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Effectiveness of Azadirachtin (NeemAzal-T/S) in Controlling Pear Psylla (*Cacopsylla pyri*) and European Red Mite *(Panonychus ulmi*)

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SUMMARY

Here we present the results of field trials conducted in Serbia to evaluate the effectiveness of a neem-based product, NeemAzal-T/S (containing azadirachtin-A as its active ingredient in the form of an emulsifiable concentrate) against pear psylla (*Cacopsylla pyri*) and European red mite (*Panonychus ulmi*).

Efficacy evaluation against *C. pyri* was carried out in a commercial pear orchard of the Williams pear cultivar, located at Borkovac (Ruma). The insecticides were applied at BBCH 09 pear growth stage, several days before the beginning of hatching of the first generation larvae. The efficacy of azadirachtin was compared to that of mineral oil, abamectin and diflubenzuron. Efficacy evaluation 18 DAT showed total termination of egg laying by *C. pyri* after treatments with azadirachtin and abamectin, while some new (white) eggs were found after treatment with mineral oil. Diflubenzuron treatment failed to fully stop egg laying, but the number of white eggs was significantly lower than it was in the control. Azadirachtin and abamectin achieved 100% efficacy, while the effectiveness of mineral oil was 97.4%, and that of diflubenzuron a mere 59%. All four insecticides significantly reduced the number of older (yellow) eggs and larvae, the efficacy being 80.5-92.6% (yellow eggs), 69.8-79.3% (larvae I-III instar) and 94.3-100% (larvae IV-V instar). In evaluation 38 DAT, azadirachtin, abamectin and mineral oil achieved 100% efficacy against white and yellow eggs, while diflubenzuron achieved 93% and 86.9% efficacy. All four insecticides were found to demonstrate high efficacy against I-III instar larvae (99.2-100%), but mineral oil treatment alone achieved high efficacy against IV-V instar larvae (92.4%) as well.

Efficacy evaluation against *P. ulmi* was carried out in a commercial orchard of the Red Chief apple cultivar located at Morović (Šid). Azadirachtin efficacy in controlling a summer population of European red mite was compared to a mineral oil, clofentezine and spirodiclofen. The acaricides were applied when the number of *P. ulmi* motile forms was below the orientational damage threshold of 3 motile forms per leaf. Azadirachtin reduced significantly the number of motile forms, leaving it well below damage threshold by the end of the trial. Good control efficacy was achieved (77.2-90.4%), the highest level of efficacy being recorded at the end of the trial 38 DAT. In mineral oil treatment 7 DAT, efficacy was 92.4%; 14 DAT and 21 DAT it was 75.2% and 78.9%, and it dropped to below 60% at the end of the trial. The efficacy of clofentezine (39.4-68.2%) was unsatisfactory, which is probably the result of a resistance developing under high selection pressure of these compounds in the Morović locality in preceding years, while the newly introduced acaricide spirodiclofen showed high and long-lasting efficacy (97.2%, 38 DAT).

The results of the trials are discussed in terms of improving management of the populations of European red mite and pear psylla.

Keywords: Azadirachtin; *Cacopsylla pyri; Panonychus ulmi;* Control

INTRODUCTION

Conventional broad-spectrum synthetic pesticides have long been used successfully for chemical control of insect and mite pests of agricultural crops. However, as a result of heavy selection pressure caused by their frequent and unrational use many arthropod pest populations have developed resistance worldwide (Whalon et al., 2008). Besides resistance evolution, most conventional pesticides are harmful to arthropod natural enemies and other non-target species. This situation has intensified research that seeks to find alternative, biorational pesticides, i.e. synthetic and/or natural compounds with novel modes of action that are more selective and ecotoxicologically safer, and therefore suitable to be included in pest management programs based on integration of biological and chemical control measures (Horowitz and Ishaaya, 2004).

Regarding botanical pesticides, research has especially focused on biological effects of derivatives (extracts, oils, powders) of seed kernels of the Indian neem tree (*Azadirachta indica*), which contain azadirachtin, a limonoid compound, as their principal insecticidal ingredient. Neem products affect a number of insect and mite pests, and their subtle activity (e.g. feeding and oviposition deterrence, growth and development inhibition, sterilization) is considered more desirable in integrated pest management programs than the quick knock-down effect of synthetic neurotoxic pesticides (Schmutterer, 1990; Koul, 2004; Isman, 2006; Copping and Duke, 2007). There are many commercial neem-based pesticide products registered in more than 40 countries against key pests in fruit, vegetables and ornamentals (Kleeberg, 2004; Koul, 2004).

