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ORIGINAL ARTICLE

Controlling *Acyrthosiphon pisum* L. infestation using products of natural origin in forage pea

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Abstract

The perspective direction in the biological regulation of insect pest populations is the combined use of different products of organic origin including fungal biological control agents. Therefore, the present study was aimed to evaluate the efficacy of products of natural origin (Aminogreen 24, Nitrogreen, Foliamin and Naturalis – strain ATCC 74040 of entomopathogenic fungus *Beauveria bassiana*) and one synthetic insecticide – standard (deltamethrin + thiacloprid), applied alone and in a mixture in the control of *Acyrthosiphon pisum* in forage pea. The study was conducted in a field experiment during the period 2017 – 2020. Infestation by leaf aphids was estimated by calculating the cumulative insect-days (*CID*). It was found that the combination of Aminogreen 24 + Naturalis had the most pronounced decrease in *CID* among products over the years from 2017 to 2020 and the average for the period. The greatest, significant reduction in the number of aphids occurred on day 5 ($F_{8.5} = 15.244$; $p < 0.033$) and day 7 ($F_{8.5} = 33.087$; $p < 0.037$) after treatment. On the 14th day, the decrease in *CID* (57.4% decrease) statistically exceeded the Proteus 110 OD standard (55.3% decrease) ($F_{8.5} = 49.841$; $p < 0.049$). Good protection against *A. pisum* was also found with Naturalis and Nitrogreen + Naturalis. There was an additive effect between Naturalis and Aminogreen 24 throughout the entire study period. The ratio of chlorophyll *a* (Chl *a*) to chlorophyll *b* (Chl *b*) and the ratio of green pigments (Chl *a* + + Chl *b*) to carotenoids determined that plants treated with Aminogreen 24 + Naturalis and Naturalis had the best physiological state. The combination of Aminogreen 24 and Naturalis gave the largest, significant, increase in stem height, followed by Nitrogreen + Naturalis. The use of Naturalis, alone and in a combination with Aminogreen 24 and Nitrogreen can be a successful alternative to conventional chemical control.

Keywords: control, naturalis, organic fertilizers, pea aphid, plastid pigments

Introduction

Synthetic insecticides have been used exclusively and extensively in the control of insect pests which are major limiting factors affecting the production and productivity of many crops. The use of chemical insecticides for controlling insects has led to several environmental problems, serious health hazards to human beings and animals, development of resistance to insecticides, destruction of natural enemies and pesticide residues. To overcome the problem of synthetic chemical risks, one of the best control measures is the use of products of natural origin. Insecticides of natural origin are more selective, ecologically friendly and suitable for pest management programs based on the integration of biological and chemical control measures (Tak and Isman 2015; Bajad and Pardeshi 2016).

The aphid species *Acyrthosiphon pisum* (Hemiptera: Sternorrhyncha, Aphididae) is considered a problematic insect pest worldwide, resulting in serious damage to different plant species, including forage pea *Pisum sativum* L. The most commonly used field strategy for the population reduction of aphids is based on chemical control. Serious problems to the environment can be minimized through the use of biological control methods of the pea aphid.

Many studies have investigated products of natural origin for pest control in organic farming (Kassimi and El watik 2012; Benelli *et al.* 2017). Considerable progress has been made in recent years in the development of environmentally benign fungal biological control agents for pest control. Entomopathogenic fungi, as promising alternatives to chemical insecticides, have reduced inputs of harmful synthetic products in agricultural systems. This is in accord with the European Commission common agricultural policy and the global consensus to reduce chemical inputs in general.

Entomopathogenic fungi play an important role in the biological regulation of insect pest populations. Among them, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) has proved to be a promising biocontrol agent against aphids and other piercing-sucking insects (Martins *et al.* 2014; Rondot and Reineke 2018), pests in Diptera, Lepidoptera and Hemiptera (Hasyim *et al.* 2017; Lorencetti *et al.* 2018), thrips in Thysanoptera (Wu *et al.* 2016), spider mites (Draganova and Simova 2010), and weevils in Coleoptera (Cox *et al.* 2016). The perspective direction in the biological regulation of insect pest populations is the combined use of different products of organic origin including fungal biological control agents. The role of mixtures in pest reduction or the decrease in feeding deterrent response in preventing habituation has been documented in some studies. For example, the combination of the entomopathogenic fungus *B. bassiana* and the aphid parasitoid *Diaeretiella rapae* McIntoch (Hymenoptera: Braconidae) was studied by Martins *et al.* (2014) against *Myzus persicae* Sulzer. The authors recommended the interaction of these two biological control agents for aphid control.

Athanassiou and Steenberg (2007) tested the insecticidal effect of the entomopathogenic fungus *B. bassiana* in combination with three diatomaceous earth formulations against adults of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). It was found that the mixtures of *B. bassiana* and diatomaceous increased weevil mortality under a broad range of temperature and radiation levels in stored wheat. The authors reported that the use of the mixtures was an effective and very appealing ecological approach for pest control. Similarly, to enhance the effectiveness of fungal spores in pollen beetles control, Kaiser *et al.* (2020) focused on the combined use of *B. bassiana* with colza oil and stone dust, which showed high mortality of insect pests as single treatments. These authors reported that the mixture of *B. bassiana* and colza oil had a strong synergistic effect and a considerably higher level of pest control than expected from single substances.

The application of various natural mixtures for crop protection from insect pests may allow for the development of more effective control agents and achieve higher levels of efficiency.

Studies related to the single and combined use of products of natural origin for pea aphid control are limited and insufficient. Therefore, the present study was aimed to evaluate the efficacy of products of natural origin (Aminogreen 24, Nitrogreen, Foliamin and Naturalis – strain ATCC 74040 of entomopathogenic fungus *Beauveria bassiana*) and one synthetic insecticide – standard (deltamethrin + thiacloprid), applied alone and in a mixture in the control of *A. pisum* in forage pea.

Materials and Methods

The action of products of natural origin applied alone and in double mixtures for pea aphid control (*A. pisum*) in spring forage pea (*P. sativum*), variety Pleven 4 was studied in a field experiment of the Institute of Forage Crops, Pleven, Bulgaria from 2017 to 2020. Synthetic insecticide was used for comparison. Trial variants and product characteristics are shown in Table 1.

