ISSN 1314-6246 Nikolova & Georgieva *J. BioSci. Biotechnol.* **2017**, 6(2): 91-98

RESEARCH ARTICLE

Ivelina Nikolova
Natalia Georgieva

Efficacy of organic products against insect pests in alfalfa grown for seeds

Authors' address:
Department Technology and ecology of forage crops, Institute of Forage Crops, 89, Gen.Vladimir Vazov Str., 5800 Pleven, Bulgaria.

Correspondence:
Ivelina Nikolova
Department Technology and ecology of forage crops, Institute of Forage Crops, 89, Gen.Vladimir Vazov Str., 5800 Pleven, Bulgaria
e-mail: imnikolova@abv.bg

Article info:
Received: 1 December 2017
Accepted: 22 December 2017

ABSTRACT
Organic pesticides can be excellent alternatives to chemical pesticides. They occupy an important place in the plant protection sector. Evaluation of the efficacy was conducted using an alginate from seaweed (organic insecticide) and two organic fertilizers (highly concentrated complex organic fertilizer and natural CO_2 fertilizer), used alone and in combinations to control major pests in alfalfa seed production such as alfalfa seed chalcid *Bruchophagus roddi* Guss., alfalfa plant bug, *Adelphocoris lineolatus* Goeze and pea aphid, *Acyrthosiphon pisum* Harr. We conducted field trials during three growing seasons. Treatments were carried out at the beginning of the flowering stage in second regrowth. The seaweed alginate had high efficacy and excellent control on the three insect species density one week after treatment (over 80%), while the complex organic fertilizer exhibited insecticidal action against pests and its effect exceeded 60% seventy days after treatment and provided a medium level of control. The mix of the two organic products provided the highest efficacy and long-lasting effects on the three insect species and additive effects were observed. The combination realized the highest seed yield and an increase of 29.7%. Treatment with organic products is suitable for organic farming.

Key words: alfalfa, organic insecticide, organic fertilizers, efficacy, productivity

Introduction

Organic pesticides can be excellent alternatives to chemical pesticides. They occupy an important place in the plant protection sector (Copping & Menn, 2000).

With increasing public awareness of health and environmental problems associated with the use of chemical products, solving problems arising from pesticide has an essential and important for many stakeholders (Saini, 2014). It is a challenge for the scientific community to provide safer methods of pest control so that agricultural production is safe to consume.

Organic pesticides are highly efficacious, safe and environmentally acceptable (Nathan et al., 2004). They have a complex of attractive properties that make them good components in integrated pest control systems. It has various insecticidal activities, including killing, deterring, anti-feeding and inhibiting the growth of over 250 species of agricultural, forestry, medical and veterinary pests, including aphids (Wang & Shen, 2007; Esparza-Díaz et al., 2010). Most of them are selective, produce little or no toxic residues, and the cost of developing them is lower than for conventional synthetic chemical pesticides (Hajek, 2004). Organic pesticides also have drawbacks, such as a slower kill rate than conventional chemical pesticides, shorter environmental sustainability, and vulnerability to adverse conditions. It has reduced the general trends over the last two decades dependence on synthetic insecticides in insect pest control in agriculture and forestry. Ecological pest control using plant extracts is not a new practice, but it needs scientific approaches to determine the effectiveness of botanical pesticides in practical terms and provide cost-effective pest control (Shiberu et al., 2013).

Since it relates the main trend in world agriculture to the impact on plant productivity to increase it (Zhelyazkova, 2007), the perspective direction in this respect is the combined use of different products of organic origin. This approach influences the plant biological manifestations. It helps to improve the plant resistance to biotic and abiotic environmental factors, solve problems with resistance to pests and environmental pollution and increases productivity and its quality (Knight & Gurr, 2010; Georgieva et al., 2014, 2015).

Despite the excellent properties of organic products and the results related to increasing the efficiency of organic feed production, the research activity in this direction is at an early stage, which causes its deployment and constant expansion and enrichment.

The aim of the present study was to estimate the efficacy of organic products, used alone and in the combination against *Bruchophagus roddi* Guss., *Adelphocoris lineolatus* Goeze, and *Acyrthosiphon pisum* Harr. and their effect on the productivity of alfalfa grown for seeds.

