

Systems of organic farming in spring vetch II: Biological response of *Aeolothrips intermedius* Bagnall and *Coccinella septempunctata* L

Ivelina Nikolova^{1*} and Natalia Georgieva¹

Institute of Forage Crops, 89 Gen. Vl. Vazov St., Pleven 5800, Bulgaria
(*innikolova@abv.bg)

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SUMMARY

The effects of four systems of organic farming of spring vetch on *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) population density and the toxicity of several products on predatory insects were studied. The variants were: Control (without using any biological products); combined treatment with Polyversum (biological foliar fertilizer) and Biofa (biological plant growth regulator); treatment with NeemAzal T/S (biological insecticide, a.i. azadirachtin) and treatment with a combination of NeemAzal with Polyversum and Biofa. Variant V was a conventional farming system in which a combination of Nurelle D (synthetic insecticide), Masterblend (foliar fertilizer) and Flordimex 420 (growth regulator) was used as a standard treatment. In the organic farming system that included treatment of plants with the biological insecticide NeemAzal (azadirachtin), the reduction in *A. intermedius* abundance was 20.7% when it was applied alone and 24.6 % in combination with the organic products Polyversum and Biofa. NeemAzal achieved a lower reduction in the counts of predatory ladybirds *C. septempunctata*, from 14.9% (alone) to 21.9% (combination). The biological insecticide, applied alone or in combination, was mostly harmless and rarely harmful to *A. intermedius*. NeemAzal manifested harmlessness to *C. septempunctata* as its toxic action did not exceed 25%. An analysis of variance regarding product toxicity to *A. intermedius* and *C. septempunctata* demonstrated that the type of treatment (the application of insecticides alone or in combination) had the strongest effect on product toxicity. The use of neem-based insecticides can be a substantial contribution towards preservation of biodiversity in ecosystems.

Keywords: Organic farming; Spring vetch; Predatory insects; Biopesticides

INTRODUCTION

Pest control in modern agro-ecosystems is largely achieved by using pesticides, but relying on them globally has led to evolution of pesticide resistance in many crop pests (Whalon et al., 2008). Current trends in

organic agriculture have therefore recognized a need for looking for alternative methods of pest control (Atanasov et al., 2005). Such organic systems, which aim for sustainability, while preserving or increasing biological diversity, and minimization of all forms of contamination, make it critical to search for alternative

techniques of control, selective and not harmful, especially not to populations of beneficial insects, such as natural enemies and pollinators (Efrom et al., 2012). Knowing the effects that biological insecticides, applied alone or in combination with other biological products, have on harmful or beneficial insects, and the use of selective products are important for the sustainability of organic systems.

NeemAzal (azadirachtin) has been the subject of a number of studies which reported its broad spectrum of action and high efficacy against pests in the orders *Lepidoptera*, *Hemiptera*, *Thysanoptera* and mites (Isman, 1993; Singh et al, 1999; Pavela, 2009; Andreev et al., 2012). A survey of current literature reveals that few data are available regarding the impact of neem (Hoelmer et al., 1990) on beneficial insects other than direct contact toxicity studies under field conditions. Because aphids and phytophagous thrips make a food source for a large number of predatory and parasitic insects, the effect of neem on non-target organisms is particularly important in the management of pest populations. Some authors have found that azadirachtin should be combined with plant oils in order to be more effective against some pest (Höhn et al., 1996; Bessin, 2008).

However, information about this product, whether used alone or in combination with other biological products, in terms of its impact on beneficial insects in the field is almost entirely missing or insufficient.

Therefore, this study aimed to evaluate the effects of some methods of organic farming that included biological products on *Aeolothrips intermedius* and *Coccinella septempunctata* density, and products toxicity to these species in a field crop of spring vetch.

MATERIAL AND METHODS

The experimental design, trial variants and product characteristics were shown in the first part of this study (Nikolova & Georgieva, 2015).

Harmful and beneficial insects were counted immediately after treatment at the budding - beginning of flowering stage (one day after treatment) and the counting continued until the aboveground biomass has dried (between mid-May and the end of July - approximately 75 days). Over the period, population density was recorded by sweepings with the entomological net once a week. The data in Table 2 regarding insect abundance in the studied variants were averaged for the indicated 75-day period. The

efficacy of insecticides and their combinations against *Acyrtosiphon pisum* Harris (*Hemiptera*, *Aphididae*), *Empoasca pteridis* Dahlb. (*Hemiptera*, *Cicadellidae*) and *Thrips tabaci* L. (*Thysanoptera*, *Thripidae*) was estimated at the budding and flowering stage (see part I). Assessments were made 1, 3, 7 and 12 days after treatment. The efficacy of the insecticides was calculated using Abbott's formula (1925) and the entomological net was used for sweepings.

