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EFFICIENCY OF THE COMPLEX BACTERIAL PREPARATION AZOGRAN APPLICATION IN PROTECTING POTATOES FROM THE COLORADO POTATO BEETLE DEPENDING ON THE STAGE OF ITS DEVELOPMENT

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The Colorado potato beetle (Leptinotarsa decemlineana Say) is a particularly dangerous potato phytophage. ***The aim*** *of this work was to study the effect of complex granulated bacterial preparation Azogran and its components on the spread of the Colorado potato beetle in the potato phytocenosis.* ***Methods.*** *The study was carried out during potato of the Slavyanka variety growing under the conditions of small-plot and laboratory experiments. The complex granulated preparation Azogran was made on the basis of interaction of nitrogen-fixing bacteria Azotobacter vinelandii IMV B-7076, phosphate-mobilizing bacteria Bacillus subtilis IMV B-7023 and clay mineral bentonite. 1 g of the preparation contains more than* 10^8 *viable bacteria of each strain. The mass of 1 granule was 0.25 g. The effect of Azogran, its components and 4-hydroxyphenylacetic acid (4-HPAA), a metabolite of B. subtilis IMV B-7023, on the survival of Colorado potato beetle larvae was estimated in laboratory conditions.* ***Results.*** *It was shown that the use of the complex bacterial preparation Azogran, B. subtilis IMV B-7023 strain and its metabolite 4-HPAA in the potato phytocenosis significantly reduces the spread of the Colorado potato beetle imago, as well as the number of egg-laying on the leaf surface of plants. Treatment of eggs clutches of the phytophage with this preparation and its specified components significantly reduces the number of larvae on the leaves. Treatment of 1–4 stages larvae with solutions of 4-HPAA at a concentration of 0.5–50.0 μg/ml, as well as a suspension of B. subtilis IMV B-7023 bacteria or their culture medium (without cells), leads to significant death of the larvae.* ***Conclusions.*** *The use of the complex bacterial preparation Azogran, as well as its component bacteria B. subtilis IMV B-7023 in the phytocenosis of potatoes stimulates plant growth, significantly reduces the spread of the Colorado potato beetle and the number of eggs clutches on the leaf surface. Treatment of Colorado potato beetle larvae of 1–4 stages of development with a suspension of B. subtilis IMV B-7023 bacteria, as well as their metabolite 4-HPAA leads to the death of more than 65 % of larvae. The results obtained indicate that the use of the complex bacterial preparation Azogran is promising for potatoes growing.*

*Keywords**:** Complex bacterial preparation Azogran, potatoes, spread of the Colorado potato beetle and its larvae.*

Potato (*Solanum tuberosum* L.) is one of the most important agricultural crops in our country. The intensive growth and productivity of these plants is harmed by the spread of phytopathogenic microorganisms and phytophages in their agrocenosis. Currently, there are over 30 of the most common diseases and types of insect pests of potato, among which the Colorado potato beetle (*L. decemlineana* Say) is especially dangerous [1–2]. The main harm to this vegetable crop is caused by larvae of 3–4 instars [2]. This phytophage is adapted to exist in various conditions and gives up to 3 generations per season. In the process of intensive spreading, it can completely destroy the leaf cover of plants, lead to a significant or complete loss of yield in conjunction with a decrease in starch and protein content in tubers [3, 4].

One of the most effective and widely used (approximately 90–95 % of the biopesticide market) means of harmful insects controlling in agroecosystems are preparations based on the gram-positive spore-forming bacterium *Bacillus thuringiensis* (Bt), which has the ability to form parasporal crystalline inclusions of a protein nature during sporulation [3]. Along with this, to limit the spread of the Colorado potato beetle, it was proposed to use the microorganisms *Photorhabdus luminescens, Chromobacterium* sp. [5], and also a biopesticide based on *Beauveria bassiana* and *B. thuringiensis* [6].

Based on the interaction of highly effective strains of phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 [7], nitrogen-fixing bacteria *Azotobacter vinelandii* IMV B-7076 [8] with the clay mineral bentonite [9] a granulated form of the preparation Azogran was created. It's using in a number of agroecosystems significantly stimulates the growth, development and productivity of plants, including potato, and protects them from a number of phytopathogens and phytophages [10–12].