Here we present the results of field trials conducted in Serbia to evaluate the effectiveness of a neem-based product, NeemAzal-T/S (containing azadirachtin-A as its active ingredient in the form of emulsifiable concentrate) against pear psylla (*Cacopsylla pyri*) and European red mite (*Panonychus ulmi*). This product is an environment-friendly biopesticide, non-toxic to pollinators and most other beneficial organisms, and compatible with organic food production (Kleeberg, 2004; Isman, 2006; Zehnder et al., 2007). The results of the trials are discussed in terms of improving management of the populations of pear psylla and European red mite.

MATERIAL AND METHODS

Efficacy evaluation against *Cacopsylla pyri*

A field trial was carried out in a commercial pear orchard of the Williams pear cultivar located at Borkovac (Ruma) (N:45°02.181' E:019°49.563') in 2007. Meteorological data for the relevant period are shown in Figure 1. The trial was conducted with the following characteristics: experimental design - randomized block; plot size - 5 trees; replications - 4; type of application - spraying until run-off. The insecticides tested in this trial are listed in Table 1. The insecticides were applied on March 16 at the BBCH 09 pear growth stage, i.e. several days before the beginning of hatching of the eggs laid by *C. pyri* winterform females; 18 and 38 days after treatment (DAT), eggs and larvae were counted on 10 marked shoots per plot. The eggs were counted as younger, 'white eggs', and older, 'yellow eggs', and the larvae were counted as L_{1-3} (I-III instar) and L_{4-5} larvae (IV-V instar). Efficacy was calculated by Abbott's formula (1).

$$Ef\,\% = [1 - (Nta/Nca)(Ncb/Ntb)] \times 100 \qquad (1)$$

N = number of eggs or larvae per shoot
t = treated plots
c = control plots
a = after treatment
b = before treatment