Complying with 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 to 50%); 3 = moderately harmful (51 to 75%); and 4 = harmful (>75%) (Hassan et al., 1994). The toxicity of insecticides and their combinations to *Aeolothrips intermedius* Bagnall (*Thysanoptera*, *Thripidae*) and *Coccinella septempunctata* L. (*Coleoptera*, *Coccinellidae*) was estimated on the first, third, seventh and twelfth day after treatment and calculated by Abbott's formula (1925). The statistical processing of experimental data was conducted using the Statgraphics Plus software.

RESULTS AND DISCUSSION

Beneficial entomofauna living on organically grown spring vetch was represented by species belonging to the orders *Thysanoptera*, *Coleoptera*, *Hymenoptera* and *Hemiptera*: suborder *Heteroptera*. The predator species *Aeolothrips intermedius* Bagnall (*Thysanoptera*: *Thripidae*) and *Coccinella septempunctata* L. (*Coleoptera*: *Coccinellidae*) were present in high numbers in the stands, which allowed the monitoring of their reaction to organic products under field conditions. The family *Coccinellidae* was represented by another 4 species: *Propylea quatuordecimpunctata* L., *Coccinula quatuordecimpustulata* L., *Adonia variegata* Goeze and *Scymnus* (*Neopulus*) *quadrimaculatus* Herb but their numbers were too low to be recorded and processed in this study.

The average number of predatory thrips differed depending on the system of production (Table 1).

Under organic production, the products Biofa and Polyversum had insignificant impact on population density of *A. intermedius* and density values insignificantly exceeded that of the control by 10.0% over the years. Probably the products slightly stimulated the density of that species.

Table 1. Mean abundance of *Aeolothrips intermedius* and *Coccinella septempunctata* predators in systems of organic and conventional production per 100 sweepings over the vegetation period

Trial variants	2012	2013	2014	Average	2012	2013	2014	Average
	<i>Aeolothrips intermedius</i>				<i>Coccinella septempunctata</i>			
Control (OS)	295.4 bc	93.8 bc	228.0 cd	205.7 cd	128.1 bc	75.0 b	158.3 c	120.5 bc
Biofa+Polyversum(OS)	310.0 c	100.0 c	268.7 d	226.2 d	135.2 c	77.8 b	216.7 d	143.2 c
NeemAzal T/S (OS)	228.8 bc	80.4 bc	180.0 bc	163.1 bc	115.4 bc	67.3 b	125.0 bc	102.6 b
NeemAzal+Biofa+Polyversum (OS)	222.5 b	75.8 b	166.9 b	155.1 b	111.4 b	62.5 b	108.3 b	94.1 b
NurelleD+Flordimex+Masterblend (CS)	113.7 a	34.8 a	78.6 a	75.7 a	70.1 a	34.0 a	48.5 a	50.8 a
LSD _{0.05%}	86.271	20.202	53.803	48.461	21.180	22.798	34.430	29.054