Materials and Methods

The trial was performed in the experimental field of the Institute of Forage crops, Pleven, Bulgaria in the period 2015 – 2017. It was studied the act of an organic insecticide Agricolle and two organic fertilizers (Nagro and Lithovit) to a control of major pests in alfalfa seed chalcid *Bruchophagus roddi* (Hymenoptera: Chalcididae); alfalfa plant bug, *Adelphocoris lineolatus* (Hemiptera, Heteroptera: Miridae) and pea aphid, *Acyrthosiphon pisum* (Hemiptera, Sternorrhyncha: Aphididae). Experiments are laid out in Randomized Complete Block (RCB) Design with four replications with alfalfa varieties "Obnova 10". The size of each net plot was 20 m^2. It made treatments with an alginate from seaweed (an organic insecticide) and two organic fertilizers (highly concentrated complex organic fertilizer and natural CO_2 fertilizer), used alone and in combinations. Trial variants and product characteristics are shown in Table 1. It carried applications out at the beginning of the flowering stage in second regrowth (from 10 to 20 June). Products were carried out at the beginning of the flowering stage in the second regrowth by hand with Mataby style 3.0 liters. It used products in a 30 000 ml water/ha. Based on the average number of each insect species, it calculated the efficacy of the products, according to the Henderson–Tilton formula (1955) on the day before application, 1, 5, 7 and 9 days after treatment. Method of sweeping with the entomological net was used in net plots.

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

Results

Agro-meteorological conditions affected the efficacy of organic products and it was more pronounced in 2016. Relatively wet and cool weather during the second half of May and the first ten days of June in 2016 compared to 2015 (when done regularly density reporting) led to established a lower efficacy of products to the previous year (Table 2). The amount of rainfall in 2016 was 33.6 mm over 2015 for that

Table 1. *Trial variants and product characteristics.*

Trial variants	Active ingredients	Producer	Application rates
1. Agricolle (natural polysaccharides for sticking small insects, organic insecticide)	Contain alginate from seaweed (28%)	Cal-Agri producta LLC, USA	3000 ml / ha (Soluble liquid)
2. Nagro (Highly concentrated complex organic nanofertilizer)	Contains humic acids - 5.6 g/l; sulfonic acids - 2.07 g/l; total nitrogen (N) - 0.28 g/l; phosphorus total (P) - 0.226 g/l; potassium total (K) - 3.073 g/l; Microelements - Mg, Co, Mn, Zn, Fe, Cu, Mo, B, Ca, Se - 1900 mg/l	Scientific Production Association "Bioplant", Russian Federation	500 ml / ha (Soluble liquid)
3. Lithovit (Natural CO_2 fertilizer)	Contains calcium carbonate from natural reserves with micronutrients: $CaCO_3$ – 79.19%; $MgCO_3$ – 4.62 %; Fe -1.31 %	ZeoVITA, Schwarzenberg, Germany	2000 ml ha^{-1} (Soluble powder)
4.Agricolle+Nagro	-		3000 ml ha^{-1}+500 ml ha^{-1}
5.Agricolle+Lithovit	-		3000 ml / ha +2000 ml / ha
6. Control	Treated with distilled water		

Table 2. *Meteorological characteristics.*

Month/ten days		Temperature, °C			Rainfall, mm			Relative humidity, %		
		2015	2016	2017	2015	2016	2017	2015	2016	2017
April	1-10	7.5	16.3	11.6	19.3*	1.5	10.1	58	62	60
	11-20	14.5	16.7	11.5	3.7	43.3	22.9	48	66	64
	21-31	14.6	13.0	13.5	20.6	28.3	4.5	56	70	60
	Average	**12.2**	**15.3**	**12.2**	**43.6**	**73.1**	**37.5**	**54**	**66**	**61**
May	1-10	18.7	13.5	15.6	3.1	37.7	42.4	68	79	75
	11-20	19.1	16.4	17.6	20	7.6	6.1	66	65	65
	21-31	18.5	19.1	17.7	7.5	31.2	106.5	64	70	73
	Average	**18.8**	**16.3**	**17.0**	**30.6**	**76.5**	**155.0**	**66**	**71**	**71**
June	1-10	20.8	19.2	21.1	17	39.3	21.6	61	72	74
	11-20	22.4	24.0	20.9	39.6	6.5	21.6	65	66	65
	21-31	19.0	25.7	27.0	39.1	0	1.6	66	63	54
	Average	**20.7**	**23.0**	**23.0**	**95.7**	**45.8**	**44.8**	**64**	**67**	**64**

* Rainfall values were presented as an amount for a ten-day period.

