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SIDE EFFECTS OF TWO PLANT INSECTICIDES ON NATURAL ENEMIES OF INSECTS IN ALFALFA (*MEDICAGO SATIVA* L.) SEED PRODUCTION

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Abstract

During the period 2012-2014, in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria, the side effect of neem and pyrethrum products on banded thrips, *Aeolothrips intermedius* Bagnall (Thysanoptera: Thripidae) and the seven-spot ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) adults was defined. Treatments were carried out at the beginning of the flowering stage of alfalfa (*Medicago sativa* L.) in the second intercut. Sweeping with an entomological net was used to collect specimens. It was found that the biological insecticides NeemAzal and Pyrethrum were nontoxic with respect to *Coccinella septempunctata* adults, and in almost 70% of cases were harmless to *Aeolothrips intermedius*. Analysis of variance (ANOVA) regarding product toxicity on predatory species demonstrated that the factor B (type of insecticide) had the strongest effect – 89.1 and 82.5% of the total variance for *C. septempunctata* and *A. intermedius*, respectively. Tested plant insecticides showed no negative impact on the predatory species and they can be applied in organic seed production of alfalfa.

KEY WORDS: NeemAzal; Pyrethrum; toxicity; *Coccinella septempunctata*, *Aeolothrips intermedius*; alfalfa

Introduction

As a consequence of agricultural intensification, biodiversity is diminishing in agricultural areas, with some species (in this case, insects) predominating in existing natural ecosystems (Kolařík *et al*., 2010). Information about the side effects of pesticides on beneficial organisms is used to appraise the possibility of their use in integrated pest management (IPM) programs (Way & van Emden, 2000; Tedeschi *et al*. 2001; Cameron *et al*., 2009). Methods of organic farming, as part of integrated control, support and improve the biodiversity of natural ecosystems because they help to create more diversified living conditions, offer better conditions for the reproduction and propagation of individual species, increase the supply of nutrients, etc. This system is focused on the problems of environmental and landscape protection. On organically managed arable land, in permanent grassland, field margins and neighboring biotopes, the biodiversity of both flora and fauna is

greater than in conventional farmed areas (Šarapatka & Urban, 2005). Additionally, the diversity of crops cultivated under organic production is higher than on conventionally managed farms (Šarapatka & Urban, 2005).

The most significant factor disrupting the biological control of arthropod pests in most cropping systems is the use of insecticides (Croft, 1990). The use of insecticides causes the mortality of target and non-target species. Toxicological studies of conventional and broad-spectrum insecticides under both laboratory and field conditions have revealed that the use of such materials typically leads to direct natural enemy mortality in the laboratory and reduced population densities and activity in the field (Simmons & Jackson, 2000). The decrease in the number of natural enemies caused by the use of non-selective insecticides may have serious consequences for pest population dynamics, such as resurgence and outbreaks of secondary pests (Gallo *et al*., 2002).

Biorational insecticides such as horticultural oils, insect growth regulators, feeding inhibitors, insecticidal soaps and microbials that are capable of targeting a specific pest are also known as selective insecticides because of this ability (Gloyd & Dickinson, 2006; Trdan *et al*., 2007). The application of selective insecticides that allow for an extended period of suppression of pests and extended control without the use of insecticides is known as bioresidual (Ellsworth & Martinez-Carrillo, 2001).

Biopesticides may be excellent alternatives to chemical pesticides. Apart from some limited laboratory experiments, there has been no extensive field research in Bulgaria to determine the deleterious impact of neem and pyrethrum products on beneficial insects like coccinellids, beneficial thrips, and others.

The predatory species, *Aeolothrips intermedius* Bagnall, was found on 30 different host plant species belonging to 16 botanical families, always in mixed populations with phytophagous or facultative phytophagous insects (Trdan *et al*., 2005a). *Aeolothrips intermedius* was the most numerous during the flowering stage of crops and has the potential to control onion thrips (*Thrips tabaci* Lindeman) when both species occur in high numbers in the open (Trdan *et al*., 2005b; Nikolova, 2015).