We have previously shown that *B. subtilis* IMV B-7023 strain synthesizes a number of phenolic compounds, among which 4-hydroxyphenyacetic acid (4-HPAA) was determined in significant amounts. This phenolcarboxylic acid can inhibit the development of some phytopathogenic micromycetes (*Fusarium culmorum* 50536, *Alternaria alternata* 16675 and *Fusarium solani* 50666) and has antioxidant potential [13]. In addition to the fact that Ph-OH are antagonists of various phytopathogens [14], it is known that these compounds also exhibit insecticidal properties, reducing the attack on potatoes.

In this regard, the **purpose** of this article is to study the effect of the complex bacterial preparation Azogran, its components and 4-HPAA on the spread of Colorado potato beetle in the potato agroecosystem.

Materials and methods. The study of the influence of the granular complex bacterial preparation Azogran and its individual components on the spread of the Colorado potato beetle in the phytocenosis of Slavyanka potato variety was carried out on the research field of the Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, as well as in laboratory conditions. 1 g of granular preparation Azogran contains over 10^8 cells of each type of bacteria. The mass of 1 granule is 0.25 g.

Potatoes were planted to the ground in the last decade of April. Experiments were done in 4 replicates, 10 plants per variant: 1. Control – soaking of potato tubers for 1 hour in sterile tap water. 2. Application of 2 granules of the Azogran per hole at potatoes planting. 3. Application of 3 granules of the Azogran per hole at potatoes planting. 4. Potato tubers soaking for 1 hour in a suspension of *A. vinelandii* IMV B-7076 diluted with sterile water 1:100 (10^7 cells/ml). 5. Soaking of potato tubers for 1 hour in a suspension of *B. subtilis* IMV B-7023 diluted 1:100 (10^7 cells/ml). 6. Soaking of potato tubers for 1 hour in a mixed suspension of *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 (in a 1:1 ratio) diluted with water 1:10, 1:100 and 1:1000 (10^8–10^6 cells/ml).

Phytophages were counted according to generally accepted methods of field research [15], starting with the appearance of the first phytophages on plants. Their number was calculated per plant.

To study the effect of Azogran and its components on the survival of Colorado potato beetle larvae, 10 larvae were introduced into Petri dishes containing sterile moist paper and potato leaves, treated with a number of the above variants of the drug or its components. The percentage of larval death was determined on the 3rd–10th days and calculated according to the Abbott equation [16]:

$$E = A - B / A \cdot 100,$$

where: E is the number of dead insects, adjusted for control, %; A – their number in the experimental version before processing; B – the number of insects in the experimental variant after treatment.

The influence of suspensions of bacterial components of the Azogran preparation, their culture media or 4-HPAA on the development of Colorado potato beetle larvae was studied using infected potato leaves. For this purpose, leaves selected from control plants containing clutches of eggs or larvae of certain developmental stages were placed in Petri dishes containing sterile moist filter paper. One Petri dish contained 4–5 clutches of eggs with the recorded number of eggs or up to 50 larvae on the leaves. 1 ml of bacterial suspensions, their culture media or 1 ml of 4-HPAA at concentrations from 0.5 μg/ml to 50 μg/ml were applied on each leaf with clutches of eggs or larvae. In the control, treatment was carried out with an appropriate volume of sterile tap water. The studies were carried out in four replicates. The results were taken into account on the 3rd, 5th and 10th days, determining the number of larvae that emerged from the clutches of eggs and their subsequent death.

The experiments were carried out according to the methods of field research [17]. Statistical processing of research results was carried out according to Lakin at a confidence level of 95 % [18].

Results. It was found that both the treatment of potato tubers with a suspension of bacterial components of the Azogran before their planting into the soil, and the introduction of this preparation into the holes had a positive effect on the growth and development of plants. So, after 16 days from the day of potato planting in the variants with the use of Azogran or bacterial suspensions, the indicators of plant height and the sprouting bush were 20–30 % higher than those in the control variant. In addition, the number of shoots increased (Table 1).