Table 3. *Efficacy of organic products (alone and in combination), 2015.*

Variants																	
								Bruchophagus roddi									
	NBT	E, % **1** DAT		NAT	Sd	E, % **5** DAT		NAT	Sd	E, % **7** DAT		NAT	Sd	E, % **9** DAT		NAT	Sd
1	24.5	**100.0**	c*	0.0	0.00	**92.3**	cd	1.4	3.81	**75.0**	d	5.0	2.82	**40.0**	d	11.2	4.24
2	22.3	**83.3**	b	3.4	3.81	**76.9**	b	3.8	2.96	**63.8**	c	6.6	3.95	**31.7**	c	11.6	2.96
3	25.0	**31.7**	a	15.6	4.67	**31.5**	a	12.7	3.53	**22.0**	a	16.0	2.82	**11.3**	a	16.8	1.83
4	23.2	**100.0**	c	0.0	0.00	**100.0**	d	0.0	0.00	**81.3**	d	3.6	2.40	**55.0**	e	7.9	2.82
5	22.9	**95.6**	c	0.9	4.24	**89.2**	c	1.8	3.96	**54.1**	b	8.6	4.38	**23.5**	b	13.3	3.53
6	23.7	-		21.6		-		17.6		-		19.4		-		18.0	
LSD 0.05%		8.476				8.256				9.049				8.185			
								Acyrthosiphon pisum									
1	192.0	**91.2**	b	15.1	2.41	**91.1**	c	17.0	2.54	**89.2**	c	17.9	3.95	**61.9**	d	68.8	2.97
2	187.2	**60.3**	a	66.3	3.18	**61.1**	b	72.5	4.10	**62.4**	b	60.9	3.39	**20.5**	b	140.0	3.25
3	194.1	**64.7**	a	6.1	3.25	**33.3**	a	128.8	4.66	**29.0**	a	119.2	2.82	**8.1**	a	167.8	2.68
4	170.5	**93.2**	b	10.3	2.54	**91.7**	c	14.1	2.40	**89.9**	c	14.9	2.68	**79.2**	e	39.8	2.54
5	172.7	**91.6**	b	12.9	2.26	**89.4**	c	18.2	6.22	**67.5**	b	48.5	4.94	**51.3**	c	79.1	3.81
6	206.5	-		184.1		-		205.5		-		178.6		-		194.3	
LSD0.05%		7.159				10.881				9.404				7.938			
								Adelphocoris lineolatus									
1	33.3	**100.0**	c	0.0	0.00	**84.6**	c	5.5	4.80	**66.7**	c	11.4	2.40	**50.0**	c	16.5	2.83
2	30.6	**70.0**	b	8.8	8.49	**71.5**	b	9.3	3.25	**55.0**	b	14.1	2.82	**35.0**	b	19.7	1.41
3	31.5	**50.8**	a	13.0	5.94	**39.6**	a	20.4	3.39	**29.2**	a	22.9	1.13	**11.5**	a	27.6	2.10
4	34.4	**100.0**	c	0.0	0.00	**100.0**	d	0.0	0.07	**78.8**	d	7.8	2.93	**57.5**	d	14.5	2.12
5	32.7	**99.9**	c	0.0	0.14	**75.1**	b	4.1	3.25	**58.6**	b	13.9	3.39	**43.8**	c	18.2	4.45
6	30.0	-		28.7		-		32.1		-		30.8		-		29.7	
LSD 0.05%		11.908				9.055				6.760				7.164			

Legend: 1–Agricolle; 2–Nagro; 3–Lithovit; 4– Agricolle+Nagro; 5– Agricolle+Lithovit; 6–Control; 1 DAT – first day after treatment; 3 DAT– third day after treatment; 7 DAT– seventh day after treatment; 9 DAT– ninth day after treatment; E– efficacy,%; NBT– number of individuals before treatment from 10 m^2; NAT– number of individuals after treatment from 10 m^2; Sd– standard deviation; *Means in each column marked by the same letter are not significantly different ($P > 0.05$)

period. In 2017 the treatment was carried out at the beginning of June as the weather conditions favored the active action of the products.

