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PHYTOPROTECTIVE AND DISINFECTIVE PROPERTIES OF BIOPREPARATION ENATIN

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Abstract

Phytoprotective, growth-stimulating and disinfecting activity of biopreparation Enatin, based on sporulating bacteria *Bacillus pumilus* BIM B-263, was investigated. Biological efficiency of Enatin in control of bacterial pathogens of plants and animals, resulting in steady decline of pathogenic microbial population and sanitary-representative bacteria of pigsties was demonstrated.

Key words: *Bacillus pumilus,* pathogens, control, barley, cucumber, bacteria

Introduction

Elaboration of biological methods for plant protection and ecologically safe technologies for farm decontamination is of great interest both from environmental and economic viewpoint. To decrease pesticide application for suppression of plant and animal pathogens in contemporary agriculture, highly ecologically safe products of vegetable and animal origin are needed. That is why a demand for efficient biological control agents increases steadily.

Currently aerobic sporulating bacteria of *Bacillus* genus appear most promising biological control agents due to inherent antagonism towards various plant and animal pathogens and high adaptability to adverse environmental conditions (Krebs et al. 1998, Leifert et al. 1995, Sherstoboeva and Sherstoboev 2002, Wulff et al. 2002, Nikitenko 1991, Tarabukina 2000, Tarabukina and Neustoev 2002, Zubov and Yagudin 1995). That is why they are being applied in several commercial biopreparations with marked antifungal activity: Epic, Kodiak, Serenade (USA), HiStick N/T (Britain), Bactophyt, Phytosporin (Russia), Bactogen, Fruitin, Phytoprotectin (Belarus) (Antipyeva 2002, Maximova et al. 2000, Romanovskaya et al. 2003, Kolomiets et al. 2002). Yet, the reports on bactericidal biological prod-

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ucts used in comprehensive systems of plant and animal protection are scarce, which triggers further research in this area.

The aim of the study was the analysis of biological efficiency of Enatin (*Bacillus pumilus* BIM B-263) against bacterial pathogens of plants and animals.

Materials and methods

A strain *Bacillus pumilus* BIM B-263, isolated by the authors from phylloplane of grapevine cv. 'Michurinskij', characterized by high antimicrobial activity against bacterial plant and animal pathogens, was chosen for the study.

Bacteria of genera *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Escherichia*, *Staphylococcus* and *Streptococcus*, originally isolated or provided by several microbial collections (Institute of Microbiology, National Academy of Sciences, Belarus; Institute of Microbiology and Virology, National Academy of Sciences, Ukraine; Institute of Plant Protection, National Academy of Sciences, Belarus; Institute of Stock Breeding, National Academy of Sciences, Belarus) served as test cultures to evaluate antagonistic potential of the examined *B. pumilus* BIM B-263 strain.

Antagonistic activity of the tested culture was estimated with wells (Voznyakovskaya 1969) and replica (Tirranen 1989) techniques.

For analysis of the bacterial antagonist phytotoxicity, development rate of seedlings grown from untreated seeds or seeds treated with the studied strains was compared. Seeds were treated for 20 h with undiluted and diluted (10, 50, 100 times) culture liquid. Afterwards the seeds were washed with sterile water, placed into sterile Petri plates with wet filter paper (10–15 seeds per each, four replications) and incubated at 24–25°C for four days. The results were expressed as seed germination rate and dry weight of seedlings (dried at 60–70°C).

Phytoprotective activity of the bacterial antagonist was tested in laboratory experiments on germination of seeds treated with the antagonist and phytopathogens, on filter paper in Petri dishes, and on plates with sterile or non-sterile soil as follows:

– germinated cucumber 'Polyanskij' cv. and barley 'Gonar' cv. seeds were treated for 3 min with cell suspension of pathogens *Pseudomonas syringae* pv. *lachrymans* 121 (titer 7×10^8) and *P. syringae* pv. *atrofaciens* 239 (titer 1.7×10^7), transferred into Petri dishes and kept for 1 h at room temperature. Then the seeds were dipped for 3 min in culture liquid of the antagonistic bacteria diluted 10, 50, 100 times, and incubated for four days at 24–25°C. Water, Meynell nutrient medium (Meynell and Meynell 1967) diluted 50 times and pathogen cell suspensions were used for seed steeping in control variants. The results were expressed as root and stem weight of tested and control plants;

