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Effect of biological products on the population of aphids and chemical components in alfalfa

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Abstract. One of the most serious pests of commercial Fabaceae crops especially alfalfa is the pea aphid *Acyrthosiphon pisum* Harris. The pea aphid damages crops directly caused chlorosis and necrosis, leading to significant alfalfa loss worldwide. Hence the need for aphid management and use of insecticides. It was studied the effect of a biological insecticide Agricolle and two biological nano–fertilizers (Nagro and Lytovit) to a control of pea aphid, *A. pisum* (Hemiptera, Sternorrhyncha: Aphididae) and changes in plastid pigment and total nitrogen contents in alfalfa. On the basis of the sweeping with the entomological net average number of aphids and mortality were calculated on the 1, 5, 7 and 9 days after treatment. It was found that the treatment with biological products Agricolle, Nagro and Litovit used alone and in combinations reduced *A. pisum* density in a different range. After application of Agricolle with Nagro combination, followed by Agricolle was found the lowest density and the highest aphid mortality. Biological products increased the total pigment content as the highest impact demonstrated used of Agricolle with Nagro–33.4 % increase, followed by treatment with Nagro–29.6 %. Chlorophyll *a* to chlorophyll *b* as well as green pigments to carotenoids ratio determined plants treated with Agricolle + Nagro and Nagro as plants with the best physiological state. Alfalfa fixed the most nitrogen (respectively more with 2.77 and 2.95 kg N/kg dry mass yield in compared to control) in the combined treatment of Aricolle with Nagro and Litovit.
Keyword: *Agricolle, nano–fertilizers, Acyrthosiphon pisum mortality, plastid pigments, nitrogen.*

Introduction

Aphid feeding causes considerable damages on plant leaves which are a plant important part since they play several important roles such as food production (photosynthesis), food storage, water transport, gas (respiration), and protection of vegetative and floral buds.

Besides the plants themselves, leaves are also necessary to other living organisms, due to oxygen release to the environment during the photosynthesis process. It is also a major source of food for human and other animals [TITIMA *et al.*, 2013]. So that the aphid–damaged leaves cannot perform their functions due to a disturbed photosynthesis process and reduced content of plastid pigments and total nitrogen [GOŁAWSKA and ŁUKASIK, 2010].

The consequences are suppressed plant growth and development and reduce yield. This causes the use of insecticide and aphid management. Insecticides have been widely and intensively used in agricultural areas to reinforce crop yield and pest control. However, many pesticides cause serious environmental problems. Natural products are an excellent alternative to synthetic pesticides to reduce negative impacts on human health and the environment, [FRANCK *et al.*, 2009]. They can contribute to reduce the pest population and increase food production. These products are safe and eco–friendly. Therefore, biological products with insecticidal action are much preferable against different pest groups, particularly against the aphids [DIAMETRY *et al.*, 2009; SAMFIRA *et al.*, 2014; BUTNARIU; 2012; BUTU *et al.*, 2014a]. In this work was determined the aphid mortality, concentrations of plastid pigments and total nitrogen in uninfested and aphid–infested leaves after used of biological products.

Material and methods

The trial was performed in the experimental field of the Institute of Forage crops, Pleven, Bulgaria. It was studied the effect of a biological

insecticide Agricolle and two biological nano–fertilizers (Nagro and Lytovit) to a control of pea aphid, *A. pisum* (Hemiptera, Sternorrhyncha: Aphididae) in alfalfa.

The experiments were laid out in Randomized Complete Block (RCB) Design with four replications.

The size of each plot was 3 x 6.50 m^2. Treatments were carried out at the beginning of the flowering stage in second regrowth (from 10 to 20 June). Trial variants and product characteristics are shown in Table 1.

Table 1.

Trial variants and product characteristics

Trial variants	Active ingredients	Producer	Application rates, per ha
1. Control	Treated with distilled water		–
2. Agricolle	natural polysaccharides for sticking small insects, bioinsecticide	Cal–Agri products LLC, USA	300 mL / 100 L water
3. Nagro	contains micro and macro elements (molybdenum, magnesium, cobalt, manganese, zinc, iron, copper, boron, nitrogen and phosphorus), meso elements, micro humates, vitamins, fulvic acid, amino acids, phytohormones, organic solvents, silicon compounds, organic calcium, antioxidants, adaptogens, metabolites, nitrogen fixators	Scientific Production Association "Bioplant", Russian Federation	500 mL ha^{-1}
4. Lytovit	contains calcium carbonate from natural reserves with micronutrients: 79.19 % $CaCO_3$; 4,62 % $MgCO_3$; 1.31 % Fe	Ctheo Vita Ltd., Germany	2000 g ha^{-1}
5.Agricol+Nagro			300 mL ha^{-1} + 500 mL ha^{-1}
6.Agricol+Lytovit			300 g ha^{-1} + 2000 mL ha^{-1}