The treatments were performed at the beginning of the flowering stage (June 3–17) by hand with Mataby style 3.0 liters. The average number of aphids was calculated on the 1st, 5th, 7th and 14 days after treatment based on sweeping with an entomological net in net plots.

Determinination of the index for the product efficacy, insect-days and cumulative insect-days makes it possible to identify both the intensity and duration of the aphid infestation. The efficacy of the products during the period between samples was based on analyses of the individual insect-days, whereas the overall efficacy of the treatments was based on the cumulative insect-days (*CID*).

Infestation by leaf aphids was estimated by calculating the *CID*. The cumulative insect-days was obtained by summing up the aphid insect-days (*ID*) in sequence (Ruppel 1983).

The insect-days were calculated by the equation:

$$\text{Insect-days} = (X_{i+1} + 1 - X_i)\,[(Y_i + Y_{i+1})/2],$$

where: X_i and X_{i+1} – adjacent points of time, Y_i and Y_{i+1} – the corresponding points of insect number.

After the treatment, the *CID* for a particular point of time was arrived at by a simple summation of insect-days of the treatment and per cent reduction to relevant *CID* in the control. Emphasizing the magnitude and

Table 1. Trial variants and product characteristics

No.	Variants	Application rates, per ha	Active ingredients	Producer
1.	Control	–	treated with distilled water	
2.	Aminogreen 24 (liquid nitrogen fertiliser with bioactive plant amino acids)	250 ml · 100 l^{-1}	nitrogen (N) – 8.5%, and free amino acids more than 24% (more than 50% amino acid and peptide in dry matter) – significantly greater proportion comparable to similar products	AGRI nova Science
3.	Nitrogreen (organic nitrogen fertiliser)	4,000 ml · ha^{-1}	nitrogen (N) – 22.0%, and free amino acids – 2.0%	Scientific Production Association "Bioplant", Russian Federation
4.	Foliamin (organic fertiliser)	200 ml · 100 l^{-1}	total nitrogen (N) – 8.0% w/w equivalent to 10.0% w/v at 20°C; organic nitrogen (N) soluble in water – 8.0% w/w equivalent to 10.0% w/v at 20°C; organic carbon (C) – 23.5% w/w equivalent to 29.3% w/v at 20°C; total amino acids – 50.0% w/w equivalent to 62.5% w/v at 20°C	Agro Proteo International, Italy
5.	Naturalis (microbiological insecticide)	1,000 ml · ha^{-1}	*Beauveria bassiana* strain ATCC 74040 (2.3×10^7 viable spores · ml^{-1})	Biogard® Italy
6.	Aminogreen 24 + Naturalis	250 ml · 100 l^{-1} + + 1,000 ml · ha^{-1}		
7.	Nitrogreen + Naturalis	4,000 ml · ha^{-1} + + 1,000 ml · ha^{-1}		
8.	Foliamin + Naturalis	200 ml · 100 l^{-1} + + 1,000 ml · ha^{-1}		
9.	Proteus 110 OD	600 ml · ha^{-1}	oil dispersion containing deltamethrin 10 g a.i. · l^{-1} + thiacloprid 100 g a.i	Nu 3 BV, Netherlands

duration of insect infestation, which is of greater importance to crop protection, Ruppel gave an alternate method of judging the insecticide efficacy based on *ID*. Insect-days are calculated from the insects surviving at various points of time after treatment. The successive build-up of *CID*, which is based on *ID*, simplifies the analysis irrespective of the population variations over time.

The experiments were set out using the long plot method with a sowing rate of 120 germinating seeds m^{-2} in three replications of the variants, plot size of 6.50 m^2 unit (2.5 m wide × 2.6 m long). The spaces between each plot were 2.5 m^2 (2.5 m wide × 1.0 m long). Each product which was used and the control were applied to the randomized replicated plots. The trial occupied an area of 200 m^2.

Calculation of productive interactions

The expected mortality (*ME*) for the combination of the insecticide with fertilizer was calculated according to Tak and Isman (2015):

$$ME = MB + MA\ (1 - MB),$$

where: *MB* – caused by fertilizer; *MA* – mortality caused by insecticides.

Results from a chi-square test, $\chi^2 = (MAB - ME)^2/ME$, where *MAB* is the observed mortality for the combination of the insecticide with fertilizer, were compared to the chi-square table value and χ^2 with $df = 1$ and $\alpha = 0.05$ is 3.84.

The interactions can be considered as synergistic when the χ^2 values > 3.84 of the mixture and have greater mortality than expected, and as antagonistic with smaller observed mortality than expected, or as an additive when χ^2 values < 3.84.

In fresh plant samples taken 10 days after treatment plastid pigments content (chlorophyll *a*, chlorophyll *b*, carotenoids, and total) (mg · 100^{-1} g FW) was determined according to Zelenskii and Mogileva (1980). Chlorophyll *a* (Chl *a*) to chlorophyll *b* (Chl *b*) ratio and the ratio of chlorophyll *a* + *b* to carotenoids were calculated. The heights of plant stems were also measured.

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

Results and Discussion

One of the main insect pests present in forage pea is pea aphid, *A. pisum*. Weather conditions affected aphid population growth. According to Grigorov (1980), warm and wet weather increased the potential for aphid outbreaks. The cool and wet weather in May of 2019 (temperatures 2.7°C lower and 35.1 mm more rainfall compared to 2018) suppressed aphid population growth and generation development and there were fewer aphids. May of both 2018 and 2020 was characterized by an optimal combination of a higher average daily temperature and an equal distribution of the amount of rainfall (Table 2). These conditions determined a higher number of aphids.

Agro-meteorological conditions affected the action of products of natural origin which was especially pronounced in 2019. Relatively wet and cool weather during the first 20 days of June 2019 compared to 2018 (temperatures 1.2°C lower and lower rainfall by 28.3 mm) resulted in a lower efficacy of products than in the previous year. The amount of rainfall during the period June 1–20, 2019 was 40.1 mm more than during the same period in 2020. In 2018 and 2020 the treatments were carried out at the beginning of June as the weather conditions favored the active action of the products.