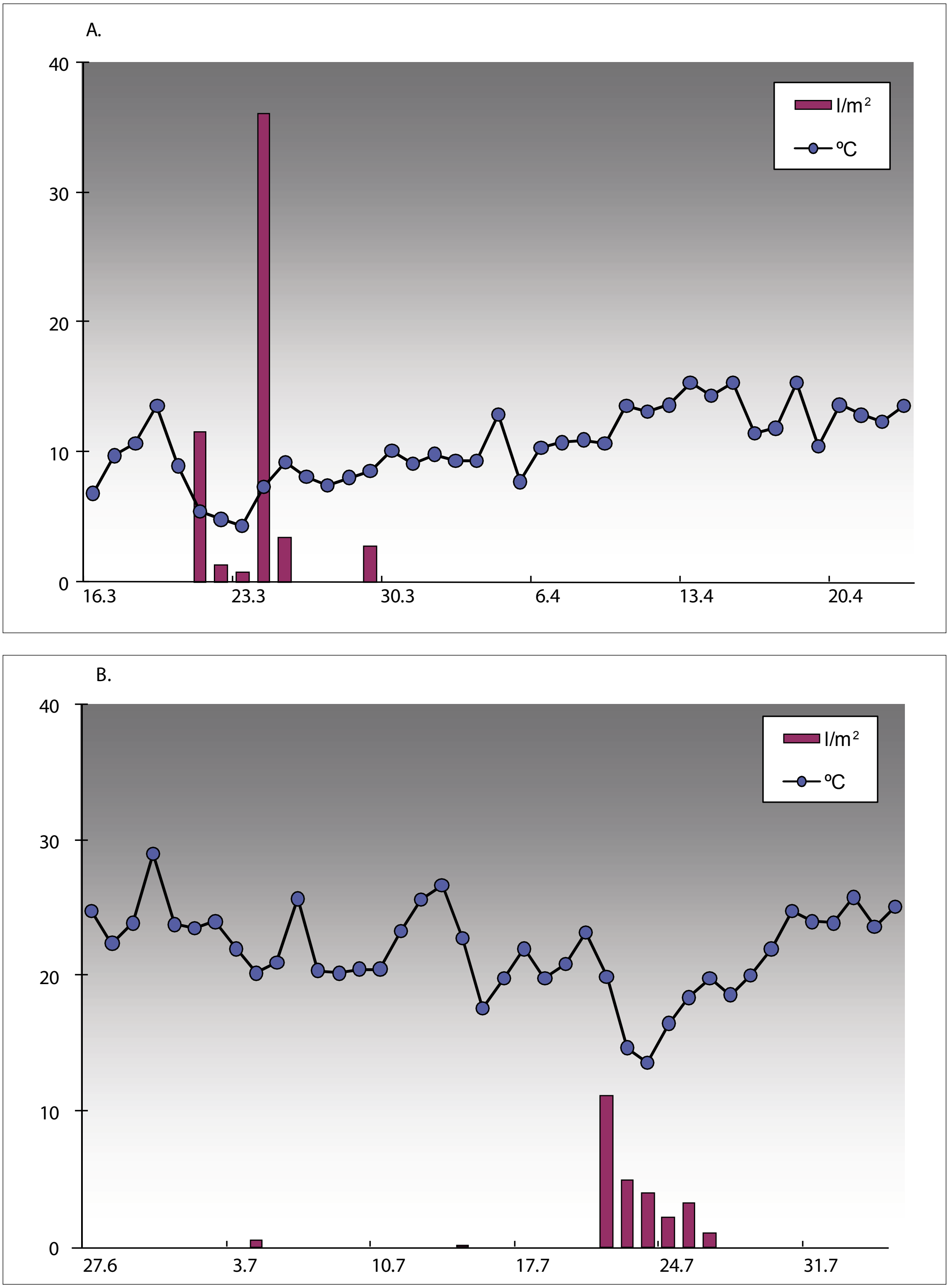

Figure 1. Mean daily temperature (ºC) and rainfall (l/m²) at Borkovac (A) and Morović (B) localities during the trials

Efficacy evaluation against *Panonychus ulmi*

Field trial was carried out in a commercial orchard of the Red Chief apple cultivar located at Morović (Šid) (N:45°00.711' E:019°15.146') in 2008. Meteorological data for the trial period are presented in Figure 1. The trial was conducted with the following characteristics: experimental design - randomized block; plot size - 5 trees; replications - 4; type of application – spraying; spray volume – 1500 l/ha. The acaricides tested in the trial are listed in Table 1. The acaricides were applied on June 27; motile forms of *P. ulmi* on 25 leaves per plot were counted just before treatment and 7, 14, 21 and 38 DAT, and efficacy was calculated by Henderson-Tilton's formula (2).

$$Ef\,\% = (1 - Nta/Nca)\times 100 \qquad (2)$$

N = number of motile pest forms per leaf
t = treated plots
c = control plots
a = after treatment

Statistical analysis

The pests (eggs and larvae of pear psyllids, motile forms of spider mites) were counted per plot, the numbers were compared using ANOVA and the means were separated by *t*-test. The data were transformed by $\sqrt{x+0.5}$ before analysis; untransformed data are presented in this paper.

RESULTS AND DISCUSSION

Control of *Cacopsylla pyri*

The efficacy of azadirachtin (NeemAzal-T/S) in controlling the first generation of pear psylla was compared to that of mineral oil, abamectin and diflubenzuron. The insecticides were applied before the hatching of the first generation larvae, when yellow eggs accounted for about 50%. Efficacy evaluation 18 DAT (Tab. 2) showed total termination of egg laying by *C. pyri* after treatments with azadirachtin and abamectin (Ew = 0), while some new eggs were found after treatment with mineral oil (Ew = 0.38). Diflubenzuron treatment failed to fully stop egg laying, but the number of white eggs was significantly lower than it was in the control. Azadirachtin and abamectin achieved 100% efficacy, while the effectiveness of mineral oil was 97.4%, and that of diflubenzuron a mere 59% (Figure 2). All four insecticides significantly reduced the number of older yellow eggs and larvae, the efficacy being 80.5-92.6% (yellow eggs), 69.8-79.3% (larvae I-III instar) and 94.3-100% (larvae IV-V instar).

Azadirachtin, abamectin and mineral oil achieved 100% efficacy against white and yellow eggs in evaluation 38 DAT (Table 2, Figure 3), while diflubenzuron achieved 93% and 86.9% efficacy. All four insecticides were found to demonstrate high efficacy against I-III instar larvae (99.2-100%), but mineral oil treatment alone achieved high efficacy against IV-V instar larvae (92.4%) as well.

