no adverse effect on predatory insect *C. perla* in our studies. Moderate toxicity of azadirachtin on *A. andersoni* was reported by Castagnoli et al. (2002), but it was not toxic on eggs, larvae and adults of lacewings (Schuster, Stansly, 2000). High toxicity of azadirachtin on *C. septempunctata* was reported. However, azadirachtin was only very toxic to *C. septempunctata* when feeding on aphids (Ahmad et al., 2003). Higher mortality of *A. andersoni* after Bioshower and Insecticidal soap treatment was recorded 3 days after treatment in 2006 compared with 2005. It was found out that higher toxicity of biopesticide like Insecticidal soap to predatory mite populations depends on meteorological factors (Hummel, Kleeberg, 1997; Bosta-

nian, Akalach, 2006; Kraiss, Cullen, 2008). Higher air temperature especially in June and August in 2006 resulted in statistically higher mortality of predatory mites after Insecticidal soap or Bioshower treatment in this study.

There was not found any published data on toxicity of Bioshower to predatory insects and predatory mites. However, both formulations of Bioshower and Insecticidal Soap are based on fatty acids and the toxicity of Bioshower and Insecticidal Soap could be similar. Both preparations had the least detrimental effect on *C. perla* and *C. septempunctata*. Insecticidal soaps did not cause any significant decrease of these predatory insects in other studies (Schuster, Stansly, 2000; Karagounis et al., 2006). Only few studies on toxicity of biopesticides to predatory mite *A. andersoni* were carried out (Castagnoli et al., 2002). The effect of insecticidal

soaps on phytoseiid predatory mites such as *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius fallacies* Garman was studied in Canada and it was found that biopesticide reduced the number of mites significantly (Bostanian, Akalach, 2006). Insecticidal soap decreased the number of other species of beneficials as *Aphidius colemani* (Viereck) and *Harmonia axyridis* (Pallas) (Bostanian et al., 2005; Kraiss, Cullen, 2008; Tremblay et al., 2008). In the present study Bioshower and Insecticidal Soap statistically resulted in lower densities of predatory mite *A. andersoni*, but the predators were not eradicated and both preparations were rated as very toxic only 3 days after treatment.

## Conclusions

1. The results of this study showed that BioNature R2000 had no adverse effects on larvae of common green lacewing *C. perla*, however, was slightly or moderately toxic to larvae of *C. septempunctata* and only slightly toxic to predatory mite *A. andersoni*.

2. Bioshower and insecticidal soap were rated as not toxic to *C. perla* and *C. septempunctata*, meanwhile they were moderately or very toxic to *A. andersoni*.

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## Biopesticidų toksiškumas žaliajam obeliniam amarui, grobuoniškiesiems vabzdžiams ir erkei obelų sode

L. Raudonis, L. Duchovskienė, A. Valiuškaitė, E. Survilienė

Lietuvos agrarinių ir miškų mokslų centro  
Sodininkystės ir daržininkystės institutas

### Santrauka

2005–2006 m. Lietuvos sodininkystės ir daržininkystės instituto obelų sode tirtas biopesticidų BioNature R2000 (v. m. *Azadirachta indica* 210 g l<sup>-1</sup>, *Pinus resinosa* 180 g l<sup>-1</sup> ir *Ricinus communis*), Bioshower (v. m. 100 % riebiųjų rūgščių) ir Insekticidinio muilo (v. m. 20 % riebiųjų rūgščių) toksiškumas dviejų rūšių grobuoniškiesiems vabzdžiams (*Chrysopa perla* L., *Coccinella septempunctata* L.) bei grobuoniškajai erkei (*Amblyseius andersoni* Chant). Visi tirti biopreparatai nebuvo toksiški paprastosios auksakės (*C. perla*) lervoms. BioNature R2000 buvo mažai arba vidutiniškai toksiškas, o Bioshower ir Insekticidinis muilas buvo netoksiškas septyntaškės boružės (*C. septempunctata*) lervoms. Bioshower ir Insekticidinis muilas buvo vidutiniškai arba labai toksiški, tačiau BioNature R2000 – tik mažai toksiškas grobuoniškajai erkei (*A. andersoni*). Biopesticidai BioNature R2000, Bioshower ir Insekticidinis muilas daugelyje šalių leidžiami naudoti ekologinėje sodininkystėje, todėl šie biopreparatai ekologiniuose obelų soduose gali būti tinkami naudoti obelų apsaugai nuo žaliojo obelinio amaro.

Reikšminiai žodžiai: *Amblyseius andersoni*, biopesticidai, *Chrysopa perla*, *Coccinella septempunctata*, toksiškumas.