Table 1

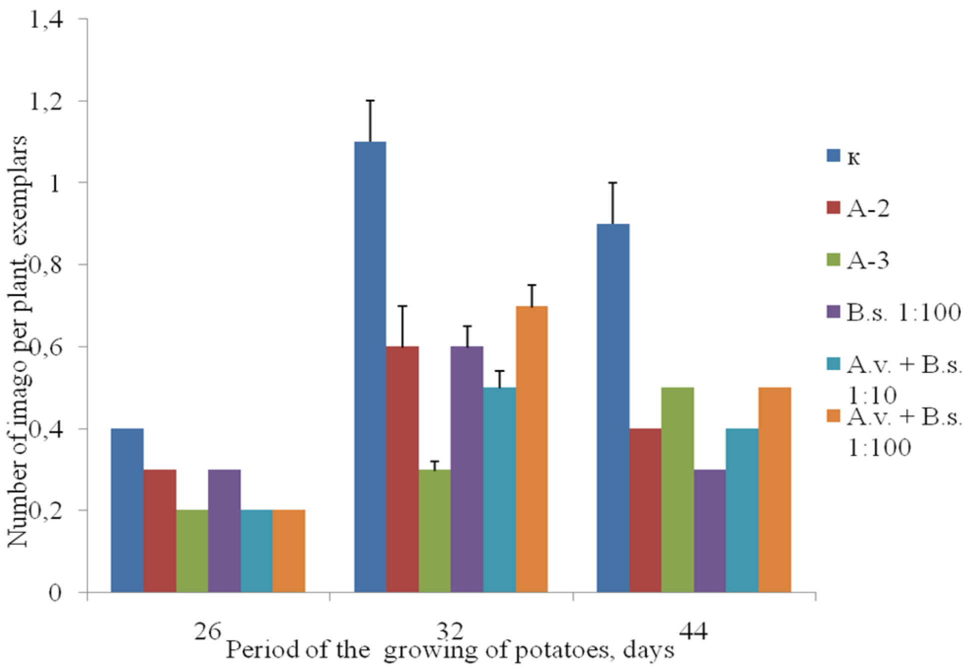
The influence of Slavyanka potato variety treatment with the Azogran and its components on the germination and morphometric parameters of the vegetative system of plants

Variant of treatment	Germination, %	Plant indicators					
		after 16 days of growth			after 26 days of growth		
		plant height, cm	number of shoots, pcs.	bush diameter, cm	plant height, cm	number of shoots, pcs.	bush diameter, cm
Control	92.0	7.7±0.7	1.7±0.1	10.8±0.2	37.0±3.0	2.5±0.2	29.0±2.0
Azogran, 2 gr.	100.0	9.8±0.5	2.2±0.1	16.0±0.1	39.8±3.1	3.0±0.2	36.4±3.0
Azogran, 3 gr.	97.0	10.4±0.9	2.7±0.2	17.2±0.5	48.5±3.9	3.5±0.3	38.5±3.0
B.s. (10^7) 1:100	95.0	9.4±0.7	2.4±0.1	18.0±0.2	35.8±2.6	3.0±0.2	30.5±2.0
A.v.+B.s. (10^7) 1:10	100.0	11.2±0.7	2.8±0.2	18.7±0.2	49.4±4.0	4.5±0.3	42.3±3.0
A.v.+B.s. (10^6) 1:100	100.0	9.4±0.8	2.6±0.1	17.8±0.6	45.6±2.9	4.0±0.2	37.4±3.0

Notes: *B.s.* – *B. subtilis*; *A.v.* – *A. vinelandii*; gr. – granules.

The preparation Azogran and its components had a significant effect on the distribution of the Colorado potato beetle in the potato phytocenosis. It should be noted that the number of imago in all variants of the experiment was insignificant and fluctuated until mid-July from 0.2 to 1.1 per plant (Fig. 1).

The greatest number of clutches of eggs of Colorado potato beetle (0.9 – 1.3 pcs.) and the number of eggs laid by the phytophage (14.6 – 26.4 pcs.) per plant was found in the control (Table 2). Other variants contained from 0.2 – 0.9 clutches with 5.6 – 17.1 eggs per plant (Fig. 2).