The effect of NeemAzal-T/S results from a combination of direct activity of egg treatment and deterrence of *C. pyri* females. Erler (2004) reported of a neem-based product having a considerable oviposition deterrence

Table 1. Pesticides applied in the trials

Active ingredient (g a.i./l)	Product	Formulation	Manufacturer	Locality
Abamectin (18)	Armada	Emulsifiable concentrate	Willowood Ltd. Hong Kong	Borkovac
Azadirachtin A (10)	NeemAzal-T/S	Emulsifiable concentrate	Trifolio-M GmbH, Germany	Mororović Borkovac
Clofentezine (500)	Apollo 50-SC	Suspension concentrate	Makhteshim Chemical Works, Israel	Morović
Diflubenzuron (480)	Dimilin SC-48	Suspension concentrate	Crompton-Uniroyal Chemical, USA	Borkovac
Mineral oil (940)	Galmin	Emulsifiable concentrate	Galenika-Fitofarmacija, Serbia	Mororović Borkovac
Spirodiclofen (240)	Envidor	Suspension concentrate	Bayer CropScience, Germany	Morović

Table 2. The number of *C. pyri* eggs (Ew = white eggs; Ey = yellow eggs) and larvae (L_{1-3} = I-III instar; L_{4-5} = IV-V instar) [1)] 18 and 38 days after treatment (DAT) at BBCH 09 pear growth stage against 1st generation eggs

Pesticide	Product rate (g/l)	18 DAT			
		Ew	Ey	L1-3	L4-5
Azadirachtin	10.0	0.00 *a*	3.10 *a*	1.95 *a*	0.00 *a*
Mineral oil	25.0	0.38 *a*	5.30 *a*	2.38 *a*	0.02 *a*
Abamectin	0.75	0.00 *a*	2.00 *a*	1.95 *a*	0.10 *a*
Diflubenzuron	0.25	6.05 *b*	2.64 *a*	2.85 *a*	0.05 *a*
Untreated	-	14.75 *c*	27.18 *b*	9.44 *b*	1.74 *b*
Pesticide	**Product rate (g/l)**	**38 DAT**			
		Ew	Ey	L1-3	L4-5
Azadirachtin	10.0	0.00 *a*	0.00 *a*	0.00 *a*	4.40 *b*
Mineral oil	25.0	0.00 *a*	0.00 *a*	0.12 *a*	1.30 *a*
Abamectin	0.75	0.00 *a*	0.00 *a*	0.05 *a*	5.85 *b*
Diflubenzuron	0.25	1.00 *a*	2.25 *a*	0.00 *a*	5.55 *b*
Untreated	-	14.20 *b*	17.20 *b*	14.50 *b*	17.10 *c*

1) per shoot (10 shoots per plot)
The means followed by the same letter are not significantly different (t-test, P<0.05)

against winterform pear psylla females 7 DAT. In our first evaluation of azadirachtin treatment there were no newly laid eggs, while some yellow eggs and I-III instar larvae were observed; in the second evaluation, only IV-V instar larvae were found, meaning that some eggs continued to develop after treatment. Larvae hatched from the treated eggs were affected due to their contact with azadirachtin residues still active on the chorion and/or plant surface. The possibility of direct effect on embryonic development should not be discarded, even though such activity has not been confirmed experimentally as yet.

Pear psylla requires season-long management because the pest has 4-5 generations per year and multiple pesticide applications are needed. Besides the compounds tested in the present trial, successful control of *C. py-*