Cumulative insect-days decreased slightly on the 1st day after treatment in 2018. Nevertheless, the differences between products of natural origin and control were statistically significant, with the exception of Foliamin ($F_{8,5} = 28.768$; $p < 0.037$) (Table 3). The single application of products reduced the aphid number by 2.8–18.7%, and the highest percentage of reduction was observed with the use of Naturalis (−18.7%). The differences between single and combined treatments with the biological insecticide were not statistically significant. The decrease in the number of *A. pisum* by 63.5% when using Proteus was significantly the highest. The outlined trend on day 5 was maintained, and a significant decrease in the number of all treatments compared to the control was established ($F_{8,5} = 40.991$; $p < 0.014$). The reduction of *CID* after the use of the organic fertilizers was weak (from 5.4 to 16.4%), while in the Naturalis variants the reduction was considerably more pronounced (27.3–34.1%). The combination of Naturalis with Aminogreen stood out with the highest, significant, reduction of *CID* between products of natural origin. In comparison, Proteus decreased the species number by two-fold higher level. There was an increasing effect of the products compared to the 1st day after treatment, seen by the greater reduction in the aphid number (Table 3).

The products of natural origin on day 7 after treatment significantly reduced *CID* compared to the control ($F_{8,5} = 44.991$; $p < 0.001$), while the tendency to increase the reducing effect in the *A. pisum* number was retained. It was particularly pronounced in the Naturalis treatments (from 46.6 to 55.9%). The Aminogreen 24 + + Naturalis combination stood out with the significantly greatest reduction in *CID*. The difference in the decrease of the insect-days between the combination (55.9%) and Proteus (76.7%) was considerably smaller than previous days since the insecticidal effects of both treatments began to be seen. A relatively higher reduction in *CID* was also observed with Aminogreen 24 than with other organic fertilizers.

Table 2. Meteorological characteristics

Month/days		Temperature [°C]			Rainfall [mm]			Relative humidity [%]		
		2018	2019	2020	2018	2019	2020	2018	2019	2020
April	1–10	14.1	11.9	8.7	14.5*	11.4	1.4	62	59	54
	11–20	16.9	10.0	13.9	0.5	42.4	11.8	68	76	51
	21–30	19.7	14.7	14.5	4.6	63.0	1.3	57	65	50
	Average	16.9	12.2	12.4	19.6	116.8	14.5	62	67	52
May	1–10	20.2	14.4	15.3	6.6	34.4	22.2	62	66	65
	11–20	18.3	17.0	21.2	40.1	15.9	1.1	72	72	67
	21–31	20.3	19.4	14.6	1.0	32.5	16.1	61	65	70
	Average	19.6	16.9	17.0	47.7	82.8	39.4	65	68	67
June	1–10	23.4	20.1	19.5	0.4	59.8	6.6	62	49	66
	11–20	22.7	23.6	20.6	39.1	8.0	21.1	72	43	74
	21–30	19.4	23.3	22.8	115.7	21.8	9.0	73	42	66
	Average	21.8	22.3	21.0	155.2	89.6	36.7	69	45	69

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

Fourteen days after treatment with the different products which were used the *CID* values decreased significantly compared to the control ($F_{8.5} = 76.464$; $p < 0.0361$). Products of natural origin had the highest reducing effect on the number of pea aphids for all reported days while the chemical insecticide had the lowest impact on the *CID* decrease over the 14-day period. The aphid decrease with Naturalis treatments was above 50% and the combination of Aminogreen 24 + + Naturalis had statistically significant the highest reduction in insect-days. The markedly increased effect of the combination (60.2%) was almost equal to that of Proteus (63.4%) and the difference was insignificant. The reducing effect in *CID* of the Aminogreen 24 application reached 29.0% while the results for Nitrogreen and Foliamin were unsatisfactory for the entire studied period.

Many authors have reported increasing efficacy, accompanied by high toxic effects of microbial insecticides the last day after treatment against several insect pests (Atanasova and Vasilev 2020; Fabrice *et al.* 2020; Abdulwahid and Mohammadali 2021). For

Table 3. Cumulative insect-days (2 m^2) in spring forage pea after treatment with products with different biological effect

Products/Dates	2018				
	1 June	6 June	8 June	15 June	Average
Control	726.6 f	1179.6 h	1914.6 h	2150.1 g	1492.7 h
	–	–	–	–	–
Aminogreen 24	[1]643.8 d	985.8 e	1407.6 e	1525.6 d	1140.7 e
	–11.4[2]	–16.4	–26.5	–29.0	–23.6
Nitrogreen	682.9 e	1066.0 f	1659.2 f	1858.0 e	1316.5 f
	–6.0	–9.6	–13.3	–13.6	–11.8
Foliamin	706.0 ef	1116.4 g	1752.8 g	1952.3 f	1381.9 g
	–2.8	–5.4	–8.4	–9.2	–7.4
Naturalis	590.6 bc	820.1 cd	946.6 c	987.8 b	836.3 c
	–18.7	–30.5	–50.6	–54.1	–44.0
Aminogreen 24 + Naturalis	570.6 b	776.9 b	844.1 b	855.2 a	761.7 b
	–21.5	–34.1	–55.9	–60.2	–49.0
Nitrogreen + Naturalis	585.0 bc	814.1 bc	961.5 c	1009.8 bc	842.6 c
	–19.5	–31.0	–49.8	–53.0	–43.6
Foliamin + Naturalis	609.0 c	857.7 d	1023.2 d	1073.0 c	890.7 d
	–16.2	–27.3	–46.6	–50.1	–40.3
Proteus 110 OD	265.2 a	317.4 a	446.9 a	786.8 a	454.1 a
	–63.5	–73.1	–76.7	–63.4	–69.6
Products/Dates	2019				
	6 June	11 June	13 June	20 June	Average
Control	717.6 de	1163.1 f	1883.1 h	2109.6 h	1468.4 h
	–	–	–	–	–
Aminogreen 24	666.6 bc	1026.6 d	1470.6 e	1594.5 e	1189.6 e
	–7.1	–11.7	–21.9	–24.4	–19.0
Nitrogreen	702.6 cde	1101.6 e	1719.6 f	1926.6 f	1362.6 f
	–2.1	–5.3	–8.7	–8.7	–7.2
Foliamin	726.6 e	1154.1 f	1817.1 g	2024.9 g	1430.7 g
	+1.3	–0.8	–3.5	–4.0	–2.6
Naturalis	657.6 b	953.1 bc	1201.1 c	1281.6 c	1023.3 c
	–8.4	–18.1	–36.2	–39.2	–30.3
Aminogreen 24 + Naturalis	660.6 b	938.1 b	1116.6 b	1172.6 b	972.0 b
	–7.9	–19.3	–40.7	–44.4	–33.8
Nitrogreen + Naturalis	678.6 bcd	977.1 c	1203.9 c	1278.2 c	1034.5 c
	–5.4	–16.0	–36.1	–39.4	–29.6
Foliamin + Naturalis	708.6 de	1040.1 d	1307.4 d	1384.1 d	1110.0 d
	–1.3	–10.6	–30.6	–34.4	–24.4
Proteus 110 OD	357.6 a	494.1 a	770.1 a	1068.600 a	672.6 a
	–50.2	–57.5	–59.1	–49.3	–54.2