On the basis of the sweeping with the entomological net average number of aphids and mortality were calculated on the 1, 5, 7 and 9 days after treatment. In fresh plant samples taken from the second harvested for seeds plastid pigments content (chlorophyll a, chlorophyll b, carotenoids, and total) (mg/100 g FW) was determined according to Zelenskii and Mogileva a one week after treatment.

Chlorophyll a/chlorophyll b and chlorophyll a+b/carotenoids ratios were calculated [ZELENSKII and MOGILEVA 1980; DIMITRIU *et al.*, 2016; GEORGIEVA *et al.*, 2018; BUTNARIU and CAUNII, 2013]. In dry plant samples, total nitrogen content by Kjeldahl (as a percentage of absolute dry matter) in aboveground mass was determined, after that nitrogen in yield of dry mass (productivity of dry mass multiplied by the percentage of nitrogen), (N, g/kg dry mass).

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 Multiple Regression Analysis of Statgraphics Plus (1995) for Windows Ver. 2.1 Software program was used.

Results and discussion

It shows the effect of biological products on alfalfa and interaction of products on the aphid density and their mortality (%) at pre– and post–spray intervals in Table 2.

Pre–spray mean aphid density was statistically similar in main plots. Mean aphid density 24 hours post–spray in control plots was significantly higher than treatment plots. The lowest aphid density was recorded in Agricolle+Nagro–treated plots which were (9.9 aphids 20 m^{-1}) with 92.7 % mortality, followed by Agricolle+Lytovit–treated plots and 89.2 % mortality. Effect between those treatments was not statistically different. It is clear from the results that post–spray (24 hours) aphid density after used of Agricolle and Agricolle with Nagro was significantly ($LSD_{0.05\%}$ 4.736).

Biological nano–fertilizer improved the protective effect of bioinsecticide and contributed to higher aphid mortality. The used of both nano–fertilizers reduced the

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insect density but there was no statistically significant effect between them.

The average aphid density five and seven days after treatment in control plots (151.9 and 140.8 number aphids m^{2-1}) was significantly higher compared to other variants. It recorded lower aphid density in the Litovit–treated plot but *A. pisum* had a low mortality and the protective effect was weak (38.2 and 25.0 % mortality). The highly concentrated complex nano–fertilizer Nagro exhibited insecticidal action against aphids such as the toxic effect was higher significantly that of Litovit. Pea aphid mortality remained high to 55% seven days after treatment and the product provided a medium level of control. It recorded the lowest aphid and maximum aphid mortality for the mix of Agricolle and Nagro. There was a significant difference to other variants. Statistically, similar mean aphid density and rate of aphid mortality was recorded for Agricolle and Agricolle+Litovit.

Table 2.

Effect of biological products (alone and in combination) on the aphid density, % mortality, on average 2015–2017

Samples	NBT	NAT **1** DAT	M, %	NAT **5** DAT	M, %	NAT **7** DAT	M, %	NAT **9** DAT	M, %
1	143.3a	18.6b	86.2	23.2b	84.7	36.3b	74.2	64.9b	53.8
2	138.5a	45.4c	66.4	51.5c	66.1	63.1c	55.2	106.2c	24.3
3	139.5a	44.0c	67.4	93.9d	38.2	105.6d	25.0	121.1c	13.8
4	139.5a	9.9a	92.7	14.6a	90.4	18.9a	86.6	35.5a	74.7
5	133.2a	14.5ab	89.2	25.5b	83.2	41.7b	70.4	54.4b	61.2
6	142.2a	135.1d	0.0	151.9e	0.0	140.8e	0.0	140.4d	0.0
LSD0.05%10.167				4.736		5.637		9.046	16.343

Legend: 1–Agricolle; 2–Nagro; 3–Lytovit; 4– Agricolle+Nagro; 5– Agricolle+Lytovit; 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; M– mortality, %; NBT– number of individuals before treatment from 10 m^2; NAT– number of individuals after treatment from 10 m^2. *Means in each column followed by the same letters are not significantly different ($P > 0.05$)

The trend was maintained nine days after treatment, as the lowest density and the highest mortality was recorded for Agricolle with Nagro, followed by Agricolle and Agricolle with Litovith with insignificant differences between the latter. On the other hand, both nano–fertilizers showed statistically similar results, low aphid mortality, and unsatisfactory protective effect.