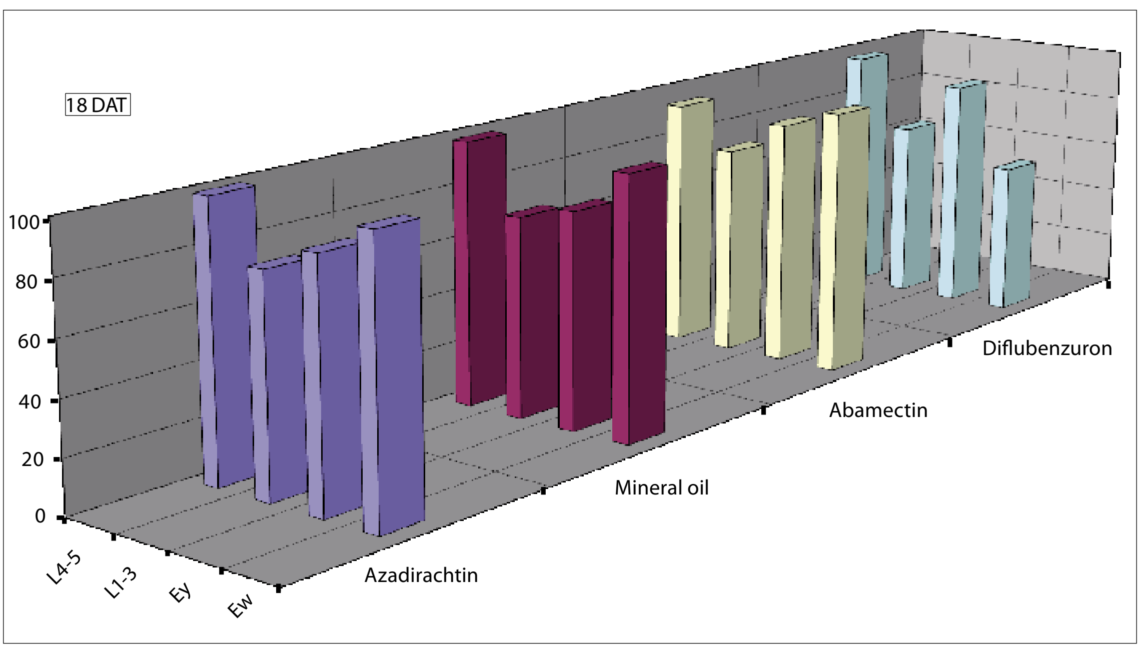

Figure 2. The efficacy of insecticides against eggs (Ew = white eggs, Ey = yellow eggs) and larvae (L_{1-3} = larvae I-III instar, L_{4-5} = larvae IV-V instar) of *C. pyr*i 18 DAT

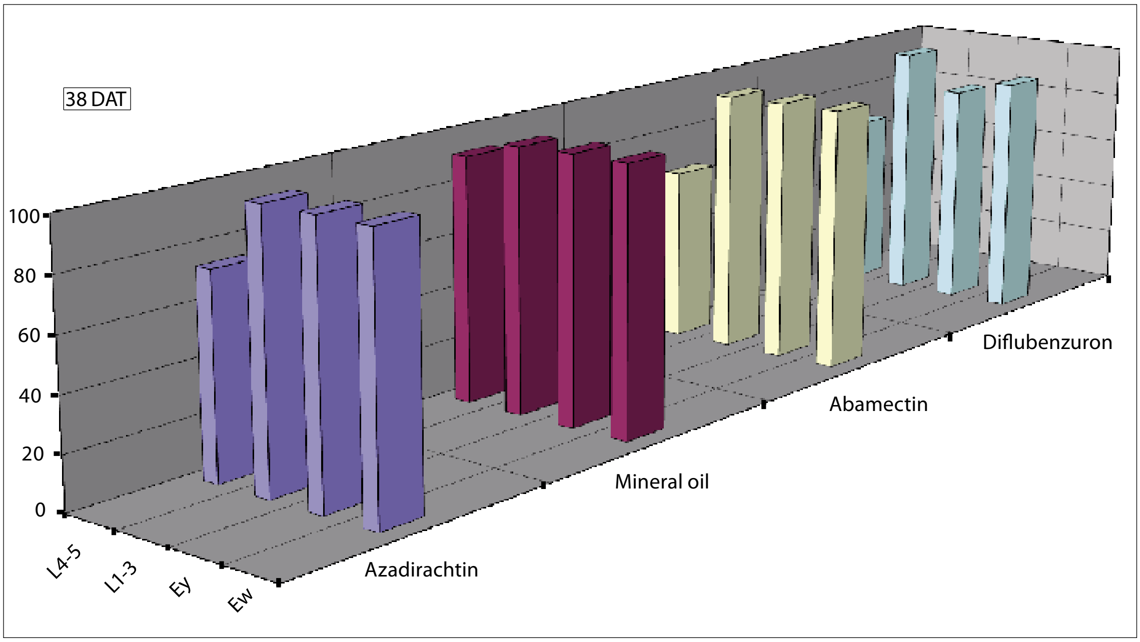

Figure 3. The efficacy of insecticides against eggs (Ew = white eggs, Ey = yellow eggs) and larvae (L_{1-3} = larvae I-III instar, L_{4-5} = larvae IV-V instar) of *C. pyr*i 38 DAT