– one-day-old culture liquid of *B. pumilus* BIM B-263 was introduced into sterile soil (70 g per Petri dish) in proportion of 1%, 2% or 4% of substrate weight (initial titer 5.7×10^8 and 5.7×10^7, respectively) and adaptation of bacteria was monitored. Soil humidity was maintained at 10–15%. Plant-protecting potential of the intro-

duced cultures was analysed in variants with soil pre-inoculated with bacteria *P. syringae* pv. *atrofaciens* 239. The bacteria inoculum was grown in beef-extract broth in flasks on shaker for two days, the culture liquid was centrifuged, biomass was washed, resuspended in sterile water to the initial volume and introduced into the soil (ratio 8.4% of soil weight). Number of antagonistic bacteria and pathogenic bacteria was recorded after 10, 20 and 30 days by plating on Meat Peptone Agar (MPA) media. Number of seeds was 10–15 per each plate (with four replications).

Field trials on biological efficiency of Enatin against pathogens responsible for bacterial canker of fruits were performed earlier on young fruit trees (Romanovskaya et al. 2002, 2003) in which stem lesions were treated with 10% suspension of Enatin in early spring or late autumn.

Efficiency of Enatin, as disinfecting agent, was evaluated *via* its impact on infectious background of farm air in the presence or absence of animals, in cooperation with the Institute of Stock Breeding, National Academy of Sciences, Belarus at Borisovsky pigsty complex, Minsk region in sections of growing piglets.

Test sections (with young stock and after raising the nurslings to the age of 106 days) were exposed to Enatin containing 2.4×10^7 spores per 1 ml by spray technique. Air samples were taken prior to spraying and 2, 7 and 14 days after treatment. Sections with piglets and empty after growing up, not treated with Enatin, served as control.

The level of air microbial contamination in pigsties was estimated by total number of bacteria, differentiated into escherichia and staphylococcal-streptococcal groups, using Koch sedimentation method (Kremlyova 2002).

Statistical processing of the experimental data envisaged calculation of arithmetic means and their standard deviations for probability rate 95% with MS Excel 2003 (Dmitriev 1995). The least significant difference (LSD) was determined with dispersion analysis.

Results

The bacterial strain *Bacillus pumilus* BIM B-263 had a broad anti-microbial spectrum and antagonistic activity towards plant pathogenic bacteria (*Erwinia* spp., *Pseudomonas* spp., *Xanthomonas* spp.) and some animal pathogens (*Escherichia* spp., *Staphylococcus* spp., *Streptococcus* spp.).

Seed treatment with bacterial culture liquid diluted 10, 50, 100 times did not cause phytotoxic effect on seedlings but, on the contrary, stimulated seed germination. Germination rate of cucumber seeds increased by 8–18% and 6–15%, and barley seeds – by 14–20% and 11–17% with respect to water or Meynell medium control, respectively.

Results of laboratory experiments on phytotoxicity of *B. pumilus* BIM B-263 to cucumber and barley seedlings and on its growth-promoting and plant-protective activity are presented in Figures 1 and 2. *Bacillus pumilus* BIM B-263 culture liquid tested on stems (A) and roots (B) of cucumber and barley seedlings did not cause