Other predatory ladybird beetles (coccinellids) have significant importance as a biological control agent because of their ability to limit many soft bodied insect pests, especially the aphids on which the larvae as well as adult stages feed vigorously. The biological control agent, *Coccinella septempunctata*, has high predatory efficacy against aphids (Milevoj, 1997; Amin Sarmad *et al*., 2015).

Therefore, the aim of the present study was to determine the side effects of neem and pyrethrum products on the natural enemies of the pest insects *Coccinella septempunctata* and *Aeolothrips intermedius* in alfalfa seed production.

Materials and Methods

Over the period 2012-2014, in the experimental field of the Institute of Forage Crops, Bulgaria, an investigation was conducted with alfalfa grown for seed production. The field trial was conducted using a long plot design with a sowing rate of 25 kg ha^{-1}, plot size of 10.4 m^2 (8 x 1.3 m), in three replications with a natural background of soil supply of major nutrients. In the long plot design, replications are arranged in an elongated strip, i.e. the replications are arranged one after the other with the aim of equalizing the soil fertility. The trial occupied an area of 130 m^2.

The action of three insecticides for controlling the lucerne seed weevil *Tychius flavus* Beck. (Coleoptera: Curculionidae), alfalfa plant bug *Adelphocoris lineolatus* Goeze (Hemiptera, Heteroptera: Miridae) and pea aphid *Acyrthosiphon pisum* Harr. (Hemiptera, Sternorrhyncha: Aphididae), major pests in alfalfa seed production, was studied. The insecticides used defined the side effect of Neem and pyrethrum products on banded thrips, *Aeolothrips intermedius* Bagnall (Thysanoptera: Thripidae) and the seven-spot ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) adults.

NeemAzal-T/S® (50 ml ha^{-1}) is a product from the Indian Neem tree *Azadirachta indica* A. Juss: Meliaceae. The active substances are 1% azadirachtin A, 0.5% azadirachtin B, W, G, D and 2.5% neem substance (Trifolio-M GmbH, Germany).

Pyrethrum FS EC (500 ml ha^{-1}) is a natural extract of *Chrysanthemum cinerariaefolium*. Its components are 32% extract from pyrethrum (25% pyrethrin), 32% sesame oil and 36% adhesives (soft potassium soap) (Andermatt Biocontrol, Switzerland).

Nurelle D (50g a.i. l-1 cypermethrin + 500g a.i. l-1 chlorpyrifos-ethyl; 400 ml ha^{-1}) is a pyrethroid insecticide. (Dow AgroSciences, Indiana, USA).

The treatments were carried out at the beginning of the flowering stage of alfalfa in the second intercut (from 10-20 June). The method of sweeping with the entomological net was used. Based on the principles of the International Organization of Biological Control (IOBC), four evaluation categories were used (% mortality or reduction in beneficial capacity): 1 = harmless (<25%), 2 = slightly harmful (25-50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan *et al*., 1994). The toxicity of insecticides to *A. intermedius* and *C. septempunctata* adults was estimated on the first, fifth, seventh and ninth day after treatment and calculated by Henderson and Tilton formula (1955).

The data were subjected to one-way ANOVA, and the means were compared by Tukey's test at 5% probability ($p \leq 0.05$). Multiple Regression Analysis of Statgraphics Plus (1995) for Windows Ver. 2.1 was used.

Results and Discussion

Beneficial entomofauna living on alfalfa was represented by species belonging to the orders Thysanoptera, Coleoptera, Hymenoptera and Hemiptera: suborder Heteroptera. The predator species *Aeolothrips intermedius* and *Coccinella septempunctata* were present in high numbers in the stands, which allowed for the monitoring of their reaction to plant products under field conditions.

The results of this study indicated that biological insecticides were harmless with respect to adults of the predatory ladybird *C. septempunctata* (Tab. I).

