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Biological Preparations Developed by Belarusian State University for Environmentally Friendly Farming

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Due to the environmental deterioration it become urgent in the world to develop and apply effective biological preparations able to regulate plant growth and protect crops from diseases, as an alternative of chemical fertilizers and pesticides applying. Advantages of microbiological preparations derived from bacteria are well known: they are environmentally friendly, do not break the biological balance in natural communities, are safe for warm-blooded, not phytotoxic and does not affect the harvesting time, while significantly promote the plant growth, have a long protective effect, increase productivity and improve the condition of the soil. The Research Laboratory of Molecular Genetics and Biotechnology of the Belarusian State University developed a range of biological products derived from *Bacillus* and *Pseudomonas* with all the above characteristics intended for ecologically friendly farming in greenhouses and open ground: Baktogen, Aurin, Nemacid, Stimul and Zhytsen.

Keywords: biological preparations, plant protection, growth promotion, environmentally friendly farming, greenhouses, Bactogen, Aurin, Nemacid, Stimul, Zhytsen, *Bacillus* sp., *Pseudomonas* sp.

Introduction

Countries with high level of industrial and agricultural intensification feel concern about the problem of the impact of technogenic and anthropogenic factors on the environment.

Switching of certain sectors of agricultural production to harmless management providing for introduction of new technologies of plant cultivation and protection by biological methods can significantly improve the environmental situation.

Biological crop protection products are environmentally friendly, do not disturb the biological balance in natural communities, are not toxic to humans and animals, have a lasting effect, so their advantages as compared to plant

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protection chemicals are indisputable. Biopreparations are particularly required for production of crops in greenhouses where high temperature, humidity and other factors contribute to focal and mass infection of vegetable crops with bacterial diseases (vascular bacteriosis, cancer tomatoes, "blackleg", etc.) and diseases of fungal etiology (fusarium blight, grey and white rot, ascochytosis, etc.).

Applying chemicals in greenhouses is not desirable for hygienic and sanitary reasons, and in some countries it is even prohibited by law. For example, in Belarus pesticides and agrochemicals in greenhouses are permitted only in exceptional cases – in the event of mass reproduction of pathogenic microorganisms (Resolution of the Chief State Medical Officer of the Republic of Belarus dd. 29.12.2005 No. 280).

In agricultural practice, the preference is given to multipurpose bioprepations that, along with protective effect, are able to promote plant growth, improve plant immunity and yield. Microbial preparations developed by the Research Laboratory of Molecular Genetics and Biotechnology of the Belarusian State University correspond to all these requirements. The list of environmentally friendly biopreparations includes: Bactogen (derived from *Bacillus subtilis* 494), Aurin (*Pseudomomas aurantiaca* B-162/498), Nemacid (*Pseudomonas putida* U), Stimul (*Pseudomomas fluorescens* S32), Gulliver (*Pseudomonas aureofaciens* A8-6) and Zhytsen (*Pseudomonas sp.-11, Bacillus sp.-49*).

Objectives: This paper describes the results of the registration tests of these biological products under field conditions.

Materials and methods

Aurin was tested in greenhouse and in the open ground on hybrids of cucumbers: Componist F_1 and Courage F_1. Bactogen was tested on varieties and hybrids of tomatoes: Ruby, Jatana, Victory, Kostroma F_1, Blagovest F_1, and cucumbers: Courage, Brigadier, Companist, Coral, Small Spring, Elize, Parisian cornichon using the method described in (Feklistova I. N. *et al,* 2010). The Biological effectiveness of Stimul was assessed using the method described in (Feklistova *et al,* 2013).

Experiments for assessment of Biological effectiveness of microbial preparation Zhytsen were conducted in two variants: during dummy field experiments the ability of Zhytsen to influence the speed of mineralization of sunflower straw, and crop and root residues was assessed (Seraya, 2012); during the stationary field experiment the effect of the preparation on the

effectiveness of decomposition of sunflower plant residues was assessed (through yield of the future crops – barley).