In a comparative study of organic products applied alone in 2015 against the main insect pests in alfalfa seed production, the alginate from seaweed showed the strongest effect with significant differences. It provided great plant protection one week after its application (Table 3). The product had a rapid initial action (91.2 – 100.0% efficacy on the first day) and a long-lasting effect (66.7 – 89.2% on the seventh day) and successfully controlled three insect species density. Organic insecticide had a higher efficacy than fertilizers against pea aphids, providing excellent control one week after treatment and a medium level of control on the ninth reporting day.

The highly concentrated complex organic Nano-fertilizer exhibited insecticidal action against pests such as its effect was higher significantly that of the natural CO_2 fertilizer. Efficacy exceeded 60% one week after treatment and provided a medium level of control on *B. roddi* and *A. pisum*. Efficacy on *A. lineolatus* approaching the 60% up to the seventh day also was evaluated as a medium level of control. The results were unsatisfactory on the last reporting day on all three insect species. Regardless of the lower values of the insecticide, the Nano fertilizer provided good protection of alfalfa plants for one week after application. In terms of its effect, depending on the insect species, it was found the most pronounced reduction of the numbers during the year in alfalfa seed chalcid, followed by the plant bugs and leaf aphids.

The application of the second fertilizer did not provide good protection and did not defend plants from insect attack. It observed an exception in pea aphid number on the first day after treatment, where the efficacy exceeded 60%, after that the results were unsatisfactory.

It associated the combined application of organic products with an increase in the efficacy compared to their used alone. Treat of the alginate from seaweed with Nano fertilizer controlled species density and efficacy, over 80% one week after application (about *A. lineolatus* the value almost reached 80% on the seventh day). The mixture remained with high efficacy on a ninth day in *A. pisum* while the other two species ranged from 60 to 80% and ensured a good (medium) level of control. Regarding *A. lineolatus* was observed a statistically higher effect of the combination compared to the organic insecticide, used alone after the first day of treatment and to Nano fertilizer throughout the reporting period. Differences in the efficacy between the mixture and the insecticidal product concerning *B. roddi* and *A. pisum* were significant on a ninth day after application, and to Nano fertilizer – from the first to the ninth day including. It was observed additive effects between insecticide and Nano fertilizer.

The efficacy of the seaweed alginate combined with the natural fertilizer was less pronounced. The toxicity was high as provided a good level of control against the three species pests five days after treatment (above 80%). It observed a medium level of control on the seventh and ninth day in pea aphid and on the seventh day – in alfalfa plant bug and seed chalcid. Efficacy values over the first five days were close to the corresponding ones when the seaweed alginate used alone as differences were insignificant (exception for *A. lineolatus* on the 5th day, where efficacy was significantly lower). The biological action of the organic insecticide, applied with the natural fertilizer during the remaining reporting days was statistically lower to the insecticide and its combination with the Nano fertilizer.

Regardless of the specific conditions in 2016, the tendency for the highest efficacy of the alginate from seaweed compared to the two fertilizers was saved with significant differences (Table 4). The keeping a good control of the species density had a different duration. The plant protection from pea aphid had the longest duration – from the first to the ninth day including after treatment, as the efficacy ranged from 59.7 to 80.6% and organic insecticide supplied a medium level of control. That level of control for *A. lineolatus* was maintained for seven days and for *B. roddi* – 5 days after treatment.

Nano fertilizer provided a good control over the three insect species for five days after treatment and exceeded significantly the effect of the other fertilizer.

The highest efficacy during the year had the mix with the Nano fertilizer, which in *A. pisum* surpassed initiation action of the organic insecticide and there was the statistically significant difference during the whole period. Combination contributed to a longer time-effect compared to both products use alone. Higher efficacy of the mix of the alginate from seaweed with the Nano fertilizer to seaweed alginate, but statistically insignificant differences were observed on the first and fifth day after treatment for alfalfa seed chalcid, and in all reporting days regarding plant bug.