F i g. 1. Dependence of imago number of the Colorado potato beetle on potato plants from the time of cultivation. *Notes*: k – control; A-2 – Azogran, 2 granules; A-3 – Azogran, 3 granules; *B.s. – Bacillus subtilis*; *A.v. + B.s. – Azotobacter vinelandii + Bacillus subtilis.*

Table 2

The number of clutches of eggs (ce) of the Colorado potato beetle and the total number of laid eggs (e) on potato plants

Variant of treatment	Number of clutches of eggs (**ce**) and laid eggs (**e**) per plant depending on the growing time, pcs.			
		26 days	32 days	44 days
Control	ce	1.3 ± 0.1	1.1 ± 0.1	0.3 ± 0.0
	e	24.2 ± 0.6	18.4 ± 0.4	6.2 ± 0.1
Azogran, 2 gr.	ce	0.8 ± 0.1	0.5 ± 0.0	0.2 ± 0.0
	e	17.1 ± 0.3	13.2 ± 0.2	4.5 ± 0.1
Azogran, 3 gr.	ce	0.7 ± 0.0	0.8 ± 0.1	0.3 ± 0.0
	e	16.4 ± 0.3	17.0 ± 0.4	5.7 ± 0.1
B.s. 1:100	ce	0.8 ± 0.1	0.7 ± 0.0	0.2 ± 0.0
	e	15.2 ± 0.4	15.3 ± 0.3	5.3 ± 0.1
A.v. + *B.s.* 1:10	ce	0.5 ± 0.0	0.9 ± 0.1	0.2 ± 0.0
	e	11.3 ± 0.1	12.8 ± 0.1	4.5 ± 0.1
A.v. + *B.s.* 1:100	ce	0.5 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
	e	12.4 ± 0.3	14.8 ± 0.5	7.2 ± 0.1

Notes: *A.v.* + *B.s.* (1:1) – *A. vinelandii* + *B. subtilis* in the ratio 1:10 and 1:100; "ce" – clutches of eggs; "e" – number of eggs; gr. – granules.

F i g. 2. Clutches of eggs of Colorado potato beetle on potato leaves

Larvae of 1–2 generations appeared on plants in the second half of June. Their number ranged from 1.4 to 6.5 specimens per plant (Table 3). The maximum number of such larvae was observed in late June – early July in the control – from 10.3 to 19.4 specimens per plant. In the variants with the using of the preparation Azogran the number of larvae of 1st and 2nd generations was significantly lower – there were from 2.5 to 11.0 specimens per plant, which was two times lower than in the control. Larvae of the older generations appeared in late June – early July. Their numbers gradually increased. The maximum number of the larvae of older generations was determined in the control – from 15.4 to 18.4 specimens per plant (Table 3). At using the preparation Azogran or its components, the number of larvae of older generations after plants growing for 32 days was almost 3 times lower than in the control. However, with a longer potato growth (44 days), this indicator slightly increased, which apparently was due to a decrease in the number of larvae of younger generation.

Considering that the bacteria *B. subtilis* IMV B-7023, a component of Azogran, is capable of limiting the spread of the Colorado potato beetle in the potato agroecosystem, it was the interest to study the role of the cultural media of bacteria and 4-HPAA – metabolite of this strain of bacilli in this process. It was shown that the treatment of Colorado potato beetle larvae of 3–4 developmental stages with solutions of 4-HPAA (0.5–50.0 µg/ml), as well as with a suspension of *B. subtilis* IMV

B-7023 bacteria or their cultural medium (without cells) resulted in significant death of larvae (Table 4).

It was shown that 8 out of 154 Colorado potato beetle larvae in the control died after 3 days of growing under experimental conditions. 84 of 179 larvae died after treatment with 4-HPAA at a concentration of 0.05 µg/ml. 92 of 170 larvae died after treatment with this phenolic compound in concentration increased to 0.5 µg/ml, and when the concentration of 4-HPAA was 50 µg/ml, 134 of 187 larvae died. Thus, most of them died when using 50 µg/ml of 4-HPAA. In this treatment variant, the biological efficiency was about 66 %.