The experiment for assessment of Nemacid effectiveness was conducted using tomatoes (Chelbas hybrid) in the area of 10 m^2, with invasive load of 480 gall eelworm larva of 2^{nd} age/100 cm^3 of soil. Experiment for cucumbers (Courage hybrid) was conducted in the area of 10 m^2, with invasive load of 1640 gall eelworm larva of 2^{nd} age/100 cm^3 of soil. Experiments were conducted under cover in accordance with the technological requirements: air temperature in greenhouse – within 25-30 ^{0}C during daylight hours and 15-20 ^{0}C at night; relative humidity – 60-65 %. Application technology included consecutive root watering of seedlings with 1 % process solution of preparation 7 days prior to planting; applying 0.1 % process solution of the preparation into the hole upon planting; root watering of plants with 0.1 % solution of the preparation in 10 days after planting.

The blank was treated with water. Biological effectiveness of Nemacid was determined by the degree of reduction in meloidogynosis infestation of plant root system as compared to the blank.

Experiments for assessment of the effect of Stimul on linen flax were conducted in accordance with (Feklistova e*t al*, 2013).

Results and Discussion

Aurin

Aurin was derived from *Pseudomonas aurantiaca* B-162/498 being the overproducers of phenazine antibiotics. Aurin is intended for protection of crops from causative agents of root rots, ascochyta-leaf spot, powdery mildew, grey and white rot, cladosporiosis, false mildew, and promotes the growth of crops. Aurin is a broad spectrum preparation for protection of plants from diseases of various etiology, as active ingredients include phenazine antibiotics and pyrrolnitrin (Feklistova *et al*, 2008).

It was established that treatment of cucumber with Aurin during vegetations allowed reducing the occurrence and development of root rot (caused by *Fusarium spp*.). Thus, Aurin application resulted in reduction in disease development by 14.3 – 33.4 %, occurrence — by 28.9 – 31.1 % (Table 1). The effective control of white rot development (caused by *Sclerotinia sclerotiorum*) in cucumber was observed: occurrence of the disease on the 1^{st} and 2^{nd} year of testing reduced by 10.0 and 30.2 %, respectively, and development of the disease reduced by 20.0 and 23.3 %, respectively (Table 1)

Table 1. Effect of Aurin on Occurrence and Development of Root and White Rot in Cucumbers (soil)

Disease	Variant	Disease occurrence, %		Disease development, %	
		2007	2008	2007	2008
Root rot	Aurin	73.2	62.2	32.2	31.7
	Blank	87.5	95.6	61.1	62.8
White rot	Aurin	40.6	19.6	86.7	42.3
	Blank	65.6	42.9	96.7	72.5

At the end of vegetation, biological effectiveness of Aurin with regard to root rot was 47.3 % in 1st year and 50.0 % in 2nd year, with regard to white rot the effectiveness was 21.4 % and 54.3 %, respectively. Moreover, Aurin application on the experimental grounds in 1st year allowed increasing of yield up to 8.1 kg/m^2 that was 26.6 % greater as compared to the blank. In 2nd year application of the biopreparation promoted the increase in cucumber yield from 8.5 to 10.4 kg/m^2, i.e. by 22.3 %.

Modern requirements to growing vegetables under cover are associated with sharp decrease in material costs and more efficient care of plants with guaranteed high amount and quality of the products produced.

At present, the system of small volume technology meets such requirements. Experiments for assessment of Aurin effectiveness in protection of cucumbers (Courage F_1 hybrid) from grey (caused by *Botrytis cinerea* Pers.) and root (caused by *Fusarium spp*.) rots upon growing plants on mineral wool using the method of small-volume hidrophonics were conducted. The data on effectiveness of preparation application are shown in Table 2.

It has been established that new method of Aurin application for protection of cucumbers cultivated on mineral wool using small-volume technology ensures high biological effectiveness in respect of root rot (71 %) exceeding the same upon the biopreparation application for protection of cucumbers cultivated in soil (biological effectiveness – 50.0 %).

It was confirmed by experiments that *P. aurantiaca* B-162/498, the basis of Aurin, have high rate of acclimation in cucumber rhizosphere upon cultivation of plants on mineral wool and have prolonged protective action.