[1]cumulative insect-days (*CID*); [2]reduction in *CID* (%)

Means in each column followed by the same letters are not significantly different ($p < 0.05$)

Table 3. Cumulative insect-days (2 m^2) in spring forage pea after treatment with products with different biological effect – *continuation*

Products/Dates	2020				
	3 June	8 June	10 June	17 June	Average
Control	645.6 d –	1113.55 f –	1995.6 g –	2336.1 g –	1522.7 g –
Aminogreen 24	[1]597.6 cd –7.4[2]	890.1 d –20.1	1298.1 d –35.0	1520.1 d –34.9	1076.5 d –29.3
Nitrogreen	636.6 d –1.4	996.5 e –10.5	1686.2 e –15.5	2023.7 e –13.4	1335.8 e –12.3
Foliamin	642.6 d –0.5	1121.3 f –8.3	1766.8 f –11.5	2074.3 f –11.2	1401.3 f –9.6
Naturalis	558.6 bc –13.5	763.8 c –31.4	873.5 c –56.2	914.6 b –60.8	777.6 c –48.9
Aminogreen 24 + Naturalis	537.6 b –16.7	720.9 b –35.3	771.9 b –61.3	780.0 a –66.6	702.6 b –53.9
Nitrogreen + Naturalis	567.8 bc –12.1	779.7 c –30.0	910.5 c –54.4	958.7 b –59.0	804.2 c –47.2
Foliamin + Naturalis	570.6 bc –11.6	782.1 c –29.8	911.6 c –54.3	961.4 b –58.8	806.4 c –47.0
Proteus 110 OD	270.6 a –58.1	315.6 a –71.7	645.6 a –67.6	1095.6 c –53.1	581.9 a –61.8
Products/Dates	2018–2020				
Control	696.6 e –	1152.1 h –	1931.1 h –	2198.6 h –	1494.6 h –
Aminogreen 24	636.0 d –8.7	967.5 e –16.0	1392.1 e –27.9	1546.7 e –29.6	1135.6 e –24.0
Nitrogreen	674.0 e –3.2	1054.7 f –8.5	1688.4 f –12.6	1936.1 f –11.9	1338.3 f –10.5
Foliamin	691.7 e –0.7	1130.6 g –4.8	1778.9 g –7.9	2017.2 g –8.3	1404.6 g –6.6
Naturalis	602.3 bc –13.5	845.7 c –26.6	1007.1 c –47.8	1061.3 c –51.7	879.1 c –41.2
Aminogreen 24 + Naturalis	589.6 b –15.4	812.0 b –29.5	910.9 b –52.8	917.3 a –57.4	807.4 b –45.7
Nitrogreen + Naturalis	610.5 bcd –12.4	857.0 c –25.6	1025.3 c –46.9	1082.2 c –50.8	893.7 c –40.2
Foliamin + Naturalis	629.4 cd –9.6	893.3 d –22.5	1080.7 d –44.0	1139.5 d –48.2	935.7 d –37.4
Proteus 110 OD	297.8 a –57.2	375.7 a –67.4	620.9 a –67.8	983.7 b –55.3	569.5 a –61.9

[1]cumulative insect-days (*CID*); [2]reduction in *CID* (%)
Means in each column followed by the same letters are not significantly different ($p < 0.05$)

example, Andreev and Vasilev (2018) studied the toxicity of Naturalis against aphid *Hyalopterus pruni* Geof. (Hemiptera: Aphididae) in concentrations of 0.3% and 0.2% under laboratory conditions. They found that on the 3rd day the efficacy exceeded 95%, and on the last, 7th day it reached 100%, which showed the possibility of successful use of Naturalis in the biological control of aphids. On the other hand, Alizadeh *et al.* (2019) under field conditions found that the mortality percentage of alfalfa weevil after Naturalis spraying was lower during the first 5 days but later, on the 14th day, it reached its highest value. Also, similar results were reported Prijović *et al.* (2012), who applied Naturalis at rates of 0.1 and 0.2% against greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), and found that the efficacy ranged from 73.2 to 87.7% 14 days after treatment (DAT) and reached 82.6–94.6% 21 DAT.

The average *CID*s used as a final indicator of the overall efficacy of the studied products in the 14-day period showed that the significantly highest reduction of products of natural origin was achieved after the combined treatment of Aminogreen 24 + Naturalis (49.0%), followed by Naturalis (44.0%) and Nitrogreen + Naturalis (43.6%) ($F_{8,5}$ = 29.290; $p < 0.033$). The use of Aminogreen 24 did not lead to a satisfactory decrease of *CID* (23.6%), but when co-treated with a biological insecticide, it helped improve the ad herence of the mixture to the leaf surface and gave more rapid absorption by the plant organism. Aminogreeen is a fertilizer based on an amino acid solution, enriched with nitrogen, that is rapidly absorbed and immediately metabolized by the plant. Thus, it quickened Naturalis absorption and improved protection against *A. pisum*. Organic fertilizers, Nitrogreen and Foliamin, are involved in the processes of photosynthesis and protein synthesis and encourage vegetative growth of plants. Applied alone, they reduced the aphid number to an unsatisfactory degree, and in combination with Naturalis fertilizers did not improve its action.