Table 3

The number of Colorado potato beetle larvae on potato plants, depending on the time of cultivation

Option	The number of larvae of 1st and 2nd generations (m), 3rd and 4th generations (**s**) per plant, depending on the time of cultivation, specimens			
	м/s	26 days	32 days	44 days
Control	м	9.4 ± 0.2	10.3 ± 0.4	3.5 ± 0.2
	s	2.4 ± 0.0	18.4 ± 0.6	16.8 ± 0.5
Azogran, 2 gr.	м	3.6 ± 0.1	6.1 ± 0.2	3.0 ± 0.2
	s	0.7 ± 0.1	6.5 ± 0.3	12.4 ± 0.7
Azogran, 3 gr.	м	4.7 ± 0.1	5.4 ± 0.2	1.4 ± 0.0
	s	1.3 ± 0.0	5.3 ± 0.1	10.5 ± 0.2
B s. 1:100	м	2.6 ± 0.1	6.9 ± 0.1	2.3 ± 0.1
	s	0.6 ± 0.1	11.0 ± 0.6	13.4 ± 0.5
A.v. + *B.s.* 1:10	м	3.6 ± 0.1	6.0 ± 0.1	1.2 ± 0.1
	s	0.5 ± 0.0	6.2 ± 0.1	9.0 ± 0.2
A.v. + *B.s.* 1:100	м	3.0 ± 0.1	6.4 ± 0.1	2.5 ± 0.1
	s	0.1 ± 0.0	3.8 ± 0.0	10.4 ± 0.4

Notes: *A.v.* + *B.s.* (1:1) – *A. vinelandii* + *B. subtilis* in the ratio 1:10 and 1: 100; gr. – granules.

Table 4

The influence of 4-hydroxyphenylacetic acid and bacterial suspensions on the survival of Colorado potato beetle larvae of 3– 4 developmental stages

Variant of treatment	Number of larvae, specimens	The number of larvae (specimens) 3 days after the moment of their processing and the biological effectiveness of the preparations action, U, %		
		alive	death	unit, %
Control	154 ± 9	146 ± 11	8 ± 1	–
4-HPAA (0.05 µg/ml)	179 ± 11	95 ± 8	84 ± 7	41.7
4-HPAA (0.5 µg/ml)	170 ± 10	78 ± 7	92 ± 8	49.0
4-HPAA (50 µg/ml)	187 ± 14	53 ± 4	134 ± 11	66.5
B. subtilis (10^7 cells/ml)	215 ± 19	61 ± 5	154 ± 13	66.4
CM *B. subtilis* (10^7 cells/ml)	197 ± 15	90 ± 8	107 ± 9	49.1
A.v.+*B*.s., (10^7 cells/ml)	195 ± 16	97 ± 8	98 ± 8	45.1
CM *A.v.*+*B.s.*, (10^7 cells/ml)	156 ± 13	135 ± 11	21 ± 1	8.3
A. vinelandii (10^7 cells/ml)	190 ± 17	178 ± 14	12 ± 1	11.2

Note: CM is a cultural medium obtained from a bacterial suspension (10^7 cells/ml).

Similar results were obtained after treatment of Colorado potato beetle larvae with a suspension of *B. subtilis* IMV B-7023 (10^7 cells/ml). The use of the cultural medium of this strain for treatment of larvae also significantly reduced the number of alive specimens. In this case, 107 specimens died out of 197 larvae. The biological efficiency in this treatment variant was about 49 %.

After larvae treatment with a mixed suspension of *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 (10^7 cells/ml), 98 out of 195 specimens died, and after their treatment with a mixed cultural media of these bacteria obtained from the above suspension, only 21 larvae of 154 specimens died.

It should be noted that the treatment of Colorado potato beetle larvae with a suspension of *A. vinelandii* IMV B-7076 (10^7 cells/ml) had little effect on their viability.

Thus, the studies carried out indicate the effectiveness of using the preparation Azogran, as well as a suspension of *B. subtilis* IMV B-7023 in the agroecosystem of Slavyanka potato variety.