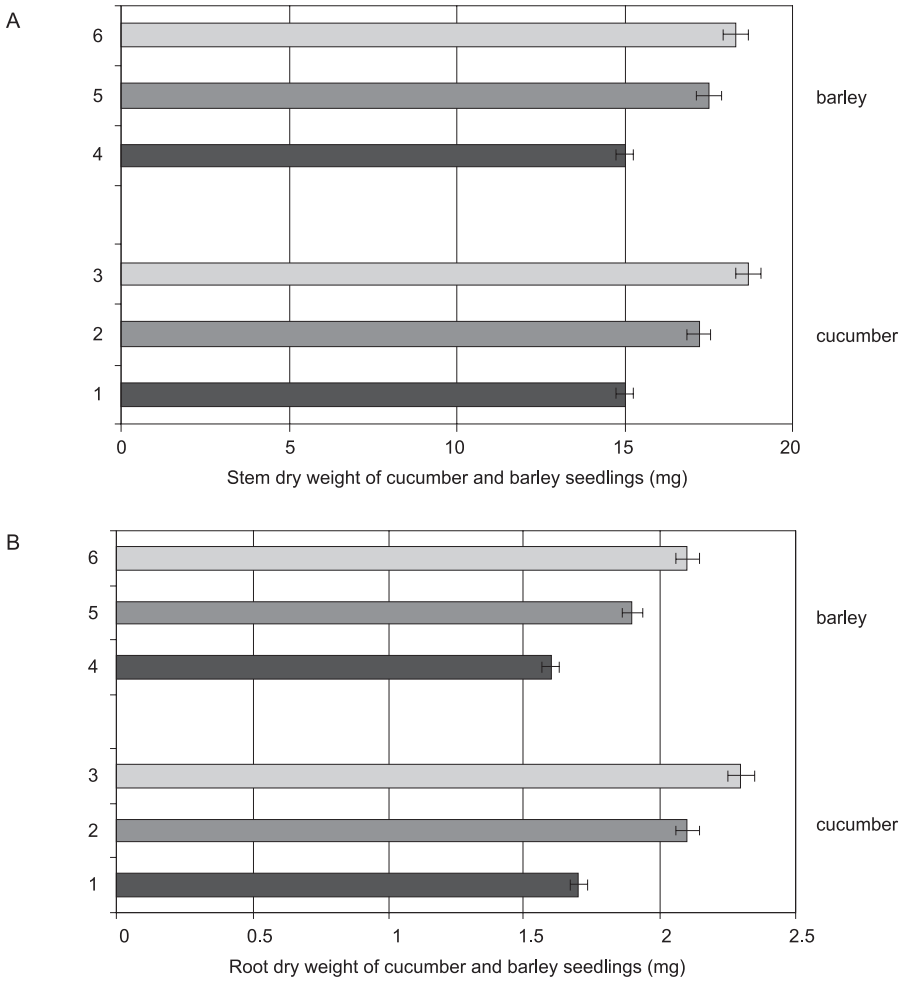

Fig. 1. Effect of *Bacillus pumilus* BIM B-263 culture liquid on development of stems (A) and roots (B) of cucumber and barley seedlings; 1, 4 – seeds were steeped in water, 2, 5, 3, 6 – in culture liquid of *B. pumilus* BIM B-263 diluted 10- and 50-fold, respectively

phytotoxic effect, but, on the contrary, promoted the growth of seedlings (average weight of a seedling increased; Fig. 1). Treating cucumber and barley seeds with culture liquid of *B. pumilus* BIM B-263 resulted in significantly higher root and stem dry weight of seedlings (Fig. 2).

The results of experiments on adaptation of *B. pumilus* BIM B-263 and on plant-protective and growth-stimulating effect of biopreparation Enatin (carried out in model systems «soil-antagonist» or «soil-antagonist-pathogen» with barley seedlings, to trace interactions of antagonistic bacteria with phytopathogens in the course of 30 days) are presented in Figures 3 and 4 and in Table 1.