OS - organic system; CS - conventional system

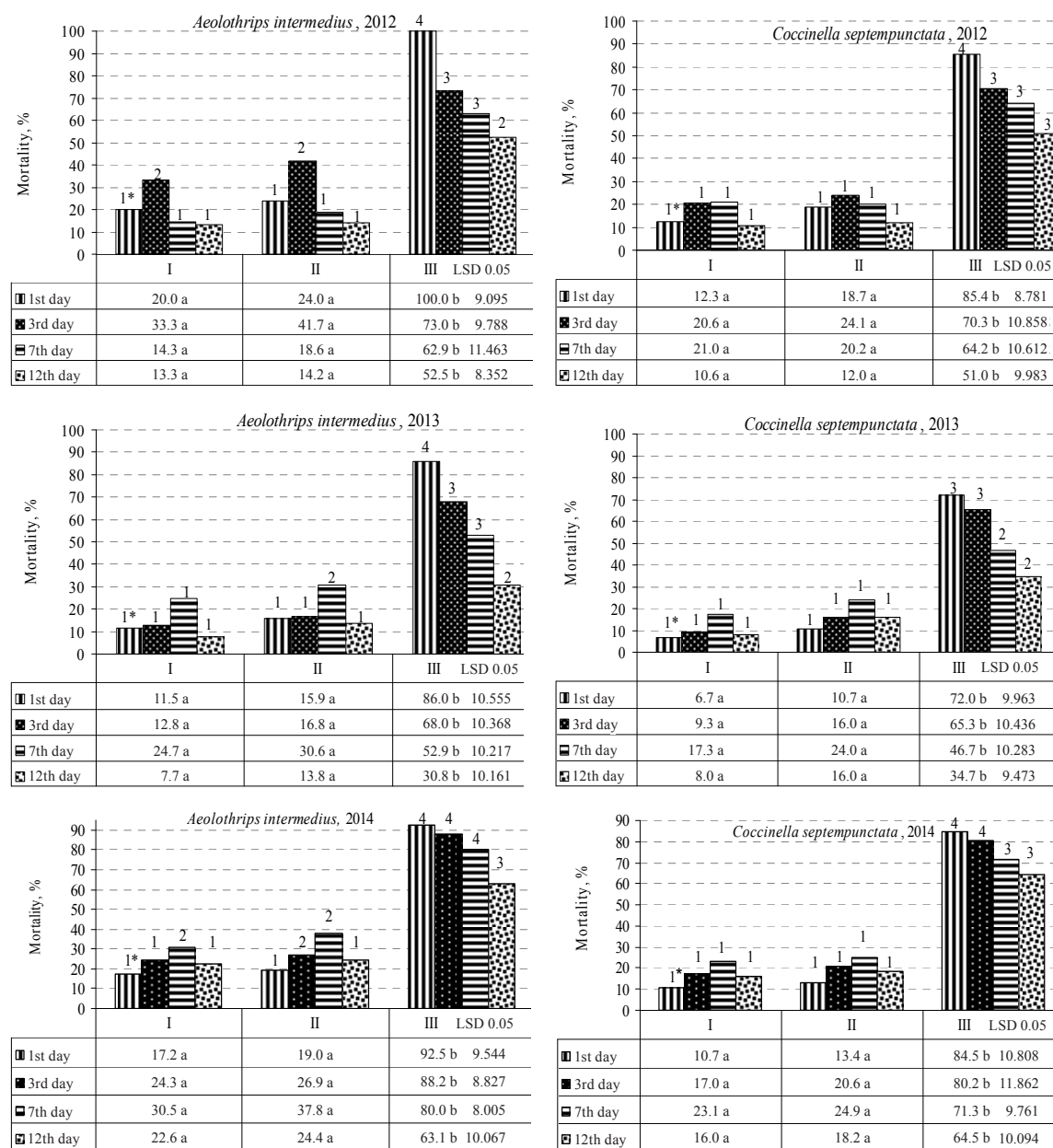
Treatment with the biological insecticide NeemAzal resulted in a lower number of species – by 20.7% on average for its use alone and 24.6% for the combination with foliar fertilizer and growth regulator, which are biological products. Despite the changeable population density of *A. intermedius* over the years, its numbers varied over a narrow range both when NeemAzal was used alone and in combination, and the difference was insignificant. Significant differences were found between the untreated control and the combined use of organic products in 2014 and on average for the entire 2012-2014 period, too. Concerning *Coccinella septempunctata*, the impact of NeemAzal on predatory density followed a similar trend and was relatively less pronounced with the reduction ranging from 14.9 (NeemAzal used alone) to 21.9% (NeemAzal in combination). The differences between these variants and the control were insignificant. Unlike NeemAzal, the synthetic products had a strong negative impact on *C. septempunctata* abundance and the reduction was 49.4%.

According to several authors, the number of predators on plants sprayed with neem may have been lower as a result of reduced aphid populations, and the number of adult coccinellids was highly correlated with aphid density (Wright & Laing, 1980; Frazer, 1988; Nijveldt, 1988; Lowery, 1992). Considering the decrease in aphid numbers and counts of predators in the variants, treatment with NeemAzal may have overestimated the damaging effect of azadirachtin. However, although an increase in host density is usually followed by increase in the counts of predators and parasitoids, the relationship is often variable and

difficult to determine (Stary, 1970; Coderre, 1988). Therefore, toxicity constitutes the most accurate measure of product impact on natural enemies.