As a result of the unfavorable meteorological conditions for the pea aphid development in 2019, its number was considerably lower (by 40.6%) than in the previous year. On the other hand, the action of the products was weaker, as they showed a pronouncedly lower toxic effect on the aphid number. Naturalis is susceptible to low temperatures and precipitation (Pavela 2009), resulting in pronouncedly less efficacy in 2019. The low average daily temperature of 18.7°C, combined with the considerably high amount of precipitation of 74.6 mm during the period May 28 – June 5, when the toxicity was reported, determined a low protective effect of the products against pea aphid. Nevertheless, the trends outlined in 2018 were confirmed.

The results of the single application of products of organic origin from the 1st to the 7th day after treatment were unsatisfactory. The reduction of *CID* varied in low limits and on the 7th day reached only 36.2% in Naturalis. Differences in comparison to control were statistically significant in most cases, but the products did not provide good plant protection against *A. pisum* (1st day: $F_{8,5}$ = 41.100; $p < 0.041$; 5th day: $F_{8,5}$ = 36.101; $p < 0.030$; 7th day: $F_{8,5}$ = 39.682; $p < 0.029$). A similar trend was observed after the combined treatment of the microbiological insecticide with organic fertilizers, only on the 7th day, the decrease in *CID* in Aminogreen 24 + Naturalis (40.7%) was significantly higher than Nimazal (36.2%).

The *CID* reduction after the use of the synthetic insecticide was significantly the highest, but the toxic effect was satisfactory over the 14-day period and did not exceed 60%. The results of the independent application of products of organic origin, as well as their combined use on day 14, were unsatisfactory, except for the mixture Aminogreen 24 + Naturalis (44.4%), where the decrease in insect-days approached that of Proteus (49.3%). In the 2nd year, the tendency for the increasing insecticidal effect of Naturalis was confirmed. In the 3rd year of the study (2020), products of organic origin showed a low reducing effect in *CID* on the 1st day after treatment. Nevertheless, significant differences were found in Naturalis and its combinations compared with control, but the differences between products were minimal ($F_{8.5}$ = 56.925; $p < 0.019$). The decrease in *CID* increased significantly on the 5th day after treatment and was particularly pronounced in the microbiological insecticide and mixtures with organic fertilizers. It was found that the combination Aminogreen 24 + Naturalis exhibited a significantly more toxic effect than Naturalis and other products of organic origin ($F_{8.5}$ = 42.207; $p < 0.027$). However, the protection against pea aphids was still not sufficiently successful. Proteus had significantly the highest reducing effect, then the effect decreased.

The evaluation of the products of organic origin, one week after treatment, showed a stronger reducing effect in the *CID* values than that of previous reports. An exception was observed for the organic fertilizers Nitrogreen and Foliamin, where the action was unsatisfactory, despite the significant differences compared to the control ($F_{8.5}$ = 43.830; $p < 0.032$).

The percentage of reduction in *CID* compared to control *CID* was an expression of the protective duration of various treatments at the desired level. For the present set of data, Naturalis approached 60%, while its mixed application with Aminogreen 24 reached the desired level in *CID* percentage reduction of 61.3% with a significant difference compared to its use alone. The use of the biological insecticide with Nitrogreen and Foliamin did not change its insecticidal effect.

Naturalis treatments (alone and in combination) reached the highest impact on the aphid numbers on the 14th day, as the reduction in *CID* significantly exceeded the Proteus 110 OD standard (53.1% decrease) ($F_{8,5}$ = 47.149; $p < 0.014$). The Aminogreen 24 + Naturalis mixture significantly stood out with the highest toxic effect and the reduction in the *CID* approached 70%. In addition, it closely followed the synthetic insecticide after the 5th day of treatment and took a significant second position of impact after Proteus, if overall mean *CID* was considered ($F_{8,5}$ = 33.336; $p < 0.033$).

Evaluation of products showed that Proteus reached its highest reduction of *CID* on the 5th and 7th days after treatment, while Naturalis and its combinations had less efficiency during the 1st day but later the *CID* decrease had considerably higher values and Naturalis treatments had the highest impact on the aphid numbers after 14 days, and thus maintained its greater lethality.

The average data for the period 2018–2020 confirmed the trend for the strongest decrease in *CID* with Aminogreen 24 + Naturalis. This combination of products of natural origin gave the greatest significant reduction of the number of aphids on the 5th ($F_{8.5}$ = 15.244; $p < 0.033$) and 7th day ($F_{8.5}$ = 33.087; $p < 0.037$) after treatment. On the 14th day, the decrease in *CID* (57.4% decrease) statistically exceeded the Proteus 110 OD standard (55.3% decrease) ($F_{8.5}$ = 49.841; $p < 0.049$). The differences between the application of Naturalis alone and its combinations with organic fertilizers, except for the 1st day ($F_{8.5}$ = 31.332; $p < 0.008$), were statistically significant.

During the 14-day period, the general trend of the aphid population in the variants was of decline as evident by the population in the control plots. The successive build-up of *CID*, which is based on insect-days, simplifies the analysis irrespective of the population variations over time. Using the average for the period, good protection against *A. pisum*, with the highest reduction in *CID* (45.7%), among products of natural origin ($F_{8.5}$ = 29.326; $p < 0.021$), was found with Aminogreen 24 + Naturalis, followed by Naturalis and Nitrogreen + Naturalis. The synthetic insecticide had the highest toxic effect and reduction of 61.9%.

Synergistic effects of complex mixtures are important in natural plant defense against insect pests. According to Hepburn (1985), the dispersion in the effectiveness of *B. bassiana* may have effects on their various proteins on most of the cuticle of the insect body wall. Henceforth, the degrading effects of proteases might result in the fast penetration of the insect's cuticle, which reproduces the *B. bassiana* virulence (Bidochka and Khachatourians 1990). In addition, Zare *et al.* (2014) found that there was a high association between the proteolytic action and virulence of many isolates of *B. bassiana* against insects. In the present study, the enhanced insecticidal activity of Naturalis and the penetration-enhancing effect of the mixture when the cuticular barrier was bypassed, might be due to reduced surface tension. That positive effect was clear when comparing the toxicities of mixtures and individual applications (Table 4). These comparisons led us to conclude that the additive effect between Naturalis and Aminogreen 24 largely resulted from the increased penetration of the compounds, especially that of *B. bassiana* in that particular combination. The positive effect of the mixture was exhibited throughout the entire study period. The interactions of Nitrogreen + Naturalis and Foliamin + Naturalis can be considered as neutral (Table 4).