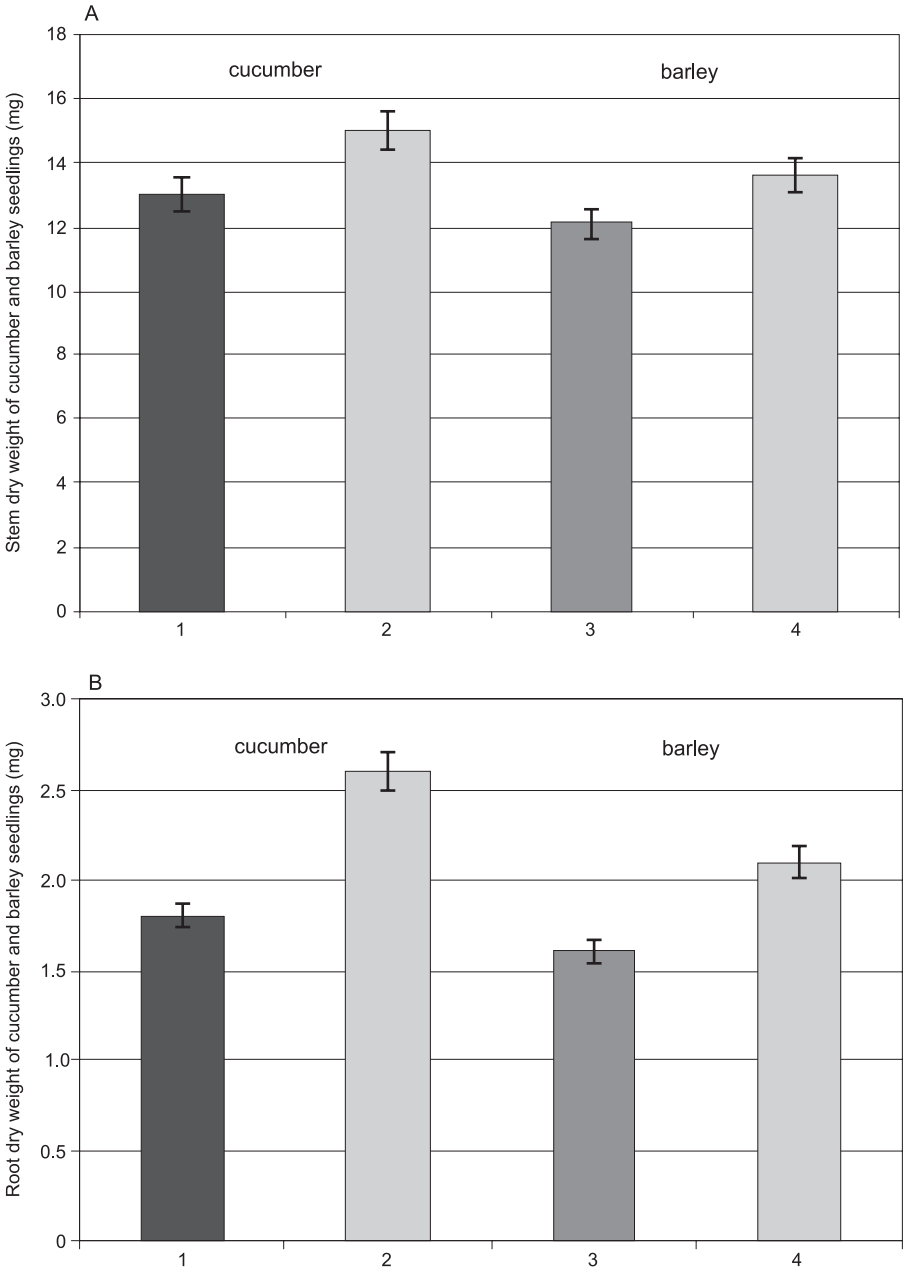

Fig. 2. Phytoprotective effect of *Bacillus pumilus* BIM B-263 culture liquid on development of stems (A) and roots (B) of cucumber and barley seedlings; 1, 2 – cucumber seeds infected with cell suspension of *Pseudomonas syringae* pv. *lachrymans* 121, 3, 4 – barley seeds infected with cell suspension of *P. syringae* pv. *atrofaciens* 239, 2, 4 – cucumber and barley seeds, respectively, treated with culture liquid of *B. pumilus* BIM B-263 diluted 50 times

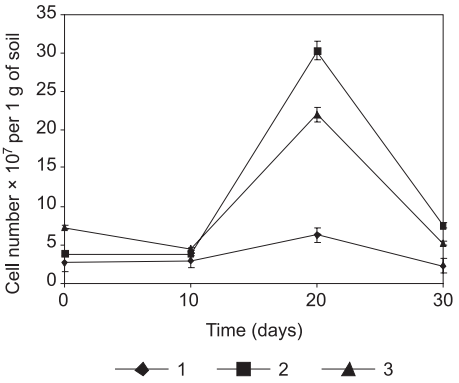

Fig. 3. Population dynamics of *Bacillus pumilus* BIM B-263 in a model ecosystem «soil-antagonist» at initial concentration of culture liquid of antagonistic bacteria 1% (1), 2% (2) and 4% (3) of soil weight

Analysis of population density in the model system «soil-antagonist» demonstrated relatively high level of the antagonistic bacteria (5×10^7–7×10^7 cells per 1 g of soil) throughout the entire observation time (30 days), proving good adaptation of the tested strain (Fig. 3).