ri can be achieved by spirodiclofen (De Maeyer et al., 2000; Marčić et al., 2007, 2008), thiacloprid (Kocourek and Stará, 2006), thiametoxam (Miletić and Tamaš, 2006) or fenoxycarb (Souliotis and Moschos, 2008), applied alone or combined with summer oils. However, following the general principles of resistance management, alternating products with different modes of action should be used, so that only one generation per season would be exposed to a class. The data acquired from our field trial proved azadirachtin (NeemAzal-T/S) to be an effective agent against the 1st generation of pear psylla. Further research is needed to test this product alone and combined with other biorational pesticides throughout the season. Product manufacturer has warned of a potential phytotoxicity of NeemAzal-T/S to several pear cultivars (Anonymous, 2009). In our trial on pear, no signs of phytotoxicity were observed on the treated plants during the trial.

Control of *Panonychus ulmi*

Azadirachtin (NeemAzal-T/S) efficacy in controlling a summer population of European red mite was compared to a mineral oil, clofentezine and spirodiclofen. The acaricides were applied when the number of *P. ulmi* motile forms was below the orientational damage threshold of 3 motile forms per leaf (Table 3). Azadirachtin reduced significantly the number of motile forms of *P. ulmi* per leaf, leaving it well below damage threshold by the end of the trial. Good control efficacy was

Table 3. The number of *P. ulmi* motile forms1) before treatment (BT) and 7, 14, 21 and 38 days after treatment (DAT)

Pesticide	Product rate (g/l)	Mean number1) of *P. ulmi*				
		BT	7 DAT	14 DAT	21 DAT	38 DAT
Azadirachtin	10.0	1.65 *a*	0.26 *a*	1.12 *a*	0.82 *a*	0.08 *a*
Mineral oil	25.0	1.70 *a*	0.15 *a*	1.47 *a*	0.78 *a*	0.35 *a*
Clofentezine	0.6	1.69 *a*	0.91 *a*	3.00 *ab*	1.17 *a*	0.52 *ab*
Spirodiclofen	0.4	2.10 *a*	0.00 *a*	0.19 *a*	0.20 *a*	0.03 *a*
Untreated		2.62 *a*	3.05 *b*	9.13 *b*	5.70 *b*	1.33 *b*

1) motile forms per leaf (25 leaf per plot)
The means followed by the same letter are not significantly different (t-test, P<0.05)

achieved (77.2-90.4%), the highest level of efficacy being recorded at the end of the trial 38 DAT (Figure 4). Azadirachtin is a slow acting pesticide: immatures react with feeding and molting inhibition and adult females show feeding inhibition and reduced progeny production. Mortality is delayed as a result of molting disruption and starvation (Kleeberg, 2004). Considering the results reported by Hiiesaar et al. (1998), the effect achieved by NeemAzal-T/S could also be the result of an ovicidal activity, i.e. a disruption of embryonic development. In mineral oil treatment 7 DAT, efficacy was 92.4%; 14 DAT and 21 DAT it was 75.2% and 78.9%, and it dropped to below 60% at the end of the trial. Considering that the mode of action of horticultural mineral oils on insects and mites is suffocation, and the fact that more than a single treatment is normally required to achieve effective control of tetranychid mites in apple orchards (Agnello et al., 1994; Fernandez et al., 2005), such a level of efficacy may be considered satisfactory. On the other hand, the efficacy of clofentezine (39.4-68.2%) was unsatisfactory, which is probably the result of a resistance developed under high selection pressure of these compounds in the Morović locality in preceding years. The newly introduced acaricide spirodiclofen showed high and long-lasting efficacy (97.2%, 38 DAT), which was expected with regard to its previous performance (Wachendorrf et al., 2000; Elbert et al., 2002; Marčić et al., 2007).

