

PESTS ON ANNUAL LEGUME CROPS AND POSSIBILITIES FOR CONTROL IN BIOLOGICAL PRODUCTION

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Abstract

Biological production of legume crops is becoming more widespread in many countries, with research aimed at increasing yields and improving product quality. Legumes are an important element of agrotechnical approaches in biological systems. They are a suitable precursor for a various vegetable crops and are actively involved in crop rotation. Annual legumes are attacked by many pests, which at high densities can compromise the yield. Experiments to determine the effectiveness of biological plant protection products to control the main pests on beans and peas grown in a certified organic field were conducted at the Maritsa Vegetable Crops Research Institute, Plovdiv during the period 2019-2020. The tested pests were: black bean aphid (*Aphis fabae* Scop.), pea aphid (*Acyrtosiphon pisum* Harris), bean weevil (*Acanthoscelides obtectus* Say), pea weevil (*Bruchus pisi* L.), pea moths (*Laspeyresia nigricana* Step. and *Laspeyresia dorsana* L.) and twospotted spider mite (*Tetranychus urticae* Koch.). Tested products are: Piretro Natura EC 75 ml/da (a. i. pyrethrins), Neem Azal T/C 0.3% (a.i. azadirachtin), Naturalis 100 ml/da (a. i. *Beauveria bassiana*), Dipel 2 X 100 g/da (a. i. *Bacillus thuringiensis* var. *kurstaki* strain ABTS) and Rapax 100 ml/da (a. i. *Bacillus thuringiensis* subsp. *kurstaki*, strain EG 2348).

Key words: pests, *Phaseolus vulgaris*, *Pisum sativum*, biological products, plant protection

1. INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) and garden pea (*Pisum sativum* L.) are the main legume vegetables in Bulgaria. They are excellent sources of dietary fiber and nutrients. At the same time, legumes are rich in plant protein, iron and zinc, which give them an important place in the group of protein foods.

Leguminous vegetable crops are used as a staple food with high biological value. They provide in easily digestible form proteins, carbohydrates, vitamins, minerals and other physiologically active substances.

Leguminous vegetable crops have a short vegetative period, and therefore they are very suitable in vegetable crop rotations. They improve soil fertility through symbiotic relations with bacteria of the genus *Rizobium* and are good precursors for all other crops except those in family *Fabaceae*.

The production of green beans and garden peas is limited by certain environmental stress factors, in particular biotic and abiotic. Each of these restrictions causes a significant yield reduction. Among the biotic factors, the attack by pests causes a significant reduction in the yield of legumes.

Damage from the bean weevil (*Acanthoscelides obtectus* Say) is of economic importance in the growing of beans for seed production. Control against this pest is difficult, demand a comprehensive approach and should start in the bean crops, targeting against adult beetles.

In recent years, efforts have focused on the use of natural plant protection products such as phytopesticides. Many plants have insecticidal properties, which are due to located in them natural alkaloids, esters, glycosides and others [1].

Natural pesticides are pesticides made by organisms usually for their own defense, or are derived from a natural source such as plant, animal, bacteria, fungi and certain mineral, use to control pest naturally. The common natural pesticides uses are neem (*Azadiracta indica*), microbial pesticide *Bacillus thuringiensis* and pyrethrins. Natural pesticides usually target specific sites in the insect such as

nervous system, resulting in knock-down, lack of coordination, paralysis and death [2]. The primary active ingredient of most neem-based pesticides is azadirachtin, a liminoid compound, which has multiple biological activities on more than 400 insect species from several orders. Azadirachtin-based compounds have insecticidal, feeding deterrent, repellent, antioviposition, and physiological properties [3].

Plant products have a number of advantages that make them preferred in modern agriculture. They are not a threat to the environment or human health. Such is pyrethrum, an extract of chrysanthemum (*Chrysanthemum cinerariaefolium* Vis.), based on natural pyrethrins. The insecticidal activity of pyrethrum is provided by six constituent esters known as pyrethrins [4]. Unique in their ability to repel most insect pests while not posing a threat to the environment, pyrethrins have been of great interest to many scientists [5]. The product Piretro Natura EC belongs to this group. The insecticidal action of pyrethrins is characterized by rapid action, hyperactivity and convulsions in most insects. These symptoms result from the neurotoxic effects of pyrethrins, which block sodium channels [6; 7].