The effect of biological products on the photosynthetic process was traced by determining the content of plastid pigments in the alfalfa leaves. It is known that aphid feeding causes considerable losses in the plastid pigment content which are an important source of nitrogen for these insect pests [GOŁAWSKA *et al.*, 2010; KERCHEV *et al.*, 2012; KUCHARIK *et al.*, 2016].

Chlorophyll content is one of the most important parameters in the relationships between plants and herbivores.

Table 3.

Plastid pigment content in alfalfa leaves, treated with biological products, on average 2015–2017

Treatment	Chl a	Chl b	Chl a+Chl b [mg/100g]	Carotenoids dry mass	Total	Chl a/Chl b	Chl a+Chl b/ carotenoids
1	55.60 b*	38.24 bc	93.84 b	15.51 bc	109.35 b	1.45	6.05
2	67.27 c	39.28 c	106.55 c	14.83 bc	121.38 cd	1.71	7.18
3	49.52 a	34.31 a	83.83 a	14.16 b	97.99 a	1.44	5.92
4	67.70 c	40.50 c	108.20 c	16.11 c	124.31 d	1.67	6.72
5	69.14 c	35.19 ab	104.33 c	14.35 bc	118.68 c	1.96	7.27
6	49.23 a	32.36 a	81.59 a	12.08 a	93.67 a	1.52	6.75
LSD $_{0.05\%}$	5.481	3.130	4.783	1.915	5.304	–	–

Legend: 1–Agricolle; 2–Nagro; 3–Litovit; 4–Agricolle+Nagro; 5–Agricolle+Litovit; 6–Control
* Means within columns followed by the same letter are not significantly different (p>0.05)

Changes in the total chlorophyll content (*Chl a* + *Chl b*) in the leaf tissue are an important indicator of chloroplast developmental disturbances and

photosynthetic disturbance in plants [SYTYKIEWICZ *et al*., 2013; BONCIU *et al.,* 2018; BONEA *et al.,* 2018a; BUTNARIU *et al.,* 2012]. Application of plant protection measures against aphids aims at creating favorable conditions for the photosynthetic process and improving the physiological status of the plants. The results of the comparative analysis (Table 3) showed that biological products increased the total pigment content.

Weaker but positive effect on chlorophyll synthesis *Chl a* + *Chl b* had Litovit treatment–an average of 2.7 % above the control, and the highest impact demonstrated used of Agricolle with Nagro–32.6 % increase, followed by treatment with Nagr –30.6 % and Agricolle with Litovith–27.9 % increase.

Carotenoid content follows a similar pattern as for chlorophylls and leaves with a high level of chlorophyll *Chl a* + *Chl b* were accompanied by a higher level of carotenoids. The carotenoid synthesis was most intense after application of Agricolle with Nagro (33.4% increase), followed by their use alone (Agricolle–28.4 % and Nagro – 22.8 % increase). Total amount of green and yellow pigments followed the trend of the respective components, as the increase to control varied from 4.6% (Litovit treatment) to 33.4 % (Agricolle + Nagro treatment).

Considerably increased synthesis of plastid pigments after Nagro used (29.6 %) was determined not only by its effect as a leaf fertilizer and better above biomass development but also by its insecticidal effect. Increased *Chl a* and *Chl b*, and carotenoid content, after foliar application of similar biological products, were reported by other authors [PARWEEN *et al*., 2011; BUTNARIU and SAMFIRA, 2012; IANCULOV *et al*., 2005].

The values of the chlorophyll *a* to *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 related to the basic activity of *Chl a*. It is relatively constant and is considered being genetically determined [TITOVA, 2010; BONEA *et al., 2018*; VARDANIAN *et al.,* 2018; BAGIU *et al., 2012*; BUTNARIU and CORADINI, 2012].

In the conditions of the present study, they ranged from 1.45 to 1.96 and from 6.05 to 7.27 respectively and determined plants treated with Agricolle + Nagro and Nagro as plants with the best physiological state.

Abdelaziz and collab. found that after *Vicia faba* treated with essential oils of rosemary (*Rosemarinus officinalis*), sage (*Salvia officinalis*) and turmeric (*Curcuma longa*) against *Aphis craccivora*, the *Chl a* content significantly decreased one hour after application [ABDELAZIZ *et al*. 2015].

For total pigments contents (*Chl a; Chl b* and carotenoids) there were no significant differences after seven days between the treatments and control.