In a comparative analysis with NeemAzal, Pyrethrum had the higher toxicity on the first day after application due to the established quick "knockdown effect". The difference between them was significant ($p<0.05$). A similar trend was observed on the fifth day in 2012 and 2014. Despite the more pronounced side effect of Pyrethrum on the ladybird (compared to NeemAzal), its toxicity did not exceed 25% according to IOBC principles and the organic products were thus classified as harmless. Only on the 1st day after treatment with

Pyrethrum in 2012 was a small deviation observed. Its effect reached 26.3% and was classified as slightly toxic.

Table I. Toxicity of three tested products on insecticides against *Coccinella septempunctata* adults.

Variants	1 DAT			5 DAT			7 DAT			9 DAT		
	E, %		Sd	E, %		Sd	E, %		Sd	E, %		Sd
2012												
1. NeemAzal	8.00	a*	2.00	15.50	a	2.28	20.60	a	1.97	22.40	a	2.25
2. Pyrethrum	26.30	b	3.22	23.60	b	2.16	20.40	a	2.51	18.00	a	2.65
3. Nurelle D	91.70	c	6.82	85.00	c	2.00	70.6	b	2.40	63.80	b	2.20
LSD0.05	9.002			4.293			4.602			4.745		
2013												
1. NeemAzal	6.20	a	1.71	10.20	a	2.71	13.10	a	2.50	11.00	a	6.20
2. Pyrethrum	16.10	b	2.61	14.50	a	3.19	9.60	a	2.16	8.30	a	16.10
3. Nurelle D	80.70	c	2.77	64.00	b	3.67	50.20	b	2.71	48.70	b	80.70
LSD0.05	4.815			6.415			4.928			5.761		
2014												
1. NeemAzal	8.50	a	2.25	10.60	a	2.62	18.30	a	3.29	20.20	a	3.37
2. Pyrethrum	20.90	b	2.80	20.60	b	2.16	16.10	a	2.80	15.40	a	2.27
3. Nurelle D	82.30	c	2.61	80.10	c	2.80	75.60	b	3.67	57.40	b	2.43
LSD0.05%	5.122			5.082			6.54			5.454		

* – means within columns followed by the same letter are not significantly different (p>0.05). 1DAT – one day after treatment, 5DAT – five days after treatment, 7DAT –seven days after treatment, 9DAT –nine days after treatment; E – efficacy, SD – standard deviation.

The data showed that the synthetic product Nurelle D (used as a standard for comparison in testing the efficacy of products against insect pest in alfalfa seed production) had the highest toxic effect. Mortality was significantly the highest on the first day after treatment, and ranged from 80.7-91.7% ($p<0.05$). Despite a decreasing toxicity 7 and 12 days after application, Nurelle again demonstrated the highest mortality rates against predatory ladybugs. The synthetic product was harmful (>75%) on the 1st and 5th days and moderately harmful (51-75%) on the 7th and 9th days after treatment.

The organic products produced more pronounced side effects on *A. intermedius* (Tab. II). NeemAzal had primarily harmless effects during the reporting days. Higher toxic effect values, slightly exceeding 25%, were found on the 5th day after application in 2012 and 2014, and on the 7th day in 2013 and 2014. This was due to the later exhibition of the active substance azadirachtin on the thrips, which resulted in certain mortality.

In comparison to NeemAzal, Pyrethrum caused significantly higher mortality of thrips only on the 1st day after treatment ($p<0.05$): differences in toxicity between the two products during the remaining days were statistically insignificant.

Table II. Toxicity of three tested insecticides against *Aeolothrips intermedius* adults.