Common pest in field growing of pea is the pea weevil (*Bruchus pisi* L.). The damage is caused by the larvae, which for its full development destroys a large part of the grain content, affecting the germ as well. Damaged seeds reach 56%, they have lower weight and reduced germination [8].

Aphids (Hemiptera: Aphididae) are a major group of pests that have a high degree of reproduction. They cause direct damages to the host plant by sucking plant sap. At the feeding, they release undigested sugars in the form of "honeydew". When aphid populations are extremely large, this honeydew can cover the leaf surface, forming an ideal substrate for the growth of sooty mold, which affect the quality of the produced beans. Sooty molds, together with honeydew, reduce the efficiency of respiration and photosynthesis of the plant and thus reduce the yield. In addition to direct damage from their feeding, aphids are vector of viruses [9].

The black bean aphid, *Aphis fabae* Scopoli is a polyphagous that attacks more than 200 legumes and can damage all parts of the plant [10]. It causes a yield loss of about 37% [11]. In peas, pea aphid, *Acyrtosiphon pisum* Harris is considered to be a serious pest because reduces both the weight and caloric content of young pea plants, depending on the number of aphids [12].

Pest management through biological control is a modern approach, using different predators, parasites and pathogens. Entomopathogenic fungi are considered unique compared to other micropathogens. Entomopathogenic fungi can be used to pest control and do not affect on other non-target organisms [13].

Bacillus thuringiensis is an aerobic Gram-positive endospore-forming bacterium which is part of the family *Bacillaceae* and it is widely used in agriculture as a biological pesticide. Commercial formulations of *B. thuringiensis* have been used in the last few decades to control insects of the order *Lepidoptera* and more recently to species of the order *Coleoptera* and *Diptera*. Many of these products are based on spore-crystal preparations [14].

The technologies of organic production of legumes are increasingly used in many countries, as the studies are directed to improving conditions, increasing yields and improving product quality [15].

The establishment of efficient, modern methods and means to pest control in the field growing of legumes is a priority in order to obtain high quality products free of pesticide residues and preserve the biological balance in agrocenoses.

The aim of the study is to determine the effectiveness of biological plant protection products against the main pests in growing of green bean (*Phaseolus vulgaris* L.) and garden pea (*Pisum sativum* L.) in the open field.

2. MATERIALS AND METHODS

The studies were conducted during the period 2019-2020 at Maritsa Vegetable Crops Research Institute, Plovdiv in the field in a natural background of pest attack. The experiments were performed on green bean varieties Tangra and Evros and garden pea varieties Skinado and Marsi.

2.1. Aphids (*Homoptera: Aphididae*)

In aphid-infested pea plants are sprayed with the appropriate concentration/dose of insecticide. The number of live individuals before spraying and at intervals after 1, 3, 5, 7, 10 and 14 days on pre-marked pea plants was counted. Test pest: black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrtosiphon pisum* Harris). Test products for plant protection: Piretro Natura EC in a dose of 75 ml/da (a. i. pyrethrins) and Naturalis 100 ml/da (a. i. *Beauveria bassiana*). Total area of the experimental plot 200 m² conventional and 200 m² biological pea production. The effectiveness (E%) was calculated by the formula [16]:

$$E\% = \left(1 - \frac{Ta \times Cb}{Tb \times Ca}\right) \cdot 100$$

Ta - number of live aphids in the variant after treatment; *Tb* - number of live aphids in the variant before treatment; *Ca* - number of live aphids in the control after treatment; *Cb* - number of live aphids in the control before treatment.

2.2. Bean weevil (*Acanthoscelides obtectus* Say)

The experiments were performed on bean varieties Tangra and Evros on an area of 200 m². The insecticides were sprayed twice at intervals of 7 days. Test plant protection products: Piretro Natura EC at a dose of 75 ml/da (a. i. pyrethrins) and Neem Azal T/C 0.3% (a. i. azadirachtin). Representative samples of 100 beans were taken from each variant. Seed damages in bean weevil are classified in the following classes [17]: 0 - without holes; 1 - with one hole from the weevil; 2 - with two holes from the weevil; 3 - with three holes from the weevil; 4 - with four or more holes from the weevil. The index of infestation (%) in seed was calculated 50 days after harvesting the beans according to the formula [18]:

$$I = \frac{\sum(x \cdot f) \cdot 100}{n \cdot 4}, \text{ where}$$

I - index of infestation (%); *x* - class value; *f* - frequency; *n* - number of seeds in the sample. Effectiveness (%) is calculated based on the index of infestation by the formula [19]:

$$E = \left(\frac{Ik - Iv}{Ik} \right) \cdot 100 \quad (\%), \text{ where}$$

E - effectiveness, %; *Ik* - index of infestation in the control (%); *Iv* - index of infestation in the variant (%).