Table 4. *Efficacy of organic products (alone and in combination), 2016.*

Variants																	
							Bruchophagus roddi										
NBT		E, % 1 DAT		NAT	Sd	E, % 5 DAT		NAT	Sd	E, % 7 DAT		NAT	Sd	E, % 9 DAT		NAT	Sd
1	19.1	**70.0**	c	6.3	2.82	**61.7**	cd	7.4	2.40	**41.2**	c	13.6	3.11	**10.0**	a	16.7	6.65
2	17.4	**56.7**	b	8.2	3.81	**49.3**	b	9.0	4.66	**28.9**	b	15.0	4.10	**2.8**	a	16.5	3.18
3	19.4	**37.0**	a	13.3	4.24	**25.8**	a	14.6	4.52	**15.3**	a	19.9	6.64	**6.5**	a	17.6	4.94
4	20.8	**77.0**	c	5.2	3.11	**70.5**	d	6.2	3.53	**58.7**	d	10.4	3.81	**32.0**	b	13.8	1.41
5	22.2	**70.8**	c	7.1	1.97	**53.5**	bc	10.5	2.12	**39.5**	bc	16.3	4.94	**12.4**	a	18.9	
6	18.5	-		20.2		-		18.8		-		22.4		-		18.0	
LSD $_{0.05\%}$		8.461				9.362				12.044				11.011			
							Acyrthosiphon pisum										
1	137.2	**80.6**	c	29.3	3.398	**76.4**	c	35.6	3.39	**70.7**	d	59.6	1.84	**59.7**	c	85.2	3.81
2	141.0	**58.0**	b	38.2	4.24	**50.3**	b	46.8	4.67	**42.3**	b	89.7	2.69	**22.6**	b	112.8	3.39
3	139.9	**25.1**	a	97.2	2.96	**21.5**	a	96.7	5.51	**11.4**	a	130.4	6.22	**4.4**	a	122.1	0.84
4	140.6	**90.8**	d	12.0	3.11	**84.0**	d	19.8	2.83	**80.6**	e	28.7	3.38	**67.8**	d	41.3	3.11
5	135.7	**82.1**	cd	22.5	3.25	**72.5**	c	39.2	2.12	**59.4**	c	50.3	3.11	**57.9**	c	52.2	2.68
6	142.7	-		132.4		-		150.1		-		130.3		-		130.3	
LSD $_{0.05\%}$		8.800				6.380				7.566				7.601			
							Adelphocoris lineolatus										
1	24.2	**82.6**	cd	3.9	2.54	**77.5**	d	4.7	2.12	**66.0**	d	6.6	2.82	**50.1**	d	11.0	6.92
2	22.7	**60.4**	b	8.4	4.80	**52.0**	b	9.4	2.82	**45.1**	b	10.1	2.96	**25.3**	b	15.5	5.23
3	23.1	**18.2**	a	17.6	3.95	**12.6**	a	17.4	3.68	**14.0**	a	16.0	4.24	**0.5**	a	21.0	0.42
4	25.1	**90.7**	d	2.2	3.25	**82.1**	d	3.9	1.56	**71.7**	d	5.7	2.40	**59.2**	d	9.4	3.11
5	20.4	**73.7**	c	5.0	3.23	**60.8**	c	6.9	3.11	**55.0**	c	8.4	4.24	**37.2**	c	11.7	2.54
6	25.5	-		23.8		-		22.0		-		20.6		-		23.3	
LSD $_{0.05\%}$		9.370				7.097				8.800				11.011			

Legend: 1–Agricolle; 2–Nagro; 3–Lithovit; 4–Agricolle+Nagro; 5–Agricolle+Lithovit; 6–Control; 1 DAT – first day after treatment; 3 DAT– third day after treatment; 7 DAT– seventh day after treatment; 9 DAT– ninth day after treatment; E–efficacy,%; NBT– number of individuals before treatment from 10 m^2; NAT– number of individuals after treatment from 10 m^2; Sd – standard deviation.

*Means in each column marked by the same letter are not significantly different ($P > 0.05$).