Discussion. It is known that nearly 10.000 species of insects, 50.000 species of fungi, 1.800 species of weeds and 15.000 species of nematodes cause significant harm to the growth, development and productivity of various plant species used by the population of our planet. Until recently the main strategy to avoid such losses was to eliminate pests with chemical pesticides such as chlorinated hydrocarbons, organophosphates and carbamates [19]. In recent years, a number of pesticides based on compounds of the neonicotinoid class have been proposed. These are preparations triamethoxam, imidacloprid and its derivatives Prestige, Confidor Extra and others [20, 21].

The use of chemical pesticides in agricultural production makes it possible to improve the growth of plants and increase their productivity. However, this approach is accompanied by environmental pollution and can harm biota and human health. Therefore, in recent decades, more and more attention has been paid to the biologization of crop production, the main method of which is the use of microbiological preparations that increase soil fertility, stimulate the growth and productivity of plants and protect them from phytopathogens and phytophages [22].

The most widely used method of protecting plants from the Colorado potato beetle was the use of preparations based on the bacteria *B. thuringiensis* in agroecosystems [23]. However, it is known that at least 27 phytophage species have developed resistance to preparations based on these bacteria [23]. These results indicate the need to search for new bioinsecticides and create complex microbial preparations that would not only inhibit the spread of phytophages in agroecosystems, but also protect plants from phytopathogenic microorganisms, stimulate the growth and development of plants, and increase their productivity.

Such requirements are met by complex microbial preparations, one of which is Azogran. We have previously shown that this preparation significantly stimulates the growth of many ornamental, flower and other plants, and also increases the yield of industrial, grain and vegetable crops by 18–37 % [24]. To a certain extent this due to the ability of the bacterial components of the Azogran preparation to fix atmospheric nitrogen, mobilize phosphate from its organic and hardly soluble inorganic compounds, antioxidant properties of strains [25] and their antagonism to phytopathogenic microorganisms [11], as well as phytophages [12].

Our results indicate that, along with the above properties of Azogran, one of the important factors that increase the yield of potatoes [10] is the ability of this preparation to inhibit the spread of the Colorado potato beetle in the agroecosystem at different stages of its development.

A significant role in this process can be played by secondary metabolites of bacterial components of the Azogran, in particular, phenolic compounds. Using the example of 4-HPAA, a metabolite of *B. subtilis* IMV B-7023, it was found that this substance effectively prevents the development of 3–4 instars of Colorado potato beetle larvae, which most severely damage potatoes. According to the literature [26, 27], the insecticidal effect of Ph-OH is in their ability to form complexes with digestive enzymes in the intestines of insects. As a result, there is a decrease in the efficiency of protein digestion, which causes a slowdown in growth and negatively affects the survival of phytophages.

Conclusions. Thus, the use of the complex bacterial preparation Azogran, as well as its component bacteria *B. subtilis* IMV B-7023 in the phytocenosis of potatoes stimulates growth, significantly reduces the spread of the Colorado potato beetle and the number of clutches of eggs on the leaf surface. Treatment of the Colorado potato beetle larvae of the 3–4 developmental stages with a suspension of bacteria *B. subtilis* IMV B-7023, as well as their metabolite 4-HPAA leads to the death of more than 65 % of larvae of this phytophage. The results obtained indicate that the use of the complex bacterial preparation Azogran is promising for potatoes growing.

ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ КОМПЛЕКСНОГО БАКТЕРІАЛЬНОГО ПРЕПАРАТУ АЗОГРАН У ЗАХИСТІ КАРТОПЛІ ВІД КОЛОРАДСЬКОГО ЖУКА В ЗАЛЕЖНОСТІ ВІД СТАДІЇ ЙОГО РОЗВИТКУ