Zarei and collab. investigated the effects of some monoterpenes (menthone and piperitone) isolated from *Dunaliella salina* on a concentration of β–carotene and *Chl a* in some cultures [ZAREI *et al*. 2016; PUTNOKY *et al.,* 2013, BUTNARIU *et al.,* 2014; BUTNARIU and GIUCHICI, 2011].

Authors found a decrease in their concentration in leaves treated with the menthone and piperitone. El–Said Salem reported that synthetic insecticides may have some side effects on the treatment of agricultural crops, such as a significant reduction in *Chl a* and *Chl b* content in maize and tomato leaves after application of malathion, chlorosane [EL–SAID SALEM, 2016], *etc*. Rehab and El–Said referred to another finding [REHAB and EL–SAID 2016]. They showed that insecticides malathion, chlorozan and Rezolex T reduce *Chl a* content by 28.2, 22.4 and 10.2 % one and ten days on treated Maize.

Nitrogen derived from the biological nitrogen fixation is the main source of nitrogen utilized directly by plants [CANFIELD *et al*., 2010; SOLOMON et al., 2012; VITOUSEK *et al*., 2013].

After carbon, hydrogen, and oxygen, nitrogen (N) is one of the essential elements in plants due to its key role in chlorophyll production, which is fundamental for the photosynthesis process [PETRACHE *et al.,* 2014, BUTNARIU *et al.,* 2014, BARBAT 2013, BUTU *et al.,* 2015].

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In addition, nitrogen is part of various enzymatic proteins that catalyze and regulate plant–growth processes [SINFIELD *et al.*, 2010]. The results of the present study showed that the nitrogen content in the dry mass increased to varying degrees depending on the products used (Figure 1).

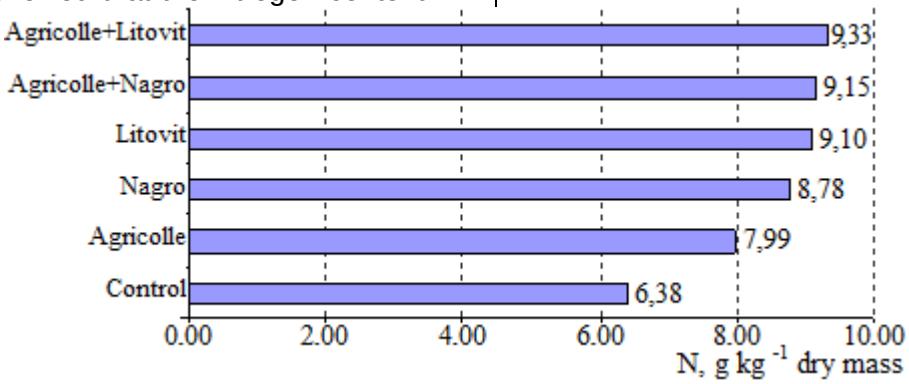

Figure 1. Nitrogen in dry mass yield in alfalfa after treatment with biological products

It changed the amount of fixed nitrogen in plants and depends on using biological products. In treatment with products, used alone alfalfa fix more nitrogen compared to control (from 1.61 to 2.72 kg N/kg dry mass yield). Alfalfa fixed the most nitrogen (more with 2.77 and 2.95 kg N/kg dry mass yield in compared to control) in the combined treatment of Aricolle with Nagro and Litovit [BUTU *et al.*, 2014c. SAMFIRA *et al.*, 2015, BUTNARIU *et al.*, 2015b; BUTU *et al.*, 2014b, PENTEA et al., 2016]. In addition, combinations had the best protective action against aphids, provided the best physiological state of the plants, and conditions for the photosynthetic process.

That fact contributed to the fixing of the highest amount of nitrogen in those treatment plants.

Conclusions

Treatment with biological products Agricolle, Nagro, and Litovit used alone and in combinations reduced *A. pisum* density in a different range. After application of Agricolle with Nagro combination, followed by Agricolle was found the lowest density and the highest aphid mortality. Biological products increased the total pigment content as the highest impact showed used of Agricolle with Nagro–33.4 % increase, followed by treatment with Nagro–29.6 %. *Chl a* to *Chl b* and green pigments to carotenoids ratio determined plants treated with Agricolle + Nagro and Nagro as plants with the best physiological state. Alfalfa fixed the most nitrogen (respectively more with 2.77 and 2.95 kg N/kg dry mass yield in compared to control) in the combined treatment of Aricolle with Nagro and Litovit.

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