2.3. Pea weevil (*Bruchus pisi* L.)

Field experiments were performed on pea varieties Skinado and Marsi. The insecticides were sprayed twice at intervals of 7 days. Test products for plant protection: Piretro Natura EC in a dose of 75 ml/da (a. i. pyrethrins), Neem Azal T/C 0.3% (a. i. azadirachtin). The first treatment was carried out during flowering and the formation of the first pods at the establishment of 10 numbers/100 plants. Total area of the experimental plot 200 m² biological pea production. After harvesting the peas from the field, average samples of 500 seeds were taken. They are placed in glass jars insulated with cheesecloth at room temperature for 50 days. The percentage of damaged seeds was then recorded and the effectiveness (%) was calculated [19]:

$$E = \left(\frac{Ik - Iv}{Ik} \right) \cdot 100 \quad (\%), \text{ where}$$

E - effectiveness, %; Ik - percentage of infested seeds in the control (%); Iv - percentage of infested seeds in the variant (%).

2.4. Pea moths (*Laspeyresia nigricana* Step. and *L. dorsana* L.)

Field experiments were performed on pea varieties Skinado and Marsi, under natural infestation by pea moths. It was sprayed twice with insecticides at intervals of 7 days. Test plant protection products: Rapax 100 ml/da (a. i. *Bacillus thuringiensis*, subsp. *kurstaki*, strain EG 2348), Dipel 2 X 100 g/da (a. i. *Bacillus thuringiensis* var. *kurstaki* strain ABTS), Neem Azal T/C 0.3% (a. i. azadirachtin). The first treatment was carried out during flowering and the formation of the first pods. Total area of the experimental plot 200 m² biological pea production. After harvesting the peas from the field, average samples of 500 seeds per variant were taken. The percentage of damaged seeds was reported and the effectiveness (%) was calculated [19].

2.5. Twospotted spider mite (*Tetranychus urticae* Koch.)

Five reports were made: First (in advance) - immediately before treatment; Second - 1 day after treatment; Third - 3 days after treatment; Fourth - 7 days after treatment; Fifth - 14 days after treatment. Effectiveness was calculated by the Henderson-Tilton formula. The mobile stages of the pest (larvae, nymphs, adults) before and at intervals after treatment are reported. Samples were taken from pre-marked plants. The reading of living individuals on the leaves taken from the marked plants is performed with the help of a stereomicroscope (binocular) in laboratory conditions. For some products, two treatments were performed at 7-day intervals. Test plant protection product: Neem Azal T / C 0.3% (a. i. azadirachtin).

The experiments with biological plant protection products were conducted in a certified biological field.

Data were processed using Duncan's multiple range test at $P < 0.05$ levels [20].

3. RESULTS AND DISCUSSION

Biological production of vegetable crops is a current direction in agriculture, which is more popular. Although the range of biological plant protection products is limited, they have been available on the market in recent years. The studies are directed at determining their biological activity against economically important pests with a view to their optimal combination for effective control.

Experiments for determining aphicide effect of the products Piretro Natura EC 75 ml/da and Naturalis 100 ml/da were made in bean varieties grown in open field. The product Piretro Natura EC 75 ml/da has the highest effectiveness in variety Evros 80.54% on the 5th day after treatment. In variety Tangra the biological activity reaches 80.01%. The product Naturalis 100 ml/da also demonstrate very good aphicide activity. It has a relatively delayed effect, as maximum values of effectiveness were reported 7th day after treatment. The highest value of this indicator was reported for variety Tangra 81.76%. In variety Evros the effectiveness reaches 81.16% (Table 1).

In the pea varieties included in the study, experiments to determine the effectiveness of the tested bioproducts also were made against pea aphid. The product Piretro Natura EC 75 ml/da shows relatively good biological activity (variety Marsi $E = 81.33\%$ on the 5th day after treatment). The bioproduct Naturalis 100 ml/da demonstrates a slower action, typical for microbial products. A maximum value of effectiveness 80.92% was reported on day 7 after treatment in variety Skinado. In variety Marsi, the highest effectiveness was also reported on the 7th day after the treatment, 79.23% respectively (Table 2).