A similar conclusion on the positive interaction of mixtures was reported by Abdulwahid and Mohammadali (2021). According to these authors, the use of pesticide mixed with bioagent showed that the treatment TIAM 25% WG + *B. bassiana* sprayed on a palm in the field was significantly different from single treatments, and the efficacy reached 82% after 7 and 14 days of treatment.

The impact of the products on the photosynthetic process was tracked by the determination of plastid

Table 4. Toxicity of product combination on pea aphids

Combination	Observed mortality [%]	Expected mortality of combination [%]	χ^2	*p*	Interactions
2018					
Aminogreen 24 + Naturalis	42.9	38.8	0.443	0.05	additive
Nitrogreen + Naturalis	38.3	38.6	0.002	0.05	–
Foliamin + Naturalis	35.0	38.6	0.323	0.05	–
2019					
Aminogreen 24 + Naturalis	28.1	25.7	0.223	0.05	additive
Nitrogreen + Naturalis	24.2	25.5	0.068	0.05	–
Foliamin + Naturalis	25.5	25.4	2.18889E-05	0.05	–
2020					
Aminogreen 24 + Naturalis	45.0	40.9	0.407	0.05	additive
Nitrogreen + Naturalis	38.9	40.7	0.081	0.05	–
Foliamin + Naturalis	35.0	40.6	0.770	0.05	–
2018–2020					
Aminogreen 24 + Naturalis	38.8	35.2	0.356	0.05	additive
Nitrogreen + Naturalis	33.9	35.1	0.037	0.05	–
Foliamin + Naturalis	31.1	35.0	0.441	0.05	–

χ^2 – interactions of the compounds; the interactions can be considered as synergistic when the χ^2 values > 3.84 of the mixture and as an additive when χ^2 values < 3.84

pigments in pea leaves. It is known that aphid feeding causes considerable losses in the photosynthetic pigment content, which is an important source of nitrogen for these insect pests (Xinzhi *et al.* 2002; Goławska *et al.* 2010). Changes in the content of total chlorophyll (*a* + *b*) in leaf tissues is an important indicator of abnormalities in the development of chloroplasts and impaired photosynthetic processes in plants (Sytykiewicz *et al.* 2013). The application of plant protection measures against them aims to create favorable conditions for the photosynthetic process and improve the physiological status of plants. The results of the comparative analysis showed that the products of natural origin increased the total pigment content (Table 5). The weakest but most positive effect on the synthesis of chlorophyll *a* + *b* was the treatment with Proteus – on average 0.8% above the control. The strongest impact with a 32.0% increase was seen with Naturalis, followed by the organic fertilizers Nitrogreen and Aminogreen 24 – with a 22.5 and 22.1% increase, respectively, with statistical differences compared with control ($F_{8,1}$ = 3.207; $p < 0.046$). Naturalis had the highest positive significant effect on chlorophyll *a* with an increase of 53.5% ($F_{8,1}$ = 3.312; $p < 0.012$) and chlorophyll *b* – 13.3% increase ($F_{8,1}$ = 1.139; $p < 0.035$). The reduction of photosynthetic pigments in attacked leaves in the control may be explained mainly by the fact that they are an important source of nitrogen for aphids (Tab. 5).

The carotenoid contents followed a similar pattern as in chlorophylls, i.e., a high level of chlorophyll *a* + *b* was accompanied by a higher level of carotenoids. The carotenoid synthesis was the most intensive after the use of Naturalis alone (46.1% increase) and Aminogreen 24 (43.1% increase) ($F_{8,1}$ = 1.152; $p < 0.040$). The total amount of green and yellow plastid pigments followed the trend of its constituent components, as the increases compared to the control were significant ($F_{8,1}$ = 3.267; $p < 0.023$) and varied from 3.7% (Proteus 10 OD variant) to 33.4% (Naturalis). Considerably increased synthesis of plastid pigments with Aminogreen 24 (24.1%) and Nitrogreen (23.5%) was determined not only by their action on organic fertilizers but also by their insecticidal effect against aphid infestation. Similar results of increased content of chlorophyll *a* + *b* and carotenoids after foliar application of organic products have been reported by other authors (El Bassiouny *et al.* 2005).

The values of the chlorophyll *a* (Chl *a*) to chlorophyll *b* (Chl *b*) ratio and the ratio of green pigments (Chl *a* + Chl *b*) to carotenoids are indicators of the physiological status of green plants. The ratio of Chl *a* to Chl *b* assessed the degree of formation of the photosynthetic apparatus. It was related to the basic activity of Chl *a*. It is relatively constant and is considered to be genetically determined. Under the conditions of the present study, the corresponding ratios ranged from 7.29 to 9.96 and from 0.87 to 1.18, respectively, and determined that plants treated with Aminogreen 24 + Naturalis and Naturalis had the best physiological state.

The results obtained indicate that products of natural origin can provide efficient pea aphid control compared to standard insecticides. Bioinsecticide efficacy would probably be higher than in this trial if the recommendation for treatment at the very beginning of infestation would be implemented, and not when the *A. pisum* population was already established. Considering that insecticide resistance in aphids is a seriously increasing phenomenon, including selective and ecotoxicological demands, application of Naturalis, alone and in combination with Aminogreen 24 and Nitrogreen can be a successful alternative to conventional chemical control.