The data showing direct impact of the insecticides on beneficial species over the years showed that the combination in the synthetic product Nurelle D had the highest toxic effect. Mortality was significantly the highest on the first day after treatment, and it ranged from 86.0 to 100.0% in *A. intermedius* and from 72.0 to 85.4% in *C. septempunctata* ($P < 0.05$) (Figure 1). Despite a decreasing toxicity of the combination seven and twelve days after application, Nurelle again demonstrated the highest mortality rates against predatory thrips and ladybugs.

The biological product NeemAzal exhibited significantly less impact as its toxicity on the first day ranged from 11.5 to 20.0% (*A. intermedius*) and from 6.7 to 12.3% (*C. septempunctata*). NeemAzal combined with Biofa and Polyversum had higher mortality values but the differences versus its use alone were statistically insignificant in each year ($P < 0.05$). Azadirachtin, applied alone and in combination, increased its activity, reaching maximum values on the 7th day after treatment. An exception was observed in *A. intermedius* in 2012 when the highest toxic effect was found on the third day after treatment. Differences between the variants, including NeemAzal, were minimal and insignificant over the reporting years and days for both predators ($P < 0.05$). Compared with the synthetic insecticide, their toxicity was three to four-fold lower. It should be noted that predatory thrips were more sensitive than predatory ladybirds.



I – NeemAzal; II – NeemAzal+Biofa+Polyversum; III - NurelleD+Flordimex 420+ Masterblend

Means in each row marked by the same letter are not significantly different ($P > 0.05$) on reporting days; *evaluation categories (% mortality or reduction in beneficial capacity) were: 1 = harmless ($< 25\%$); 2 = slightly harmful (25 to 50%); 3 = moderately harmful (51 to 75%); and 4 = harmful ($> 75\%$)

Figure 1. Toxicity of some products to *Aeolothrips intermedius* and *Coccinella septempunctata*

According to categorization by the International Organization of Biological Control (IOBC), NeemAzal, used alone or in combination, was harmless (non-toxic) in 75% of the cases, and slightly harmful in only 25% [NeemAzal, applied alone - on the 3rd day (2012) and 7th day (2014); NeemAzal, applied in combination: on the 3rd

day (2012), 7th day (2013), 3rd and 7th days (2014)] regarding *A. intermedius*. It is necessary to note that the biological insecticides were classified as harmless to the predatory ladybird *C. septempunctata* as their toxic action was not more than 25%. Our results support those of Kaethner (1991). He found that neem was not toxic to adults of *C. septempunctata*.

Table 2. Analysis of variance for toxicity of products

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Influence of factor. %	Mean square (MS)
<i>Aeolothrips intermedius</i>				
Total	105	51406	100.0	489.6
Variants	35	51068	99.3*	1459.1
Factor A - Year	2	2042	4.0*	1021.0
Factor B - Type of treatment	2	39181	76.2*	19590.5
Factor C - Days after treatment	3	3075	6.0*	1025.0
A x B	4	452	0.9*	113.0
A x C	6	1363	2.7*	227.2
B x C	6	4136	8.0*	689.3
A x B x C	12	819	1.6	68.3
Pooled error	71	338	0.7	4.8
<i>Coccinella septempunctata</i>				
Total	105	46151	100.0	439.5
Variants	35	45776	99.2*	1307.9
Factor A - Year	2	1223	2.6	611.5
Factor B - Type of treatment	2	39435	85.4*	19717.5
Factor C - Days after treatment	3	1243	2.7*	414.3
A x B	4	722	1.6	180.5
A x C	6	206	0.4	34.3
B x C	6	2648	5.7*	441.3
A x B x C	12	300	0.7	25.0
Pooled error	71	374	0.8	5.3

Means in each column marked by the symbol “*” are significantly different ($P > 0.05$)

Similar results of low impact of azadirachtin on beneficial ladybirds were also reported by Schmutterer (1997). *Azadirachtin* may cause a certain level of mortality of larval instars, especially under laboratory conditions, but no or only slight side-effects are, as a rule, observed under semi-field or field conditions (Schmutterer, 1997).

On the other hand, Nurelle D was harmful (in 41.7% of the cases) and moderately harmful (in 41.7%) to *A. intermedius*. Regarding *C. septempunctata*, Nurelle D was mainly moderately toxic (in 58.3% of the cases) and less highly toxic (in 25.0%) according to IOBC categorization.