The number of pathogenic bacteria *P. syringae* pv. *atrofaciens* 239 tended to decline in all variants with tested concentrations of the *B. pumilus* strain (1, 2, 4% of soil weight; Fig. 4 A). The initial titer of *P. syringae* pv. *atrofaciens* 239 was 1.7×10^7 and ranged from 3×10^7 after 10 days to 2.1×10^7 after 30 days in sterile soil, whereas in the presence of the antagonist the titer decreased by the end of monitoring period to 5×10^6 and 3×10^6 at culture liquid concentration of antagonist 1%, 2% and 4%, respectively, that is four–seven times below the control values. The antagonist titer in model system «soil-antagonist-pathogen» was increasing during the first 20 days. This was followed by minor titer decrease in variants with the antagonist concentrations 1% and 4% and with stabilization at the level of 4×10^7 cells per 1 g of soil at 2% antagonist concentration by 30 days (Fig. 4 B). The titer of *B. pumilus* BIM B-263 exceeded that of *P. syringae* pv. *atrofaciens* 239 in the course of observation period, indicating high competitive ability of the antagonistic strain. The decline of infection load caused by the antagonist culture promoted development of barley seedlings (Table 1). Average

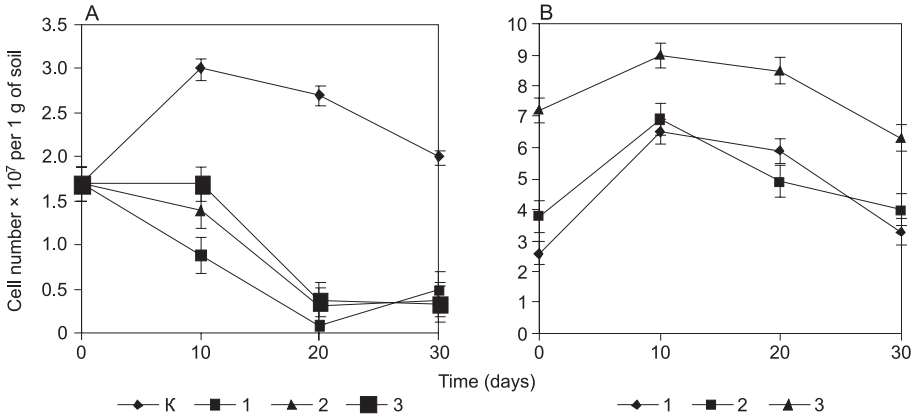

Fig. 4. Population dynamics of *Pseudomonas syringae* pv. *atrofaciens* 239 (A) and *Bacillus pumilus* BIM B-263 (B) in a model system «soil-antagonist-pathogen» with supply of culture liquid of *B. pumilus* at concentration 1% (1), 2% (2) and 4% (3) of soil weight. K – the pathogen population dynamics without the antagonist

Table 1

Effect of biopreparation Enatin based on strain *Bacillus pumilus* BIM B-263 on development of barley seedlings in model systems

Variant	Concentration of culture liquid (% of soil weight)	Weight of seedlings (calculated per 1 plant)		
		mg	% of control	
			K_1	K_2
Soil (K_1)	–	12.9	100.0	105.7
Soil + *P. syringae* pv. *atrofaciens* 239 (K_2)	–	12.2	94.6	100.0
Soil + *B. pumilus* BIM B-263*	1	13.0	100.8	
	2	14.0	108.5	
	4	14.7	114.0	
$LSD_{0.05}$		0.2	0.7	
Soil + *B. pumilus* BIM B-263 + *P. syringae* pv. *atrofaciens* 239**	1	13.1	101.6	107.4
	2	13.4	103.9	109.8
	4	15.1	117.1	123.8
$LSD_{0.05}$		0.3	1.2	2.5

*Soil was used as the control (K_1).
**Soil + pathogen served as the control (K_2).

weight of seedlings in soil inoculated with *P. syringae* pv. *atrofaciens* 239 increased by 17–24% at 2–4% supply ratio of the antagonist culture liquid.

The results of trials on antagonistic activity of *B. pumilus* BIM B-263 against total and sanitary-indicative bacteria of pigsty complex are summed up in Table 2.

The degree of growth suppression of pathogen test cultures by the antagonist reached 92–98% (the replica method). Tests on antagonistic activity of *B. pumilus* BIM B-263 by wells method resulted in growth inhibition zone of 26.0–31.5 mm in diameter.