Table 2. Biological Effectiveness of Aurin in Protection of Cucumbers from Grey and Root Rot (mineral wool)

Variant	Disease occurrence, %	Biological effectiveness, %
Grey rot		
Aurin	11.3	44.6
Blank	18.8	-
Root rot		
Aurin	19.0	71.0
Blank	26.8	-

Bactogen

Microbiological preparation Bactogen was derived from *Bacillus subtilis* (Maksimova N.P. *et al*, 2008). Bactogen is intended for protection of tomatoes from bacteriosis, blackleg, grey rot, cladosporiosis, powdery mildew; protection of cucumbers from root rots, ascochyta-leaf spot, false mildew, powdery mildew; protection of cabbage from black rot and bacterial soft rot, blackspot.

It was established that treatment of tomatoes with Bactogen during vegetation resulted in reduction in affection by cladosporiosis and powdery mildew by 9.7 – 15.1 %, and by bacteriosis and grey rot by 27 and 53.4 %, respectively. Moreover, Bactogen application on experimental grounds allowed preservation and increasing of yield, the volume of gathered products was by 20 % greater than that of blank plots, and reached 7.6 kg/m^2 .

Bactogen application also allowed reducing the level of affection of plants by cladosporiosis (appearance of greenish brown deposit, browning and withering of leaves), grey rot (appearance of brown necrotic spots, sliming and decaying of leaves), powdery mildew (appearance of brown spots and grey deposit of leaves and raised brown spots on tomatoes) and bacteriosis (withering and necrosis of leaves).

Results of experiments on cucumbers showed that in case of consecutive treatment of plants with Bactogen, the level of affection of plants by powdery mildew and false mildew was reduced by 13-27 %, and by ascochyta-leaf spot and root rot by 44 and 52 %, respectively. At the same time, the increase in cucumber yield from 6.4 to 7.9 kg/m^2, i.e. by 23.4 %, was observed.

Zhytsen

Upon ploughing of plant residues as fertilizers and their further decomposition, almost all essential fertilizer elements are supplied to soil. When ploughing 1 ton of sunflower straw at standard humidity (16 %), 6 kg of nitrogen, 2.5 kg of phosphor, 29 kg of potassium which are the source of mineral nutrition for plants will be produced in average. (Seraya, 2012).

However, in order to optimize the period of producing organic fertilizers from stubble remains, it is required to increase the factor of plant residues humification (Kuznetsov *et al*, 2006).

On the other hand, when getting into soil during harvesting, affected plant residues are the main sources of phytopathogens accumulation, and further infestation of the future plants. In recent years, progressing deterioration of phytosanitary condition of crops, accumulation of a wide range of pathogenic microorganisms, *Fusarium*, *Ophiobolus*, *Gibellina*, *Rhizoctonia*, *Phomopsis*, *Verticillium*, *Rhizopus*, *Pythium*, *Alternaria*, *Cercosporella*, etc., takes place.

In order to address the existing problems more efficiently, we developed a complex microbiological preparation, Zhytsen, intended for acceleration of stubble remains decomposition in the fields, improvement of soil and increase of yield of the future crops. The preparation consists of the mixture of live cell cultures of natural cellulolytic strains *Pseudomonas* sp.-11 and *Bacillus* sp.-49.

The effectiveness of the preparation has been proved during field tests on sod-podzol sabulous soil. It was established that application of the complex microbial preparation Zhytsen in the amount of 3 l/ha allowed reducing of the number of phytopathogenic strains in soil samples from 5 (titre 2.0×10^{6} CFU/g of soil) to 1 (titre $3{,}50\times10^{3}$ CFU/g of soil). (Maslak *et al*, 2014).

Analysis of data received during the dummy field experiment conducted in order to study the effect of Zhytsen on sunflower stubble remains showed that in 7 months after starting of the experiment on sod-podzol sabulous soil, 65 % of sunflower straw decayed as compared to 55% in the blank (Table 3).