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Резюме

Колорадський жук (*Leptinotarsa decemlineana* Say) особливо небезпечний фітофаг картоплі. **Метою** даної роботи було дослідження впливу комплексного гранульованого бактеріального препарату Азогран і його компонентів на поширення колорадського жука в фітоценозі картоплі. **Методи.** Дослідження проводили при вирощуванні картоплі сорту Слов'янка в умовах дрібноділянкових і лабораторних експериментів. До складу комплексного гранульованого препарату Азогран входять азотфіксувальні бактерії *Azotobacter vinelandii* IМВ B-7076, фосфатмобілізувальні бактерії *Bacillus subtilis* IМВ B-7023 і глинистий мінерал бентоніт. В 1 г препарату міститься понад 10^8 життєздатних бактерій кожного штаму. Одна гранула препарату – 0,25 г. Вплив препарату Азогран, його компонентів і метаболіту *B. subtilis* IМВ B-7023 4-гідроксіфенілоцтової кислоти (4-ГФУК) на виживання личинок колорадського жука проводили в лабораторних умовах. **Результати.** Показано, що застосування комплексного бактеріального препарату Азогран, бактерій *B. subtilis* IМВ B-7023 і їх метаболіту 4-ГФОК в фітоценозі картоплі значно знижує поширення в ньому імаго колорадського жука, а також кількість яйцекладок на листковій поверхні рослин. Обробка яйцекладок цього фітофага даним препаратом і зазначеними його компонентами істотно знижує кількість личинок на листках. Обробка личинок 1–4 стадії розвитку розчинами 4-ГФУК в концентрації 0,5 – 50,0 мкг/мл, а також суспензією бактерій *B. subtilis* IМВ B-7023 або їх культуральним середовищем (без клітин) призводить до значної загибелі личинок. **Висновки.** Застосування комплексного бактеріального препарату Азогран, а також його компонента – бактерій *B. subtilis* IМВ B-7023 в фітоценозі картоплі стимулює ріст рослин, істотно знижує поширення в ньому колорадського жука і кількість яйцекладок на листковій поверхні. Обробка личинок колорадського жука 1–4 стадії розвитку суспензією бактерій *B. subtilis* IМВ B-7023, а також їх метаболітом 4-ГФОК призводить до загибелі понад 65 % личинок цього фітофага. Отримані результати свідчать про перспективність застосування комплексного бактеріального препарату Азогран при вирощуванні картоплі.

Ключові слова: комплексний бактеріальний препарат Азогран, картопля, поширення колорадського жука і його личинок.

1. Awang Maharijaya. Ben Vosman. Managing the Colorado potato beetle; the need for resistance breeding. Euphytica (2015); 204:487–501. DOI 10.1007 / s10681-015-1467-3
2. Voronkova MV, Sycheva II. The use of biologically active plant components in the protection of potatoes from *Leptinotarsa decemlineata* Say. 2017. DOI https://doi.org/10.18551/rjoas.2017-11.54
3. Dobritsa AP, Koretskaya NG, Gaitan VI, Kolomben LV, Derbyshev VV, Zhigletsova SK. [Development of biopesticides against the Colorado potato beetle]. Grew up chem g. J of the Russian Chemical Society named after D. I. Mendeleev. 2001; XLV (5–6):174–184. Russian.
4. Gusev GV, Koval AG, 1990. [Biological method of combating the Colorado potato beetle: Plant protection]. M.: Agropromizdat; 1990. 63 p. Russian.
5. Phyllis AW Martin, Michael Blackburn, Ashaki DS Shropshire. Two New Bacterial Pathogens of Colorado Potato Beetle (Coleoptera:

Chrysomelidae) Journal of Economic Entomology. 2004; 97(3):774–780. https://doi.org/10.1093/jee/97.3.774
6. Atef MM Sayed, Robert W Behle. Evaluating a dual microbial agent biopesticide with *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana* blastospores. Journal Biocontrol Science and Technology. 2017; 27 (4):461–474.
7. Patent of Ukraine №54923A [The bacterial strain *Bacillus subtilis* for bacterial fertilizer for crop production]. Kurdish IK, Roy AA. Publ. 2003. Bul. № 3. Ukrainian.
8. Patent of Ukraine № 72856 [Strain of bacteria *Azotobacter vinelandii* for bacterial fertilizer obtaining for plant-growing]. Kurdish IK, Bega ZT. Publ. 2006. Bul. № 8. Ukrainian.
9. Declarative patent of Ukraine No. 57269. The method of obtaining granular bacterial preparations. Kurdish IK, Roy AO, Bega ZT. Bul. 2003. No. 6.
10. Shirokonos AM, Tsvey YaP, Roy AO, Kurdish IK. [Efficiency of storage of granular bacterial preparations in case of viral cartoplasty]. Zbirnik of the Uman State Agrarian University (special edition). Uman. 2003. p.217–220. Ukrainian.
11. Roy AA, Pasichnik LA, Tserkovnyak LS, Khodos SF, Kurdish IK. [Influence of bacteria of the genus *Bacillus* on causative agents of tomato bacterial cancer]. Mikrobiol Z. 2012; 74 (5):74–80. Ukrainian.
12. Roy AO, Matselyukh OV, Zubko PD, Varbanets LD, Kurdish IK. [Proteolytic activity of phosphate mobilizuval bacteria of the genus *Bacillus* and their infusion on some phytophages]. Silskogospodarska Microbiology. 2014; 20:66–73. Ukrainian.
13. Tserkovniak LS, Kurdish IK. Phosphate-mobilizing Bacteria *Bacillus subtilis* as Phenolic Producers. Appl Biochem Microbiol. 2009; 45 (3):279–284.
14. Christina E Maddox, Lisa M Laur, Li Tian. Antibacterial activity of phenolic compounds against the phytopathogen *Xylella fastidiosa*. Curr Microbiol. 2010; 60(1):53–58. doi: 10.1007/s00284-009-9501-0.
15. Omelyuta VP, Grigorovich IV, Chaban VS, et al. [Oblik shkidnikiv and ailment of agricultural cultures]. Kiev: Urozhay; 1986. 294 p. Ukrainian.
16. Triebel SO, Sigarova DD, Sekun MP, Ivashchenko OO. [Methods of viprobation and consumption of pesticides]. K.: Svit; 2001. 448 p. Ukrainian.
17. Dospekhov B.A. [Field experiment technique (with the basics of statistical processing of research results)]. M.:Agropromizdat; 1985. 416 p. Russian.
18. Lakin G.F. [Biometry]. Moskow: Vysshaia shkola; 1990. 352 p. Russian.
19. Opender Koul. Microbial biopesticides: opportunities and challenges. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2011; 6 (056). doi: 10.1079 / PAVSNNR 20.116056
20. Kurdish IK. Interaction of microorganisms with nanomaterials as a basis for creation of high-efficiency biotechnological preparations. In: Prasad R, et al. editors. Nanobiotechnology in Bioformulations, Nanotechnology in the Life Sciences. Springer Nature Switzerland AG; 2019. p. 259–287.
21. List of pesticides and agrochemicals permitted for use on the territory of the Russian Federation in 2012. Appendix to the journal “Plant Protection and Quarantine”. M.; 2012. 578 p.
22. Glez VM, Zeyruk VN, Vasilyeva SV, Derevyagina MK. The effectiveness of neonicotinoid insecticides on potatoes. Agriculture. 2015; 7:43–46.
23. Bravo A, Likitvivatanavong S, Gill S, Soberon M. *Bacillus thuringiensis*: a story of a successful bio-insecticide. Insect Biochem Mol Biol. 2011; 41:423–431. doi: 10.1016 / j.ibmb.2011.02.006.
24. Kurdish IK. [Introductions of microorganisms in the agroecosystem]. Kyiv: Naukova Dumka; 2010. 253 p. Ukrainian.
25. Skorochod I, Roy A, Kurdish I. Antioxidant potential of the phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 and *Bacillus subtilis* IB-22. The Journal of Free Radicals and Antioxidants. Photon. 2014; 141:371–377.

26. Schoonhoven LM, JJA van Loon M Dicke. Plants as insect food: not the ideal. Insect plant biology. Oxford University, New York: USA; 2005. 115 p.

27. Schaller A. Induced plant resistance to herbivory. In: Bernards MA, L Bastrup-Spohr, editors. Phenypropanoid metabolism induced by wounding and insect herbivory. Springer, New York: USA; 2008. p. 189–208.

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