It was found high efficacy and a good control of level with the longest duration (one week) at the pea aphid, followed by the plant bug – a five-day good control when applied to that mix. During the remaining days, the combination provided a medium level of control of the two species (60 to 80% efficacy) and to *B. roddi* – medium level from the first to the seventh day.

Lower efficacy and a shorter duration of a medium level of control of insect pests characterized the combined application of the organic insecticide and natural fertilizer.

Application of organic products in 2017 confirmed the outlined trend (Table 5). Seaweed alginate had higher efficacy during the reporting period with significant differences to fertilizers against the pests and provided a good level of control five days after application (over 80% efficacy). The effect on the seventh day showed a medium level of control and on a ninth day – a limited control.

Nano fertilizer exhibited good insecticidal activity and a medium level of control one week after use. Efficacy at *A. pisum* was lower than 60% (56 – 58%) but values approached that percentage and the product had a satisfactory effect.

Treatment with the alginate from seaweed with Nano fertilizer had the highest efficacy. The combination provided excellent control of *B. roddi* and *A. lineolatus* five days after use, and for *A. pisum* – for seven days. Its efficacy was highest with significant differences to other treatments on the seventh and ninth day in seed chalcid; on the fifth, seventh and ninth day – in pea aphid and on the first, seventh and ninth day after application – in plant bugs.

The efficacy in combining use the organic insecticide with the natural fertilizer had not statistically different from the respective at the separate use of the insecticide, as, in *B. roddi* and *A. pisum*, the established values were lower significantly on the ninth and seventh, and ninth days respectively.

In a comparative analysis of the results, depending on the species of insect pest was found that used products and their combinations exhibited the highest efficacy against *A. pisum*, followed by *A. lineolatus* and *B. roddi*.

Table 5. *Efficacy of organic products (alone and in combination), 2017.*

Variants																	
	Bruchophagus roddi																
	NBT	E, % **1** DAT		NAT	Sd	E, % **5** DAT		NAT	Sd	E, % **7** DAT		NAT	Sd	E, % **9** DAT		NAT	Sd
1	21.0	**86.1**	c	2.6	4.52	**83.9**	cd	2.9	3.60	**69.3**	c	6.6	1.90	**44.0**	c	12.3	1.41
2	19.4	**70.2**	b	5.2	2.96	**63.1**	b	6.2	3.95	**59.3**	b	8.0	2.33	**31.0**	b	14.1	2.96
3	21.2	**30.0**	a	13.3	3.53	**24.0**	a	13.9	3.46	**25.9**	a	16.0	1.76	**12.3**	a	19.5	1.62
4	22.7	**93.6**	c	1.3	2.26	**90.1**	d	1.9	0.14	**76.4**	d	5.5	2.54	**60.5**	d	7.8	2.40
5	18.7	**83.9**	bc	2.7	10.25	**78.5**	c	3.6	2.12	**58.5**	b	7.9	3.53	**33.8**	b	13.0	2.54
6	22.1	-		19.8		-		19.0		-		22.5		-		23.2	
LSD $_{0.05\%}$		14.175				7.729				6.420				5.830			
	Acyrthosiphon pisum																
1	100.6	**86.2**	b	11.5	2.75	**81.8**	c	17.1	1.90	**70.6**	c	31.3	1.27	**55.2**	c	40.6	0.84
2	87.4	**56.1**	a	31.8	3.32	**56.9**	b	35.2	4.24	**58.2**	b	38.6	3.53	**16.3**	b	65.9	3.39
3	84.6	**60.4**	a	28.7	3.11	**29.0**	a	56.1	4.52	**24.7**	a	67.3	2.68	**3.8**	a	73.3	2.54
4	107.5	**91.7**	b	7.4	2.68	**90.2**	d	9.8	2.54	**88.4**	d	13.2	2.82	**73.7**	d	25.5	2.68
5	91.2	**89.1**	b	8.2	1.90	**77.5**	c	19.2	2.12	**72.7**	c	26.3	3.25	**61.1**	c	32.0	4.03
6	107.3	-		88.9		-		100.2		-		113.4		-		96.7	
LSD $_{0.05\%}$		7.197				8.377				7.263				7.466			
	Adelphocoris lineolatus																
1	22.4	**84.1**	c	4.3	1.06	**78.4**	cd	4.4	5.51	**63.0**	c	8.5	2.40	**54.7**	c	11.5	1.76
2	20.3	**63.3**	b	9.0	8.48	**64.8**	b	6.4	3.25	**52.9**	b	9.8	2.19	**41.3**	bc	13.5	1.37
3	22.2	**45.1**	a	14.7	0.07	**27.9**	a	14.4	9.40	**20.7**	a	18.1	2.61	**13.7**	a	21.7	1.83
4	18.8	**95.1**	d	1.1	0.14	**86.3**	d	2.3	1.27	**70.1**	d	5.8	2.96	**52.6**	c	10.1	2.26
5	18.1	**79.0**	c	5.4	1.41	**70.4**	bc	4.6	2.54	**61.1**	c	6.9	2.68	**38.9**	b	13.1	11.24
6	19.0	-		21.8		-		16.3		-		18.6		-		20.5	
LSD $_{0.05\%}$		9.965				13.482				6.650				13.658			