Table 5. Plastid pigments in the leaves of forage pea treated with products of natural origin, on average (2018–2020)

Products	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	Carotenoids	Total	Chlorophyll *a*/*b*	Chlorophyll *a* + *b*/ Carotenoids
	[mg · 100 g^{-1} fresh biomass]						
Control	75.50 a	86.50 b	162.00 a	17.18 a	179.18 a	0.87	9.43
Aminogreen 24	103.88 d	93.86 e	197.74 d	24.58 d	222.32 f	1.11	8.04
Nitrogreen	100.58 d	97.92 f	198.50 d	22.86 c	221.36 f	1.03	8.68
Foliamin	83.08 b	85.92 b	169.00 b	21.28 b	190.28 c	0.97	7.94
Naturalis	115.92 e	98.00 g	213.92 e	25.10 d	239.02 g	1.18	8.52
Aminogreen 24 + Naturalis	82.88 b	93.26 e	176.14 c	17.68 a	193.82 d	0.89	9.96
Nitrogreen + Naturalis	86.12 b	90.56 d	176.68 c	21.76 c	198.44 e	0.95	8.12
Foliamin + Naturalis	89.86 c	88.96 c	178.82 c	22.50 c	201.32 e	1.01	7.95
Proteus 110 OD	85.62 b	77.74 a	163.36 a	22.42 c	185.78 b	1.10	7.29

Means in each column followed by the same letters are not significantly different ($p < 0.05$)

In conclusion, the combination of Aminogreen 24 + Naturalis had the most pronounced decrease in cumulative insect-days. The greatest reduction of the number of aphids occurred on the 5th ($F_{8.5}$ = 15.244; $p < 0.033$) and 7th days ($F_{8.5}$ = 33.087; $p < 0.037$) after treatment. On the 14th day, the decrease in *CID* (57.4% decrease) statistically exceeded the Proteus 110 OD standard (55.3% decrease) ($F_{8.5}$ = 49.841; $p < 0.049$). Good protection against *A. pisum* was also found in Naturalis and Nitrogreen + Naturalis. It was determined that there was an additive effect between Naturalis and Aminogreen 24 throughout the whole study period. The values of the chlorophyll *a* to chlorophyll *b* ratio and the ratio of green pigments (Chl *a* + Chl *b*) to carotenoids determined that plants treated with Aminogreen 24 + Naturalis and Naturalis had the best physiological state.

Treatment with products of natural origin on forage pea had a positive effect on the development and plant height, which increased 1.7–59.9% compared to the control (Table 6). The combination of Aminogreen 24 and Naturalis was characterized by significantly the largest increase in stem height (by 59.9%), followed by Nitrogreen + Naturalis (by 41.1%) ($F_{9,8}$ = 4.555; $p < 0.001$). The application of organic fertilizers alone showed significantly lower height values than Naturalis. However, the treatment with insecticides alone was associated with an insignificant increase in plant height.

Organic fertilizers are formulations of amino acids in a readily assimilable form. They are beneficial to the metabolic functions of plants (according to manufacturers). Fertilizers are recommended as biostimulants and they can be used for promoting vegetative growth, since they are carriers of nutrients (particularly micronutrients). They favor growth and flowering and increase the photosynthetic potential of the plants. Due to their stimulating effect, the height of the treated plants increased considerably, which can help to achieve higher productivity.

Table 6. The average influence of products of natural origin on forage pea height (2018–2020)

Products	Height [cm]	SD
Control	58.6 a	11.1
Aminogreen 24	74.8 c	3.4
Nitrogreen	71.7 bc	5.4
Foliamin	68.0 b	4.9
Naturalis	61.8 a	5.6
Aminogreen 24 + Naturalis	93.7e	9.4
Nitrogreen + Naturalis	82.7 d	9.5
Foliamin + Naturalis	79.2 c	7.7
Proteus 110 OD	59.6 a	4.5

SD – standard deviation
Means in each column followed by the same letters are not significantly different ($p < 0.05$)

It is known that the use of inorganic fertilizer for crops is not very good for health because of residual effects. In addition, some studies have suggested that the excessive use of these agrochemicals may actually increase pest problems in the long run (Altieri and Nicholls 2003). On the other hand, the application of organic fertilizer does not cause such problems since it increases the productivity of soil as well as crop quality and yield.

The use of Naturalis, alone and in a combination with Aminogreen 24 and Nitrogreen can be a successful alternative to conventional chemical control.

References

Abdulwahid A.N., Mohammadali M.T. 2021. Evaluation of the effectiveness of some pesticides and bioagents against Parlatoria date scale *Parlatoria blanchardi* Targ (Homoptera: Diaspididae) on date palm *Phoenix dactylifera*. IOP Conference Series Earth and Environmental Science 735 (1): 012031. DOI: 10.1088/1755-1315/735/1/012031

Alizadeh E., Valizadegan O., Aramideh S., Safaralizadeh M.H., Sadeghi S.E. 2019. Evaluation of the effect of some biopesticides on alfalfa weevil larvae, *Hypera postica* (Gyllenhal.) in laboratory and field conditions. International Commission of Agricultural and Biosystems Engineering 21 (4): 161–168.

Altieri M.A., Nicholls C.I. 2003. Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. Soil and Tillage Research 72: 203–211.

Andreev R., Vasilev P. 2018. Efficacy ot chemical and biological insecticides against *Hyalopterus pruni* Geof. (Hemiptera: Aphididae). Scientific Works of Agricultural University, Plovdiv 61 (2): 193–198. (in Bulgarian)

Atanasova D., Vasilev P. 2020. Efficacy of some bioinsecticides against the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) under laboratory conditions. Journal of BioScience and Biotechnology 9 (2): 61–64.

Athanassiou C.G., Steenberg T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40 (3): 411–416. DOI: https://doi.org/10.1016/j.biocontrol.2006.12.001

Bajad P.N., Pardeshi A.B. 2016. Bioinsecticidal effect of natural saponin containing plant extract of *Acacia concinna* on pulse beetle, *Callosobruchus chinensis* (Linn.) (Coleoptera: Bruchidae). International Journal of Current Research 8 (10): 39698–39701.