Populations of European red mite have an exceptional natural potential for rapid pesticide resistance evolution: this mite is ninth on a list of top 10 resistant arthropod pests with data showing its resistance to 38 compounds developed in the period 1914-2007 (Whalon et al., 2008). Because of its new mode of action – inhibition of lipid synthesis – spirodiclofen is a solution for controlling mite populations resistant to clofentezine and other acaricides (Nauen et al., 2000; Elbert et al., 2002). On the other hand, in order to prevent/delay development of resistance to an acaricide, only one treatment per cropping cycle is recommended for compounds of the same mode of action (Wege and Leonard, 1994). As a result, there is a constant need for having new acaricides of different modes of activity available in order to expand their choice over the season. Tested against spider mites in laboratory, greenhouse and field trials, neem-based products have shown di-

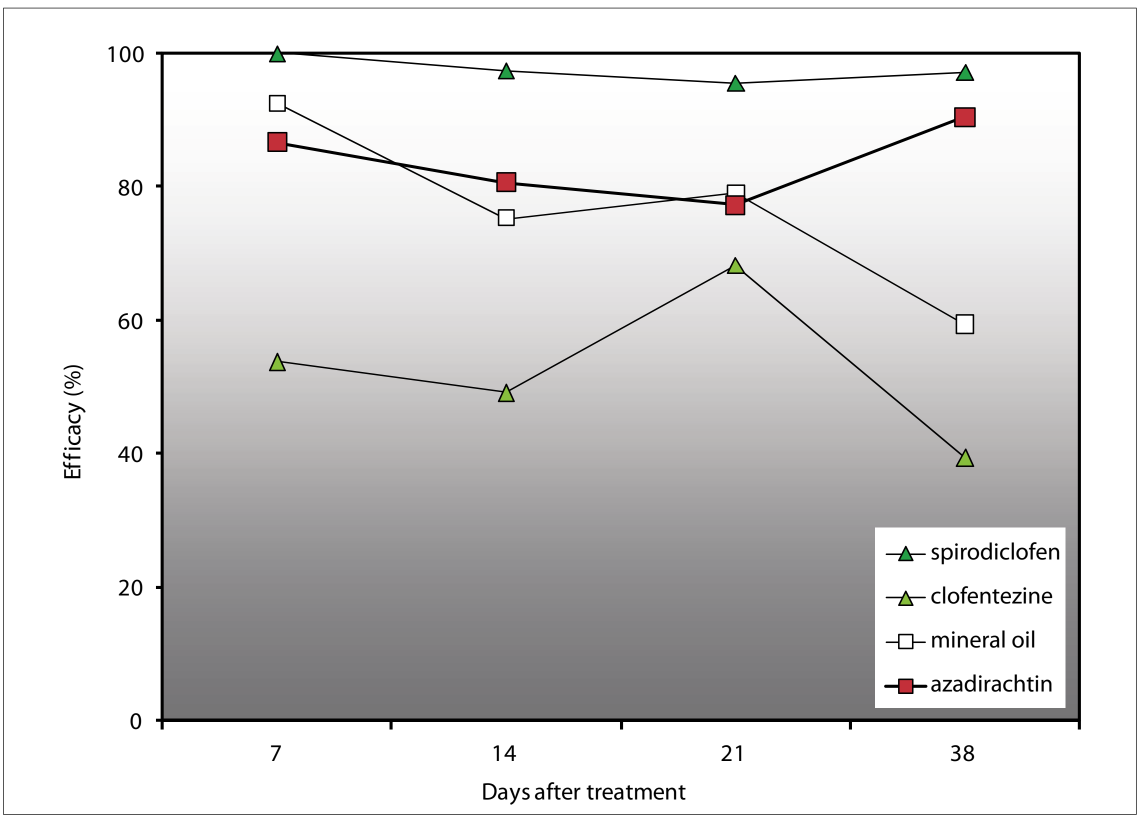

Figure 4. The efficacy of acaricides against motile forms of *P. ulmi* 7, 14, 21 and 38 DPT

rect toxic effects on different development stages, feeding and oviposition deterrence, reduction in fecundity, fertility and longevity (Sundaram and Sloane, 1995; Mansour et al., 1997; Hiiesaar et al., 1998; Kashenge and Makundi, 2001; Kleeberg and Hummel, 2001; Chiasson et al., 2004; Martinez-Villar et al., 2005). The extent of these effects was dependent on the type of formulations of the neem products. Kleeberg (2004) emphasized that the success of application of neem-based products against spider mites depended on progress of pest infestation and usually required repetitive application. The data acquired from our field trial indicate that a single treatment with NeemAzal-T/S could be sufficient for effective control of *P. ulmi* summer populations on condition that it is applied at a low infestation level. Due to the specific and complex mode of action of azadirachtin (Isman, 2006) there is no risk of cross-resistance, so that this product is potentially a good alternative for successful control of resistant mite populations.