Legend: 1–Agricolle; 2–Nagro; 3–Lithovit; 4– Agricolle+Nagro; 5– Agricolle+Lithovit; 6–Control; 1 DAT – first day after treatment; 3 DAT– third day after treatment; 7 DAT– seventh day after treatment; 9 DAT– ninth day after treatment; E–efficacy,%; NBT– number of individuals before treatment from 10 m^2; NAT– number of individuals after treatment from 10 m^2; Sd– standard deviation;

[*] Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 6. *Seed yield in alfalfa after treatment with organic products, average for the period 2015-2017.*

Variants	Seeds kg ha^{-1}		Differences to C, %
Control	549.4	a	-
Agricolle	683.3	d	24.4
Nagro	656.0	c	19.4
Lithovit	627.3	b	14.2
Agricolle+Nagro	712.7	e	29.7
Agricolle+Lithovit	680.7	d	23.9
LSD $_{0.05\%}$		20.32	

* Means in each column marked by the same letter are not significantly different ($P > 0.05$).

Table 7. *Variation analysis depending on seed yield.*

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Influence of factor, %		Mean square (MS)
Total	53	217420.00	-		4102.3
Variants	17	208828.74	96.0		12284.0
Factor A – Year	2	53373.10	24.5	*	26686.6
Factor B –Product	5	150641.00	69.3	*	30128.2
A x B	10	4814.44	2.2	*	481.4
Pooled error	36	8591.26	4.0		238.7

* There are significantly different ($P > 0.05$).

Results of the present study showed a high and statistically significant positive effect of products on the generative development of alfalfa (Table 6). Organic insecticide treatment led to an increase in the seed yield by 24.4% and exceeded productivity after fertilizer use with significant differences. The yield of plants treated with the mix of the organic insecticide and Nano fertilizer reached the highest value of 29.7% with statistical differences compared to other variants. The act of the active ingredients of the fertilizer determined that and also its insecticidal effect. It was also important the high content of macro and micronutrients including pine, whose positive influence on the processes of flowering, pollination and seed development of alfalfa. Several authors established that. The effect of natural fertilizer using was the least pronounced, but sufficiently high and statistically significant compared to the control variant (+ 14.2%). Its mix with the seaweed alginate ensured a yield close to that of the organic insecticide.

Analysis of variant regarding the seed yield (Table 7) showed that the factors years of study, respectively weather conditions during the treatment years and the type of used product had a significant impact on the yield variation. The dominant effect had the applied product (69.3% of the total variation) due to differences in mechanism of action and the active substances, followed by treatment years (24.5%). An interaction between products and weather over the years (A × B – 2.2%) had a significant but a lesser impact on yield variation.

Discussion

Tested organic products had a different influence. The alginate from seaweed is an innovative product with a natural mechanism of action. On the properties of some natural polysaccharides from seaweed to selectively stick of a small insect body alginate based. It acts as a coating and causes suffocation for harmful insects like aphids, whiteflies, fleas, etc. (according to Producer).