Results of microbial air contamination analyses in piglet sections, before and after disinfecting with Enatin and caustic soda treatment, are presented in Tables 3 and 4, respectively.

Application of Enatin resulted in reduction of air contamination in piglet section by 100–87% for escherichia bacteria, by 84–68% for bacteria of staphylococcal-streptococcal group, by 78–58% for total bacteria (two and seven days after

Table 2

Antagonistic activity of *Bacillus pumilus* BIM B-263 towards total and sanitary-indicative bacteria

Test culture	Diameter of test culture growth inhibition zone (wells method) (mm)	Degree of test culture growth suppression (replica method) (%)
Escherichia coli	26.0±0.5	95±0.5
Staphylococcus sp.	28.5±0.3	98±0.7
Total bacteria	31.5±0.6	92±0.3

Table 3

Growth of total and sanitary-indicative bacteria upon Enatin treatment

	Total bacteria			Escherichia group			Staphylococcal-streptococcal group		
	N (cfu/plate)	M (th/m^3)	treatment effect (%)	N (cfu/plate)	M (th/m^3)	treatment effect (%)	N (cfu/plate)	M (th/m^3)	treatment effect (%)
Before treatment	942±9.5	120		36.8±1.7	4.69		65.2±1.9	8.3	
After treatment									
2 days	207.2±4.7	26.4	78	–	–	100	10.5±0.7	1.34	84
7 days	393.3±4.2	50.1	58	0.9±0.2	0.12	87.5	21.2±1.2	2.7	68
14 days	934.2±8.0	119	–	6.0±0.8	0.75	84	130.0±1.9	16.6	–

M – microbial titer (th/m^3), $M = \frac{100 \cdot N}{S} \cdot 100$

N – arithmetic mean value (cfu/plate),
cfu – colony forming units,
S – area of Petri plate.

Table 4

Growth of total and sanitary-indicative bacteria after caustic soda treatment

	Total bacteria			Escherichia group			Staphylococcal-streptococcal group		
	N (cfu/plate)	M (th/m^3)	treatment effect (%)	N (cfu/plate)	M (th/m^3)	treatment effect (%)	N (cfu/plate)	M (th/m^3)	treatment effect (%)
Before treatment	865±11.1	110.2		47.4±1.7	6.04		53.6±1.5	6.8	
After treatment									
2 days	562.2±16.2	71.6	45	21.3±0.9	2.7	55	31.6±1.4	4.02	41
7 days	778.5±10.9	99.2	10	44.1±1.9	5.6	7	50.9±1.4	6.5	5
14 days	971.3±6.4	123.7	–	65.8±1.6	8.4	–	87.2±2.3	11.1	–

M – microbial titer (th/m^3), $M = \frac{100 \cdot N}{S} \cdot 100$

N – arithmetic mean value (cfu/plate),
cfu – colony forming units,
S – area of Petri plate.

treatment, respectively). Growth of escherichia bacteria was not detected even 14 days after Enatin treatment, in contrast to caustic soda treatment requiring a repeated disinfection procedure after two–four days.

30-day-old piglets were raised up to 100 days age in sty section disinfected with Enatin. Growth and development of animals ranged within physiological limits. Health complications were not found in young stock of the tested age group.

Discussion

Sporulating bacteria of genus *Bacillus* find wide application in control of phytopathogenic microorganisms (Krebs et al. 1998, Leifert et al. 1995, Sherstoboeva and Sherstoboev 2002, Wulff et al. 2002) and animal pathogens (Nikitenko 1991, Tarabukina 2000, Tarabukina and Neustoev 2002, Zubov and Yagudin 1995). Technology of producing biopreparation Enatin (with a broad spectrum of antibacterial action) was elaborated, basing on studies on physiological-biochemical properties, antimicrobial activity, effect of cultural conditions on antimicrobial potential of the examined culture *B. pumilus* BIM B-263.

The increase of germination rate after treatment with bacterial culture liquid (cucumber seeds by 8–18% and 6–15%, and barley seeds – by 14–20% and 11–17% with respect to water or Meynell medium control, respectively) was superior to the results achieved in Russia by Semynina (2005), where barley seed germination rate increased only by 5.4–7.8% under the impact of biopreparations and growths-promoters.