Based on the results presented in this study, it is possible to conclude that NeemAzal, applied alone or in combination with the organic products Biofa and Polyversum, overall showed no significant harmful effects on the two predatory species, although it manifested a slightly harmful effect on *A. intermedius*. In other words, the use of neem-based insecticides can substantially contribute towards preservation of biodiversity in ecosystems in spite of the fact that they are not completely safe to beneficial insects.

An analysis of variance regarding product toxicity to *A. intermedius* and *C. septempunctata* demonstrated

that the factor B - type of treatment (application of insecticides alone or in combination) had the strongest effect on product toxicity - 76.2 and 85.4% of the total variance in the variants for *A. intermedius* and *C. septempunctata*, respectively (Table 2). The influence of the years (factor A) and reporting days after treatment (factor C) was considerably weaker than factor B, while the impact of year was statistically significant (4.0%) regarding *A. intermedius*. The highest significant interaction was between the type of treatment and the reporting days after treatment - BxC - 8.0% in predatory thrips and 5.7% in ladybugs. The strength of the interaction between AxC was considerably lower and a significant effect was found for *A. intermedius* - 2.7%.

The biological insecticide NeemAzal used in combination with the growth regulators Biofa and Polyversum in organic production provided the best protection of plants against a complex of sucking pests and higher productivity. These biological products were also found to be harmless to *C. septempunctata* and *A. intermedius*. The possibility of managing sucking pests in vetch is thus maximized, and farmers are becoming aware of its benefits as an eco-friendly botanical pesticide that causes no residue problems in vetch.

CONCLUSIONS

Under conditions of organic farming, the tested biological insecticide caused a low reduction in the number of predatory ladybirds *Coccinella septempunctata*, from 14.9 (used alone) to 21.9% (in combination), and had a less pronounced influence than the conventional system.

NeemAzal applied alone and in combination was mostly harmless and rarely slightly harmful to *A. intermedius*. The biological insecticide was harmless to *C. septempunctata*, as its toxic action did not exceed 25%.

The analysis of variance for the species *A. intermedius* and *C. septempunctata* showed that factor B - type of treatment (application of the insecticide alone or in combination) had the strongest effect on product toxicity.

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Sistemi organske proizvodnje obične grahorice II: Biološki odgovor *Aeolothrips intermedius* Bagnall and *Coccinella septempunctata* L

REZIME

Proučavano je delovanje četiri sistema organske proizvodnje obične grahorice na gustinu populacija *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) i toksičnost nekoliko preparata za predatore. Varijante oglada su bile: kontrola (bez korišćenja bioloških preparata); kombinovana primena Polyversum (biološko folijarno đubrivo) i Biofa (biološki regulator rasta biljaka); tretman preparatom NeemAzal T/S (biološki insekticid (a.s. azadirahthin) i tretman kombinacijom NeemAzal sa Polyversum i Biofa. Varijanta V sastojala se od klasičnog sistema uzgajanja u kojem su primenjeni preparati Nurelle D (sintetički insekticid), Masterblend (folijarno đubrivo) i Flordimex 420 (regulator rasta) kao standardni tretman. U sistemima organske proizvodnje u kojima je primenjen biološki insekticid NeemAzal (azadirahthin), smanjenje broja *A. intermedius* bilo je 20,7% kada je primenjen samo insekticid i 24,6 % u kombinaciji sa organskim preparatima Polyversum i Biofa. NeemAzal je postigao manje smanjenje brojnosti predatora *C. septempunctata*, od 14,9 (sam) do 21,9% (u kombinaciji). Biološki insekticid, bilo da je primenjen sam ili u kombinaciji, bio je uglavnom bezopasan za *A. intermedius* u retkim slučajevima slabo štetan. NeemAzal se pokazao bezopasnim za *C. septempunctata* pošto toksično delovanje u tom slučaju nije prelazilo 25%. Analiza varijanse kod toksičnosti preparata za *A. intermedius* i *C. septempunctata* pokazala je da je vrsta tretmana (primena insekticida samog ili u kombinaciji) imala najsnažniji efekat na toksičnost preparata. Korišćenje insekticida na bazi nim drveta može značajno doprineti očuvanju biodiverziteta ekosistema.

Ključne reči: Organska proizvodnja; Obična grahorica; Predatori; Biopesticidi