Table 3 – Effect of Microbiological Fertilizer Zhytsen on Decomposition of Sunflower Straw and Stubble

Variant	Decomposition, % to initial weighted portion		Increase in mineralization level for December-April, %
	For October-November	For October-April	
Sunflower straw	45	55	10
Straw + $N_{carbamide}$ (standard)	57	68	11
Straw + Zhytsen, 3 l/ha	48	65	18
Sunflower stubble	41	50	9
Stubble + Zhytsen, 3 l/ha	42	58	16

In variant with applying the compensating dose of mineral nitrogen (standard), the degree of straw decomposition was about 68 % which was comparable to the similar rate for sunflower by-products treated with microbial preparation. It was established that during the same period, the degree of sunflower stubble decomposition was 50 %. Treatment of the stubble with Zhytsen (3 l/ha) ensured the increase of decomposition rate up to 58 %.

During the field experiment, the effect of the complex microbial preparation Zhytsen on decomposition of sunflower plant residues was assessed indirectly through the yield of the future crops (barley). Crop yield upon cultivation of barley against the background of ploughing sunflower stubble remains (blank) amounted to 31.7 dt/ha with dry matter yield 27.3 dt/ha (Tables 4 and 5).

Table 4 – Effect of Treatment of Sunflower Stubble Remains with Microbiological Fertilizer Zhytsen on Barley Yield, dt/ha

Variant	Yield	Extra yield to the blank
Sunflower stubble remains (blank)	31.7	–
Stubble remains + Zhytsen, 3 l/ha	38.4	6.7
Sunflower stubble remains + N_{48} in autumn (standard)	32.8	1.1

Application of Zhytsen (3 l/ha) had a positive effect on formation of barley yield with considerable addition of grain at the level of 6.7 dt/ha (21 %) as compared to the variant where stubble remains were ploughed in without treatment. Extra yield in the experimental variant, as compared to the standard, amounted to 5.6 dt/ha (17 %).

Application of Zhytsen allowed achievement of better results in grain quality during the experiment. The higher figures were received upon production of crude protein, feed (FU) and feed-protein units (FPU), 356.7 kg/ha, 42.7 and 46.5 dt/ha, respectively. At the same time, additional 75.9 kg/ha of crude protein, 8.1 dt/ha of feed units and 8.2 dt/ha feed-protein units were produced.

Table 5 – Effect of Treatment of Sunflower Stubble Remains with Microbiological Fertilizer Zhytsen on Quality Indicators of Ataman Barley Grain

Variant	Crude protein, on dry basis	Crude protein, kg/ha	FPU	FU	Dry matter
			dt/ha		
Sunflower stubble remains (blank)	10.3	280.8	34.5	38.4	27.3
Sunflower stubble remains + N_{48} in autumn (standard)	11.2	315.1	37.0	39.6	28.2
Stubble remains + Zhytsen, 3 l/ha	10.8	356.7	42.7	46.5	33.0

Nemacid

Nemacid derived from *P. putida* U is intended for suppression of gall eelworms of tomatoes and cucumbers under cover.

The results of experiments showed high biological and economic effectiveness of biological nematocide Nemacid. The consecutive application of the preparation reduces the level of plant affection by meloidogynosis: by 45.4 % for tomatoes, by 54.8 % for cucumbers. This allows to increase the yield of tomatoes by 43.8 % and cucumbers by 41.5 % (Tables 6-7).

Table 6 - Effectiveness of Nemacid in Respect of Gall Eelworms for Cucumbers Grown under Cover

Experiment variant	Meloidogynosis development, %	Biological effectiveness, %	Yield, kg/m^2
Nemacid	41.9	53.8	5.4
Blank	90.6	-	3.5

Table 7 – Effectiveness of Nemacid in Respect of Gall Eelworms for Tomatoes Grown under Cover

Experiment variant	Meloidogynosis development, %	Biological effectiveness, %	Yield, kg/m^2
Nemacid	35.0	50.4	6.9
Blank	70.6	-	4.8

The studies showed that the preparation did not affect the plant growth and development, at the same time the improvement of resistance in treated plants both to pathogenesis of gall eelworms and other causative agents of diseases existing in soil (root rots, vascular disease and fusarium blight) was observed. Experimental plants were ahead of blank samples by biometric (stem height) and reproductive (period of blossoming of the first truss, number of blooms and set of seed or fruit) indicators. The fading of plants on hot and sunny days was observed in blank variant. It was caused by intensive affection of root system by meloidogynosis.