Vitamins, amino acids, growth regulators produced by biocontrol agents influence plant development, stimulate seed germination and increase yield (Maximova et al. 2000). Growth-promoting effect of bacteria *B. pumilus* BIM B-263 was noted on cucumber and barley seedlings, whose stems and root system mass increased by 13–24% and 25–35%, respectively, when treated with the antagonist culture liquid diluted 10–50 times. The results obtained are similar to data of other authors (Maximova et al. 2000).

Bacillus pumilus BIM B-263 culture liquid diluted 50 times displayed also considerable plant-protecting effect on cucumber and barley seeds, due to active growth suppression of phytopathogenic bacteria *P. syringae* pv. *lachrymans* 121 and *P. syringae* pv. *atrofaciens* 239 (an increase of seedling root and stem biomass by 13–15% and 32–47%, respectively).

Investigation of bacterial antagonists in laboratory experiments, vital for understanding various biological aspects, can not explain their behaviour in soil *in vivo*. Testing the promising strains in model ecosystems to evaluate their adaptation capacity and impact on make-up of soil microbial cenosis and on crop development is an essential research stage (Dyatlova 2001).

The results of laboratory experiments are supported by results of field trials. Biological efficiency of Enatin against the pathogen responsible for bacterial canker of fruits – *P. syringae* pv. *syringae*, was 54.3% (Romanovskaya et al. 2002).

Biological efficiency of Enatin towards canker pathogens of fruit cultivars (54.3%) matches that of recognized biopreparations Fruitin (Belarus), Integral (Russia), and exceeds some of them (Pentaphag, Belarus), as well as chemical preparations (Beileton), at least two-times (Romanovskaya et al. 2002, Grigortsevich et al. 2002, Romanovskaya et al. 2003).

One of the ways to reduce density of microbial infestation in sty complexes and to prevent spread of infectious animal diseases is to launch a series of veterinary-sanitary measures. A key point in this strategy is effective air disinfection in stock farms (Ibragimova 2000).

Chemical decontamination methods based on disinfecting chemicals are often applied for sanitation of various objects and sites. Principal disadvantage of the methods is lack of ecological safety since they engage chemical agents hazardous to humans, animals and microorganisms, which are decomposed slowly in the environment. Long-term use of chemical disinfectants generates problem of microbial resistance and can lead to partial or total loss of their efficiency. It appears therefore that the efforts should be focused on biological agents characterized by ecological safety, non-toxicity to animals, effective preventive action aimed at elimination of infectious agents in farm stock, particularly of escherichia and staphylococcal-streptococcal bacteria (Ibragimova 2000).

Enatin antimicrobial activity towards sanitary-indicative bacteria – escherichia, staphylococci, streptococci and total bacteria, investigated at piglet section of Borisovsky sty complex, proved superior to bacteriophag preparation (Ibragimova 2000) and chemical decontaminating agents (caustic soda, combined surface disinfectant) currently used at stock farms.

It seems that the new ecologically safe biopreparation Enatin, developed on the basis of sporulating bacteria *B. pumilus* BIM B-263, showing phytoprotective and disinfecting potential, has good prospects for application as microbial disinfectant to control bacterial pathogens of plants and to prevent infectious diseases of young farm animals.

Streszczenie

OCHRONNE WOBEC ROŚLIN ORAZ DEZYNFEKCYJNE WŁAŚCIWOŚCI BIOPREPARATU ENATIN

Przebadano antybakteryjne działanie biopreparatu Enatin, opartego na szczepie zarodnikującej bakterii *Bacillus pumilus* BIM B-263, w odniesieniu do patogenów roślin i zwierząt. Enatin wykazał biologiczną aktywność przeciwko patogenowi jęczmienia *Pseudomonas syringae* pv. *atrofaciens*, przeciwko sprawcy kanciastej plamistości ogórka *P. syringae* pv. *lachrymans* oraz przeciwko sprawcy raka bakteryjnego drzew owocowych *P. syringae* pv. *syringae*. Okazał się także skuteczny w ograniczaniu ogólnej liczby bakterii oraz bakterii wskaźnikowych dla stanu sanitarnego chlewni (bakterie *Escherichia* oraz grupa *Staphylococcus-Streptococcus*).

Literature

Antipyeva N.I., 2002: Novel microbial product showing antifungal activity. Grain Storage Process. 3: 25–29.

Dmitriev E.A., 1995: Matematicheskaya statistika v pochvovedenii. MGU, Moskva.