Variants	1 DAT			5 DAT			7 DAT			9 DAT		
	E, %		Sd	E, %		Sd	E, %		Sd	E, %		Sd
2012												
1. NeemAzal	19.15	a*	4.45	27.20	a	3.11	24.10	a	1.27	11.00	a	1.41
2. Pyrethrum	36.50	b	2.69	28.10	a	2.69	22.20	a	1.31	13.70	a	1.84
3. Nurelle D	92.80	c	3.96	77.80	b	3.11	67.90	b	2.97	57.00	b	4.24
LSD0.05	12.013			9.473			6.29			8.884		
2013												
1. NeemAzal	10.50	a	2.12	14.55	a	1.48	25.73	b	1.03	14.95	a	4.17
2. Pyrethrum	23.47	b	2.59	20.35	a	2.48	16.74	a	2.07	13.03	a	2.87
3. Nurelle D	79.91	c	2.11	71.90	b	2.40	57.35	c	2.19	39.10	b	1.84
LSD0.05	7.266			6.902			5.853			9.899		
2014												
1. NeemAzal	18.50	a	0.71	25.65	a	1.34	29.13	a	2.64	24.02	a	1.44
2. Pyrethrum	35.62	b	2.24	30.70	a	3.25	23.70	a	2.69	21.57	a	2.15
3. Nurelle D	89.52	c	2.29	84.95	b	2.62	75.50	b	3.54	57.58	b	3.65
LSD0.05%	6.031			8.065			9.500			8.220		

* – means within columns followed by the same letter are not significantly different (p>0.05). 1DAT – one day after treatment, 5DAT – five days after treatment, 7DAT –seven days after treatment, 9DAT –nine days after treatment; E – efficacy, SD – standard deviation.

The synthetic insecticide Nurelle D showed a similar effect as Pyrethrum with a quick knockdown effect, but was distinguished by a significantly high toxic effect on predatory thrips. Compared with the synthetic insecticide, the toxicity of the organic products was 3- to 4-fold lower. It should be noted that the predatory thrips were more sensitive to the tested insecticides than predatory ladybirds.

According to IOBC categorization, NeemAzal was harmless (non-toxic) to *C. septempunctata* as its toxic action was less than 25%, and harmless in 67% of the cases regarding *A. intermedius*. NeemAzal was classified on the 5th and 7th day after treatment as slightly harmful (not exceeding 30% mortality) with regard to thrips. Pyrethrum was defined as harmless for predatory ladybugs and in 67% of the cases to *A. intermedius*. Because of its rapid initiation action, the biological insecticide caused certain mortality in predatory thrips on the 1st and 5th days after application, categorizing it as a slightly harmful. Nurelle D was defined as harmful in 50% of cases, and in the remaining 50%, moderately harmful to both prey species.

A number of authors reported that neem products did not exhibit harmful effects on common predators, including different ladybug species, which occupy a principal place (Banken & Stark, 1997; Angeli &. 2005; El Shafie, 2001; Kraiss & Cullen, 2008). Qi *et al*. (2001) found that neem treatments were not toxic to adult *Harmonia conformis* Boisduval (Coleoptera: Coccinellidae) and the larvae of *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae) exposed via feeding. Other authors indicated that azadirachtin-containing products were harmless for older predators of the family Coccinellidae, but were toxic to their larvae (Hoelmer *et al*., 1990; Ossiewatsch, 2000). Similar results to the present study were reported by Hamd *et al*.

(2005), Cosme *et al*. (2007) and Broglio *et al*. (2014), where azadirachtin-based products were non-toxic to *C. septempunctata* adults.

There was insufficient information in the literature about the impact of NeemAzal on predatory thrips. Efíl *et al*. (2010) reported that the use of azadirachtin had no harmful effects on predatory thrips of the genus *Aeolothrips*, which supports the current results.

Studies on Pyrethrum toxicity against predatory ladybugs were contradictory. According to some authors pyrethrins were highly toxic to first instars of *Harmonia axyridis* (98% mortality), but had no effect on third instars, pupae or adults (Kraiss & Cullen, 2008). Other authors found that the plant product had a toxic action against predators from the Coccinellidae family and the best time to apply pyrethrum is in the late afternoon (6:00 PM) when aphid populations can be effectively reduced without affecting the natural enemy community (Wagner *et al*., 2014). Singh *et al*. (1985) determined that pyrethrins were effective against the aphids *Myzus persicae* Sulz. and *Brevicoryne brassicae* L., and less harmful to the predator *C. septempunctata* L. (Coleoptera: Coccinellidae).