Application of the preparation inhibited the meloidogynosis development during the entire period of plant vegetation. This was confirmed by absence of water and nutrients deficit signs in hot, sunny days. It should be noted that smaller fruits were formed and ripened in the blank, the main portion thereof was out-of-standard. Application of nematocide allowed considerable increase in the standard products yield.

Stimul

Stimul derived from rhizobacteria *Pseudomonas fluorescens* S-32 is intended for promotion of plant growth and development.

Registration tests of Stimul were conducted with linen flax, Blakit variety. During testing, it was established that height of haulm stand and plant population in the variant with Stimul application were at the level of the blank. According to the results of assessment of yield structure shown in Tables 8 and 9, application of Stimul promotes improvement of indicator of flax fiber quality of linen flax by 8 % which results in increase of yield of long fiber. Indicator of flax fiber quality means ratio of technical length of stem to its diameter (describes the technological value of straw). Upon analysis of measurements in the variant with Stimul as compared to the variant without treatment with the preparation, the reliable extra yield of flax straw (+16.5 %) and seeds (+59.7 %) was observed.

Table 8 – Effect of Stimul Application on Morphological Characters and Yield Structure of Linen Flax, Blakit variety (field experience)

Variant	Technical length of stem, cm	Stem thickness, mm	Indicator of flax fiber quality	Number of capsules, pcs/plant
Without preparation	86.2	1.5	574	5.6
Stimul	80.6	1.3	620	6.3

Table 9 - Effect of Stimul Application on Biological Yield of Linen Flax, Blakit variety (field experience)

Variant	Weight of 1000 seeds, g	Yield, dt/ha		Extra yield, %	
		flax straw	seeds	flax straw	seeds
Without preparation	4.4	53.8	5.7	-	-
Stimul	5.2	62.7	9.1	16.5	59.7

Analysis of yield structure gives evidence that double spraying of linen flax with Stimul with the rate of application of 3 l/ha has improved the quality of seeds: weight of 1000 seed increased by 18.2 % as compared to the variant without application of the preparation and by 10.6 % as compared to the standard.

Conclusions

It was established that Aurin efficiently inhibits causative agents of grey, white and root rot of cucumber upon cultivation of plants in soil, and on mineral wool using the method of small-volume hidrophonics. The developed method of Aurin application ensures high biological effectiveness in respect of prevention of diseases development, extra yield up to 26.6 %, and production of environmentally friendly products.

Thus, it was established that Bactogen not only have antagonistic effect against a number of diseases of tomatoes (bacteriosis, grey rot, powdery mildew, cladosporiosis) and cucumbers (root rot, ascochyta-leaf spot, powdery mildew, false mildew) but also reduce the risk of development of such diseases. Consecutive Bactogen application ensured increase in plant height, number of leaves, ovaries and fruits, and increase of yield of ecologically pure vegetables by 20-23 %.

Results of Zhytsen studies give evidence concerning the possibility of replacement of applying compensating dose of mineral nitrogen along ploughed stubble remains with treatment of the same with microbial preparation Zhytsen with the rate of application of 3 l/ha in order to activate the rates of decomposition of by-products and get extra yield of the future crops.

High biological and economic effectiveness of Nemacid was demonstrated. For cucumbers it amounted to 54.8 % and 41.5 %, and for tomatoes it was equivalent to 32.6 % and 32.6 %, respectively.

Treatment of linen flax with Stimul ensured the production of 8.9 dt/ha of high quality flax straw and 3.4 dt/ha of seeds as compared to the variant without treatment.

The Research Laboratory of Molecular Genetics and Biotechnology of the Belarusian State University developed the range of biopreparations intended for improvement of efficiency of environmentally friendly farming. Application of preparations allows to protect crops from diseases and pests, ensure growth promotion and increase in their yield, and enable restoration of soil fertility.

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