The biological activity of the tested products significantly decreased on the 14th day after treatment. This trend was reported and begins 10 days after treatment. This suggests that for relatively good crop

protection by aphids, it is recommended to perform two or three treatments with the bioproducts Piretro Natura EC 75 ml/da and Naturalis 100 ml/da at intervals of 7 days.

Table 1. Results of biological test of plant protection products against black bean aphid (*Aphis fabae* Scop.) in field growing of bean varieties

Treatment	Days after treatment	Variety/ Effectiveness, %	
		Tangra	Evros
Piretro Natura EC 75 ml/da	1	62.60 b	61.74 ab
Naturalis 100 ml/da		50.18 b	52.89 c
Piretro Natura EC 75 ml/da	3	79.86 a	71.09 ab
Naturalis 100 ml/da		57.11 ab	66.18 abc
Piretro Natura EC 75 ml/da	5	80.01 a	80.54 a
Naturalis 100 ml/da		69.00 ab	74.35 ab
Piretro Natura EC 75 ml/da	7	69.01 ab	69.69 ab
Naturalis 100 ml/da		81.76 a	81.16 a
Piretro Natura EC 75 ml/da	10	65.75 ab	60.67 b
Naturalis 100 ml/da		67.74 ab	75.38 ab
Piretro Natura EC 75 ml/da	14	54.30 b	53.35 b
Naturalis 100 ml/da		64.91 ab	61.54 bc

a, b, c ... – Duncan's multiple range test ($p < 0.05$)

The bioproducts Piretro Natura EC 75 ml/da and Neem Azal T/C 0.3% were studied to control weevils in beans and peas grown in the open field. The phytopesticide Piretro Natura EC shows low biological activity against bean weevil in both bean varieties (variety Tangra E = 60.58%, variety Evros E = 55.85%). The product Neem Azal T/C has good effectiveness in both bean varieties, in variety Tangra E = 73.72% and in variety Evros E = 73.87%, respectively (Table 3). The same bioproducts have been tested against pea weevil in pea varieties Skinado and Marsi. The same trend was reported here. In the bioproduct Piretro Natura EC 75 ml/da, the reported effectiveness against pea weevil is unsatisfactory ($E < 65\%$). The phytopesticide Neem Azal T/C 0.3% showed very good biological activity ($E > 65\%$) in both cultivars included in the study (Table 4). The bioproduct Neem Azal T/C 0.3% can be used to control this pest in the open field, as the treatments will limit the attack of weevils in the field and will provide a better protected production of seeds until they are brought into storehouse.

The control of moths in garden pea is difficult due to the hidden lifestyle of the caterpillars. Treatments should be carried out at the appropriate time before nibbling at the pods and grains. Good effectiveness $> 70\%$ from the tested bioproducts demonstrated Dipel 2 X 100 g/da, followed by Rapax 100 ml/da in both pea varieties. The phytopesticide Neem Azal T/C 0.3% has unsatisfactory biological activity (Table 5).

Table 2. Results of biological test of plant protection products against pea aphids (*Acyrtosiphon pisum* Harris) in field growing of pea varieties

Treatment	Days after treatment	Variety/ Effectiveness, %	
		Skinado	Marsi
Piretro Natura EC 75 ml/da	1	68.11 ab	55.65 b
Naturalis 100 ml/da		44.73 d	45.09 b
Piretro Natura EC 75 ml/da	3	79.11 a	73.37 ab
Naturalis 100 ml/da		58.54 cd	61.14ab
Piretro Natura EC 75 ml/da	5	79.16 a	81.33 a
Naturalis 100 ml/da		68.35 abc	65.59 ab
Piretro Natura EC 75 ml/da	7	66.14 ab	78.47 ab
Naturalis 100 ml/da		80.92 a	79.23 a
Piretro Natura EC 75 ml/da	10	53.43 b	57.24 ab
Naturalis 100 ml/da		77.39 ab	75.21 ab
Piretro Natura EC 75 ml/da	14	45.60 b	52.98 b
Naturalis 100 ml/da		61.28 bc	65.05 b

a, b, c .. – Duncan's multiple range test ($p < 0.05$)

Table 3. Effectiveness of plant protection products against bean weevil (*Acanthoscelides obtectus* Say) in field growing of bean varieties Tangra and Evros