Coccinella septempunctata is one of the most intensively studied predators, but there is still no consensus about the toxicity of pyrethrum-based products on this species. In the present study, Pyrethrum was harmless to adults of *C. septempunctata* and mainly had a non-toxic effect on *A. intermedius*.

The presented results clarify some of the side effects of plant products on predatory species and the possibility of their application in the organic cultivation of alfalfa, as well as in the protection of biodiversity in ecosystems.

ANOVA of product toxicity to *A. intermedius* and *C. septempunctata* demonstrated that the factor B (type of insecticide) had the strongest effect on product toxicity – 89.1 and 82.5% of the total variance for *C. septempunctata* and *A. intermedius*, respectively (Tab. III). The influence of reporting days after treatment (factor C) was considerably weaker than factor B and it was statistically significant (6.4%) only with regard to *A. intermedius*. The highest significant interaction (5.9%) was found between the type of insecticide and reporting days after treatment, B x C, in predatory thrips. The strength of the interactions between A x B x C, A x B and A x C were considerably lower and a significant effect was not found.

Conclusions

The plant insecticides NeemAzal and Pyrethrum were non-toxic with respect to adults of *Coccinella septempunctata*, and in almost 70% of cases were harmless to *Aeolothrips intermedius* adults.

Analysis of variance regarding product toxicity for predatory species demonstrated that factor B (type of insecticide) had the strongest effect on product toxicity – 89.1 and 82.5% of the total variance for *C. septempunctata* and *A. intermedius*, respectively.

Tested plant insecticides showed no negative impact on the predatory species and they can be applied in organic seed production of alfalfa.

Table III. Analysis of variance on product toxicity.

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Influence of factor, %		Mean square (MS)
Coccinella septempunctata					
Total	105	82512.3	100.0		785.83
Variants	35	81926.7	99.3	*	2340.76
Factor A - Year	2	2341.93	2.8		1170.97
Factor B -Insecticide	2	73494.0	89.1	*	36747.00
Factor C – Days after treatment	3	1109.9	1.3		369.97
A x B	4	397.522	0.5		99.38
A x C	6	217.834	0.3		36.31
B x C	6	3969.17	4.8		661.53
A x B x C	12	396.315	0.5		33.03
Pooled error	72	585.6	0.7		8.13
Aeolothrips intermedius					
Total	105	68792.10	100.0		655.16
Variants	35	68541.98	99.6	*	1958.34
Factor A − Year	2	2377.00	3.5		1188.50
Factor B − Insecticide	2	56743.50	82.5	*	28371.75
Factor C − Days after treatment	3	4434.95	6.4	*	1478.32
A x B	4	205.613	0.3		51.40
A x C	6	254.90	0.4		42.48
B x C	6	4062.77	5.9	*	677.13
A x B x C	12	463.23	0.7		38.60
Pooled error	72	250.12	0.4		3.47

* Significant at 5% level of probability.

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НЕЖЕЉЕНИ ЕФЕКТИ ДВА БИЉНА ИНСЕКТИЦИДА НА ПРИРОДНЕ НЕПРИЈАТЕЉЕ ИНСЕКАТА У ПРОИЗВОДЊИ СЕМЕНА ЛУЦЕРКЕ *MEDICAGO SATIVA* L.

ИВЕЛИНА НИКОЛОВА

Извод

У периоду 2012-2014 на огледном пољу Института за крмно биље, Плевен, Бугарска истраживан је ефекат производа Neem и Pyrethrum на адултима трипса, *Aeolothrips intermedius* Bagnall (Thysanoptera: Thripidae и бубамара, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Третирања су изведена на почетку цветања луцерке (*Medicago sativa* L.). За сакупљање коришћена је ентомолошка мрежа.

Пронашли смо да биолошки инсектициди Neemazal and Pyrethrum нису токсични за адулте *Coccinella septempunctata* и у око 70% случајева нису штетни за предатора- *Aeolothrips intermedius.*

Употреба инсектицида који нема негативан утицај на предаторске врсте омогућава њихову примену у условима органског гајења луцерке.

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