Dyatlova K.D., 2001: Microbial products in plant breeding. Sorosovskiy Obrazovat. Zh. 5: 17–22.

Grigortsevich L.N., Kolomiets E.I., Molchan O.V., 2002: Antagonistic action of *Bacillus* and *Streptomyces* strains against fruit crop pathogens. Bull. Pol. Acad. Sci. 50, Biol. Sci. 2: 95–97.

Ibragimova D.M., 2000: Razrabotka rezhimov aehrozol'noy sanacii vozdukha dlya profilaktiki infekcionnykh bolezney norok v promyshlennom zverovodstve. Vserossiyskiy nauchno-issledovatel'skiy veterinarnyy institut, Kazan'.

Kolomiets E.I., Popov F.A., Romanovskaya T.V., Chikileva A.E., Baeva S.V., Lobanok A.G., 2002: Rhizospheral strain *Bacillus subtilis* 12A and its influence on vegetable pathogens. Bull. Pol. Acad. Sci. 50, Biol. Sci. 2: 99–103.

Krebs B., Hoding B., Kubart S., 1998: Use of *Bacillus subtilis* as biological control agent. J. Plant Dis. Prot. 105: 181–197.

Kremlyova E.P., 2002: Laboratornyy praktikum po kursu «Ehkologiya». GGU, Grodno.

Leifert C., Chidburee H., Hampson S., 1995: Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. J. Appl. Bacteriol. 78, 2: 97–108.

Maximova N.P., Lysak V.V., Komarova M.S., Baeva I.A., Sadovskaya L.E., Maslak D.V., 2000: Prospects of applying microbial biopreparations for biological control in plant cultivars. In: Microbiology and biotechnology on the eve of XXI century. Proceedings of the International Conference, Minsk, Belarus, June 1–2, 2000. Ed. A.G. Lobanok. Nova-Print Press, Minsk: 183–184.

Meynell D., Meynell E., 1967: Experimental microbiology. Mir, Moscow.

Nikitenko V.N., 1991: Bacteria as drug substitutes. Nauka v SSSR 4: 117–121.

Romanovskaya T.V., Kolomiets E.I., Zdor N.A., Lobanok A.G., 2002: Biopreparation "Enatin" displaying a broad range of antimicrobial action. Prikl. Biokhim. Microbiol. 38, 6: 669–676.

Romanovskaya T.V., Molchan O.V., Kolomiets E.I., Supranovich R.V., 2003: Biological activity of biocide «Fruitin» in the control of some plant pathogens. Bull. Pol. Acad. Sci. 51, Biol. Sci. 3: 187–190.

Semynina T.V., 2005: Prospects of applying biological preparations, immune- and growth-stimulators for comprehensive protection of grain crop seeds from phytopathogens complex. In: Phytosanitary health of agroecosystems. Proceedings of the second Russian congress on plant protection, Saint-Petersburg, Russia, December 5–10, 2005. Ed. V.A. Pavlushin. Pavel Press, Saint-Petersburg: 191–193.

Sherstoboeva E.V., Sherstoboev N.K., 2002: An alternative to chemical fungicides. Grain Storage Process. 3: 16–19.

Tarabukina N.P., 2000: Nauchnoe obosnovanie i razrabotka sistemy veterinarno-sanitarnykh meropriyatiy v zhivotnovodstve Kraynego Severa. Vserossiyskiy nauchno-issledovatel'skiy institut veterinarnoy sanitarii, gigieny i ehkologii, Moskva.

Tarabukina N.P., Neustoev M.P., 2002: Methods of optimizing microbial cenoses in stock farms of northern regions. Nauka Obrazov. 1: 102–104.

Tirranen L.S., 1989: Role of volatile metabolites in microbial interactions. Nauka, Novosibirsk.

Voznyakovskaya Yu.M., 1969: Plant microflora and harvest. Kolos, Leningrad.

Wulff E.G., Mguni C.M., Mansfeld-Giese K., Fels J., Lubeck M., Hockenhull J., 2002: Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Bacillus pumilus* isolated with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. Plant Pathol. 51, 5: 574–584.

Zubov N.D., Yagudin R.G., 1995: Mikrobnaya i pylevaya zagryaznennost' vozdukha korovnika. Veterinariya [Moskva] 3: 27–29.

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