Variety	Total number of seeds	Infested seeds		Index of infection	Effectiveness
		number	%	%	%
Biological production – treatment with Piretro Natura EC 75 ml/da					
Tangra	443	20	4.51	2.31	60.58
Evros	514	71	13.81	6.37	55.85
Biological production – treatment with Neem Azal T/C 0.3%					
Tangra	456	15	3.29	1.54	73.72
Evros	510	45	8.82	3.77	73.87
Biological production – control					
Tangra	397	42	10.58	5.86	-
Evros	466	122	26.18	14.43	-

Table 4. Effectiveness of plant protection products against pea weevil (*Bruchus pisi* L.) in field growing of pea varieties Skinado and Marsi

Variety	Total number of seeds	Infested seeds		Effectiveness
		number	%	%
Biological production – treatment with Piretro Natura EC 75 ml/da				
Skinado	500	87	17.40	61.50
Marsi	500	91	18.20	60.61
Biological production – treatment with Neem Azal T/C 0.3%				
Skinado	500	68	13.60	69.91
Marsi	500	72	14.40	68.83
Biological production - control				
Skinado	500	226	45.20	-
Marsi	500	231	46.20	-

Table 5. Effectiveness of plant protection products against pea moths (*Laspeyresia nigricana* Step. and *L. dorsana* L.) in growing of pea Skinado and Marsi in two production systems

Variety	Total number of seeds	Infested seeds		Effectiveness
		number	%	%
Skinado				
Rapax 100 ml/da	500	6	1.20	70.00
Dipel 2 X 100 g/da	500	5	1.00	75.00
Neem Azal T/C 0.3%	500	7	1.40	65.00
Control	500	20	4.00	-
Marsi				
Rapax 100 ml/da	500	7	1.40	69.57
Dipel 2 X 100 g/da	500	6	1.20	73.91
Neem Azal T/C 0.3%	500	9	1.80	60.87
Control	500	23	4.60	-

The twospotted spider mite (*Tetranychus urticae* Koch.) is found in many vegetable crops, including beans. The highest population density of it was found in the period from the second ten days of July to the second ten days of August, when relatively highest average daily temperatures were registered during the vegetation of field beans [21]. The products with the active ingredient azadirachtin have a specific action - they suppress and stop feeding, reduce molting, reduce the fertility of females [22; 23]. The action of Neem Azal T/C was defined as "insecticide-static" [22]. In our experiments we observe good biological activity of this product. The maximum value of effectiveness against the

mobile forms of *T. urticae* was reported on the 7th day after treatment in both cultivars included in the study, in variety Tangra - 90.36% and in variety Evros - 88.10%, respectively (Table 6).

Table 6. Effectiveness of the product Neem Azal T/C 0.3% against twospotted spider mite (*Tetranychus urticae* Koch.) in field growing of bean varieties

Treatment	Days after treatment	Effectiveness, %/Variety	
		Tangra	Evros
Neem Azal T/C 0.3%	1	68.99 b	72.34 b
Neem Azal T/C 0.3%	3	84.01 a	86.72 ab
Neem Azal T/C 0.3%	7	90.36 a	88.10 a
Neem Azal T/C 0.3%	14	81.56 a	78.92 ab

a, b, c .. – Duncan's multiple range test ($p < 0.05$)

In the growing of beans in the open field, in addition to mentioned pests in certain years can be observed attack by greenhouse whitefly and tobacco thrips. In biological production systems, treatments with plant protection products Piretro Natura EC 75 ml/da and Neem Azal T/C 0.3% can lead to a reduction in their population density. Pests often appear at the same time and treatments against aphids and weevils can limit their attack.

The timely application of bioproducts ensures well-preserved production of beans and peas in the open field.

4. CONCLUSIONS

Piretro Natura EC 75 ml/da and Naturalis 100 ml/da have good afficide activity ($E > 75\%$) and can be used to control aphids in field growing of beans and peas.

The bioproduct Neem Azal T/C 0.3% showed good biological activity ($E > 65\%$) against weevils in beans and peas in the open field. The phytopesticide Piretro Natura EC 75 ml/da has unsatisfactory effectiveness ($E < 65\%$) against these pests.

The bioproduct Dipel 2 in a dose of 100 g/da is efficient against pea moths (*Laspeyresia nigricana* Step. and *L. dorsana* L.) and can be included in biological systems for pea production.

The phytopesticide Neem Azal T/C 0.3% has a very good effectiveness $> 85\%$ against twospotted spider mite (*Tetranychus urticae* Koch.) in field growing of beans and can be successfully applied in biological production systems.

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