Marine algae are the renewable living resources which are a rich source of an important novel and biologically active secondary metabolites. It was found that marine algae had insecticidal activities and offer a novel approach to pest control and management (Sahayaraj & Mary Jeeva, 2012; Bantoto & Dy, 2013; Syed Ali et al., 2013).

In our study, the organic insecticide had high efficacy against insect pests and provided a good level of control five days after application (over 80% efficacy). Therefore, it is an excellent tool for strategies for biological and integrated pest control.

Similar results associated with high efficacy and excellent control of that bio-insecticide against adults of greenhouse whitefly in cucumbers (*Trialeurodes vaporariorum* Westv) for 14 days reported Yankova & Masheva (2010). Authors found that the product provided good protection against powdery mildew (*Sphaerotheca fuliginea* Pollaci) and adults of cotton aphid (*Aphis gossypii* Glov). Pilkington and colleagues (2013) also found a high efficacy of the alginate from seaweed and a significant reduction in the number of greenhouse whitefishes and *Tetranychus urticae* Koch in greenhouse cucumbers and tomatoes. In addition, Piattoni et al. (2010) identified the fungicidal activity of the product against *Alternaria* and *Peronospora* genus and the increased viability of the plants.

Nanotechnology in the agricultural sector offers several novel approaches in the pest management and crop production. It can use nanoparticles in preparing new formulations of pesticides and fertilizers. According to Khan & Rizvi (2017), nanoencapsulation is a most promising technology to step down the chemical release under controlled situations, reducing the current application dosage and improving the efficiency of a fertilizer or pesticide.

The Nano-fertilizer is a highly concentrated complex liquid bio-organic fertilizer that has bio-insecticide properties with anti-pheromone action. Because of the extremely rich content of macro and microelements, natural plant hormones, etc., the Nano-fertilizer not only increases the resistance to unfavorable climatic factors (drought, low temperatures) but also increases the leaf area, and subsequently the intensity of photosynthesis. The Nano-product enhances the natural immune response of plants by contributing to better natural resistance to insect pests (according to Producer).

The highly concentrated complex organic Nano fertilizer exhibited insecticidal properties and provided a medium level of control against the three species pests from the first to the seventh day after treatment in 2015 and 2017, and from the first to the fifth day in 2016. Because of its properties, the product led to the increased efficiency of the combination (with the organic insecticide), which provided the highest efficacy and long-lasting effect (in 2015 and 2017 – seven days after treatment at *B. roddi* and *A. lineolatus*, and nine days at *A. pisum*; in 2016 five days in *B. roddi*, seven days at *A. lineolatus*, and nine days at *A. pisum*). The combined application of organic products is a suitable alternative to plant protection. That approach has a lot of success in the last years. The combined use of insect pathogens and botanical agents has received too some interest in recent years (Mascarin & Delalibera, 2012). Several studies have shown additive effects at low doses of botanical insecticides and viruses. For example, previous studies have shown increased mortality of lepidopteran larvae by the combined use of nucleopolyhedrosis virus and azadirachtin (Nathan et al., 2005; Nathan & Kalaivani, 2006). Fei et al. (2012) discovered a synergistic effect or additive effects of destruxins and three botanical insecticides, rotenone, azadirachtin and paeonolum against the cotton aphid, *Aphis gossypii*.

In the present study, application of organic products alone and in mix provided great protection against insect pest (except natural fertilizer, used alone) and high seed productivity. That allows treatment with those products in terms of organic farming.

Conclusions

The alginate from seaweed had high efficacy and excellent control of the three insect species density one week after treatment (over 80% efficacy). Nano fertilizer exhibited insecticidal action against pests and its effect exceeded 60% seven days after treatment as provided a medium level of control.

The mix of the seaweed alginate and Nano fertilizer provided the highest efficacy and long-lasting effect against three insect species. It was observed an additive effect.

In a comparative analysis, depending on the species of insect pest was found that used products and their combinations exhibited the highest efficacy against *A. pisum*, followed by *A. lineolatus* and *B. roddi*.

Organic products positively influenced the generative development of alfalfa as in the mix of the seaweed alginate and it realized Nano fertilizer the highest seed yield and an increase by 29.7%.

The dominant effect regarding the seed yields had factor type of applied product (69.3% of the total variation) followed by treatment years (24.5%).

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