Benelli G., Canale A., Toniolo C., Higuchi A., Murugan K., Pavela R., Nicoletti M. 2017. Neem (*Azadirachta indica*): towards the ideal insecticide? Natural Product Research 31 (4): 369–386. DOI: 10.1080/14786419.2016.1214834

Bidochka M.J., Khachatourians G.G. 1990. Identification of *Beauveria bassiana* extracellular protease as a virulence factor in pathogenicity toward the migratory grasshopper, *Melanoplus sanguinipes*. Journal of Invertebrate Pathology 56 (3): 362–370. DOI: https://doi.org/10.1016/0022-2011-(90)90123-N

Cox P.D., Wakefield M.E., Price N., Wildey K.B., Chambers J., Moore D., Aquino de Muro M., Bell B.A. 2016. The potential use of insect-specific fungi to control grain storage pests in empty grain stores. Available on: https://cereals.ahdb.org.uk/media/363584/pr341-final-project-report.pdf [Accessed: 19 July 2016]

Dannon H.F., Dannon A.E., Douro-Kpindou O.K., Zinsou A.V., Houndete A.T., Toffa-Mehinto J., Elegbede I.A.T., Olou B.D., Tamó M. 2020. Toward the efficient use of *Beauveria bassiana* in integrated cotton insect pest management. Journal of Cotton Research 3: 1–24. DOI: https://doi.org/10.1186/s42397-020-00061-5

Draganova S., Simova S. 2010. Susceptibility of *Tetranychus urticae* Koch. (Acari: Tetranychidae) to isolates of entomopathogenic fungus *Beauveria bassiana*. Pesticides & Phytomedicine (Belgrade) 25 (1): 51–57. DOI: https://doi.org/10.2295/PIF1001051D

El Bassiouny H.M.S., Gobarah M.E., Ramadan A.A. 2005. Effect of antioxidants on growth, yield and favism causative agents in *Vicia faba* L. plants grown under reclaimed sandy soil. Journal of Agronomy 4 (4): 281–287. DOI: 10.3923/ja.2005.281.287

Goławska S., Krzyżanowski R., Łukasik I. 2010. Relationship between aphid infestation and chlorophyll content in fabaceae species. Acta Biologica Cracoviensia Series Botanica 52 (2): 76–80. DOI: 10.2478/v10182-010-0026-4

Grigorov S. 1980. Aphids and their Control. Zemizdat, Sofia, Bulgaria, 285 pp. (in Bulgarian)

Hasyim A., Setiawati W., Jayanti H., Hasan N., Syakir M. 2017. Identification and pathogenicity of entomopathogenic fungi for controlling the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). AAB Bioflux 29 (1): 34–46.

Hepburn H.R. 1985. Structure of the integument, p. 1–58. In: "Comprehensive Insect Physiology, Biochemistry and Pharmacology" (G.A. Kerkut, L.I. Gilbert, eds.). Vol. 3. Pergamon Press, Oxford, UK, 625 pp.

Kaiser D., Handschin S., Rohr R.P., Bacher S., Grabenweger G. 2020. Co-formulation of *Beauveria bassiana* with natural substances to control pollen beetles – synergy between fungal spores and colza oil. Biological Control 140: 104–106. DOI: https://doi.org/10.1016/j.biocontrol.2019.104106

Kassimi A., El watik L. 2012. Comparison of insecticide effect of plant extracts on aphids of watermelon and green alfalfa. Sustainable Agriculture Research 1 (2): 301–307. DOI: 10.5539/sar.v1n2p301

Lorencetti G.A.T., Potrich M., Mazaro S.M., Lozano E.R., Barbosa L.R., Schmatz Menezes M.J., Goncalves T.E. 2018. *Beauveria bassiana* Vuill. and *Isaria* sp. efficiency for *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae). Ciencia Florestal 28 (1): 403–411. DOI: https://doi.org/10.5902/1980509831612.

Martins I., Silva R.J., Alencar J., Silva K.P., Cividanes F.J., Duarte R.T., Agostini L.T., Polanczyk R.A. 2014. Interactions between the entomopathogenic fungi *Beauveria bassiana* (Ascomycota: Hypocreales) and the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) on *Myzus persicae* (Hemiptera: Aphididae). Journal of Economic Entomology 107 (3): 933–938. DOI: http://dx.doi.org/10.1603/EC13542

Pavela R. 2009. Effectiveness of some botanical insecticides against *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). Plant Protection Science 45 (4): 161–167. DOI: 10.17221/16/2009-PPS

Prijović M., Drobnjaković T., Marčic D., Perić P., Petronijević S., Stamenković S. 2012. Efficacy of insecticides of natural origin in whitefly (*Trialeurodes vaporariorum*) control in tomato. Acta Horticulturae 960: 359–364. DOI: 10.17660/ActaHortic.2012.960.52

Rondot Y., Reineke A. 2018. Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduces infestation with piercing-sucking insects. Biological Control 116: 82–90. DOI: https://doi.org/10.1016/j.biocontrol.2016.10.006

Ruppel R.F. 1983. Cumulative insect-days as an index of crop protection. Journal of Economic Entomology 76 (2,1): 375–377. DOI: https://doi.org/10.1093/jee/76.2.375

Sytykiewicz H., Czerniewicz P., Sprawka I., Krzyzanowski R. 2013. Chlorophyll content of aphid-infested seedlings leaves of fifteen maize genotypes. Acta Biologica Cracoviensia Series Botanica 52 (2): 51–60. DOI: 10.2478/abcsb-2013-0023

Tak J.-H., Isman M.B. 2015. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in *Trichoplusia ni*. Scientific Reports 5: 12690. DOI: 10.1038/srep12690

Wu S., Gao Y., Smagghe G., Xu X., Lei Z. 2016. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the predatory mite *Neoseiulus barkeri* and biological control of their shared prey/host *Frankliniella occidentalis*. Biological Control 98: 43–51. DOI: https://doi.org/10.1016/j.biocontrol.2016.04.001

Xinzhi N., Quisenberry S.S., Heng-Moss T., Markwell J.P., Higley L.G., Baxendale F.P., Sarath G., Klucas R. 2002. Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomologia Experimentalis et Applicata 105: 43–53. DOI: 10.1023/A:1021754831841

Zare M., Talaei-Hassanloui R., Fotouhifar K.B. 2014. Relatedness of proteolytic potency and virulence in entomopathogenic fungus *Beauveria bassiana* isolates. Journal of Crop Protection 3 (4): 425–434. Available on: http://jcp.modares.ac.ir/article-3-1245-en.html

Zelenskii M., Mogileva G. 1980. Comparative evaluation of the photosynthetic ability of agricultural crops by the photochemical activity of chloroplasts. VIR, Leningrad, 1980, 36 pp. (in Russian)