In our trials, NeemAzal-T/S achieved effective control of the 1st generation of *C. pyri* and summer population of *P. ulmi*. Further investigation should be carried out to evaluate this product alone and in combination with other biorational pesticides throughout the season, with a special emphasis on its compatibility with the key natural enemies of these pests, and (in pear orchards) on its possible phytotoxic effects on different cultivars.

ACKNOWLEDGEMENT

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Efektivnost azadirahtina (NeemAzal-T/S) u suzbijanju kruškine buve (*Cacopsylla pyri*) i crvene voćne grinje (*Panonychus ulmi*)

REZIME

Prikazani su rezultati poljskih ogleda realizovanih u Srbiji u cilju ocene efektivnosti nim-derivata NeemAzal-T/S (koji sadrži azadirahtin-A kao aktivnu materiju u formi koncentrata za emulziju) u suzbijanju kruškine buve (*Cacopsylla pyri*) i crvene voćne grinje (*Panonychus ulmi*).

Ogled ocene efektivnosti suzbijanja *C. pyri* izveden je u komercijalnom zasadu kruške sorte Viljamovka u Borkovcu (Ruma). Insekticidi su primenjeni u fenofazi BBCH 09, nekoliko dana pre početka piljenja larvi prve generacije. Efikasnost azadirahtina upoređena je sa efikasnošću mineralnog ulja, abamektina i duflubenzurona. Ocena efekata 18 DPT pokazala je da je polaganje jaja *C. pyri* potpuno zaustavljeno u tretmanima azadirahtinom i abamectinom, dok je u tretmanu mineralnim uljem ipak registrovan izvestan broj novih (belih) jaja. U tretmanu diflubenzuronom polaganje jaja nije zaustavljeno, ali je broj belih jaja bio značajno niži u poređenju sa kontrolom. Efikasnost azadirahtina i abamektina iznosila je 100% a efikasnost mineralnog ulja 97,4%, dok je efikasnost diflubenzurona bila svega 59%. Sva četiri insekticida značajno su redukovala broj starijih, žutih jaja i larvi, a efikasnost je iznosila 80,5-92,6% (žuta jaja), 69,8-79,3% (larve I-III stupnja) i 94,3-100% (larve IV-V stupnja). U oceni 38 DPT u tretmanima azadirahtinom, abamektinom i mineralnim uljem zabeležena je 100% efikasnost redukcije broja belih i žutih jaja, dok je diflubenzuron ostvario efikasnost od 93% i 86,9%. Sva četiri insekticida ispoljila su visoku efikasnost u suzbijanju larvi I-III stupnja (99,2-100%), ali je samo u tretmanu mineralnim uljem zabeležena visoka efikasnost i protiv larvi IV-V instar stupnja (92,4%).

Ogled ocene efektivnosti suzbijanja *P. ulmi* izveden je u komercijalnom zasadu jabuke sorte Red Chief u Moroviću (Šid). Efikasnost azadirahtina u suzbijanju letnje populacije crvene voćne grinje upoređena je sa efikasnošću mineralnog ulja, klofentezina i spirodiklofena. Akaricidi su primenjeni u vreme kad je brojnost *P. ulmi* bila ispod orijentacionog praga štetnosti od 3 pokretne forme po listu. Azadirahtin je značajno redukovao broj pokretnih formi, koji je do kraja ogleda ostao znatno ispod praga štetnosti. Postignuta je dobra efikasnost suzbijanja (77,2-90,4%), s tim da je najviši nivo efikasnosti registrovan na kraju ogleda, 38 DPT. U tretmanu mineralnim uljem, 7 DPT ostvarena je efikasnost 92,4%; 14 DPT and 21 DPT efikasnost je bila 75,2% i 78,9%, da bi na kraju ogleda pala ispod 60%. Efikasnost klofentezina (39,4-68,2%) bila je nezadovoljavajuća, što je verovanto rezultat rezistentnosti nastale pod snažnim selekcionim pritiskom ovim akaricidom prethodnih godina, dok je efikasnost novog akaricida spirodiklofena bila visoka i dugotrajna (97,2%, 38 DPT).

Ostvareni rezultati razmatrani su u kontekstu unapređenja suzbijanja populacija ove dve štetne vrste.

Ključne reči: Azadirahtin; *Cacopsylla pyri*; *Panonychus ulmi;* suzbijanje