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THE EFFECT OF ENDOPHYTIC BACTERIA *Bacillus thuringiensis* W65 AND *B. amyloliquefaciens* P20 ON THE YIELD AND THE INCIDENCE OF POTATO RHIZOCTONIOSIS AND LATE BLIGHT

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Abstract

Chemical fungicides are chemicals used to combat late blight and potato rhizoctoniosis. However, due to repeated treatments, the resistance of plant pathogens to fungicides increases. Biofungicides serve as an alternative to chemical fungicides. The use of strains of endophytic bacteria of the genus *Bacillus* is promising for the development of novel biofungicides. Endophytes, being inside plants, have an advantage in interactions with the plant compared to bacteria occupying other ecological niches. In this work, for the first time, the effectiveness of experimental samples of preparations based on strains of endophytic bacteria of the genus *Bacillus* was established when growing potato (*Solanum tuberosum* L.) varieties differing in resistance to late blight in the conditions of the North-West of the Russian Federation. It is known that the effectiveness of the use of endophytes with biocontrol activity differed in the field when growing potatoes, while the varietal responsiveness of potatoes to biocontrol agents has not been sufficiently studied. The aim of the research was to study the effect of experimental samples of preparations of endophytic bacteria *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 on the yield and infection of potato plants with rhizoctoniosis and late blight. Strains of endophytic bacteria *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 isolated from potatoes had antagonistic activity to phytopathogens-pathogens of late blight *Phytophthora infestans* (Mont. de Bary) and rhizoctoniosis *Rhizoctonia solani* (Kuhn.) when growing on agar media. Small-scale field experiments (2020-2021) were conducted at the experimental field of the Leningrad Research Agriculture Institute Belogorka. The experiment scheme included the following options: clean control — no treatments; chemical control — treatment with chemical fungicides: CELEST® Top, SC (Syngenta, Russia), Mankoceb, WP (AgroRus and Co., Russia), Rapid Duo, WP (AgroRus and Co., Russia), Infinito, SC (Bayer Crop Science, Germany), Buzzer, SC (CROPEX, Russia) and desiccant Golden Ring (Agro Expert group, Russia); biological control — BisolbiSan (BISOLBI INTER, Russia), the biofungicide based on rhizospheric bacteria *Bacillus subtilis* Ch-13; experimental sample of the preparation *B. thuringiensis* W65, experimental sample of the preparation *B. amyloliquefaciens* P2. Two potato varieties, Charoit (resistant to late blight) and Gusar (susceptible to late blight), were used. In the experiments, the dynamics of plant growth and development, yield and infection of potato plants with rhizoctoniosis and late blight were evaluated. Statistical treatment of the obtained results (calculations of averages and their standard errors, ANOVA analysis of variance, Duncan's test, was carried out using the Statistica 10 program («StatSoft, Inc.», USA). When inoculated with experimental samples of *B. amyloliquefaciens* P20 and *B. thuringiensis* W65, the duration of flowering of potato plants increased by 8-13 days compared to the control. The potato tuber harvest also increased by 7.9-14.6 % ($p < 0.05$). The largest increase in yield was registered on the Gusar variety in 2020. It was found that responses of potato varieties to inoculation with experimental samples of *B. amyloliquefaciens* P20 and *B. thuringiensis* W65 preparations differed. The yield of potato tubers of the Charoit variety mainly increased due to an increase in the average weight of one tuber while the yield of the Gusar variety increased due to an increase in the number of tubers per plant. When inoculated with experimental samples of *B. amyloliquefaciens* P20 and *B. thuringiensis* W65, the crop structure changed, the yield of the large tuber fraction increased by 22.5-30.6 % ($p < 0.05$) in Charoit variety. The use of experimental samples of *B. amyloliquefaciens* P20 and *B. thuringiensis* W65 did not have a significant effect on the development of rhizoctoniosis in small-scale experiments. The *B. amyloliquefaciens* P20-based preparation showed 42.8 % biological efficacy in reducing the development of late blight on the potato variety Charoit. Preparation of endophytic bacteria based on *B. amyloliquefaciens* P20 can be recommended for further testing in commercials field trials when growing potatoes in an integrated protection system together with chemical fungicides and inducers of systemic plant resistance.

Keywords: endophytic bacteria, *Bacillus thuringiensis* W65, Bacillus amyloliquefaciens P20, biofungicides, tuber harvest, potatoes, rhizoctoniosis, late blight

Late blight and rhizoctonia are the most common and aggressive mycoses among diseases of potato (*Solanum tuberosum* L.), which can lead to loss of 30-50% yield and impares the realization of the potential productivity of the crop [1]. Oomycete Phytophthora infestans (Mont.) de Bary, the causative agent of late blight with asexual reproduction, spreads due to the formation of sporangia which germinate and form motile zoospores at low air temperatures from +4 to +15 °C, then encyst and their growth tubes penetrate into the plant tissue. At elevated temperatures (20-25 °C), zoospores are not formed. The sexual process is possible only if there are two types of mating in the population. As a result of mating, oospores appear, which, after overwintering, germinate into growth tubes. Sporangia are formed at the end of the growth tubes [2]. The main source of infection is diseased tubers and contaminated plant debris [3]. During infection, the pathogen synthesizes protein effector molecules (apoplastic and cytoplasmic) which affect the structure and function of the plant cell [4-7]. EPIC1 is one of the best characterized apoplastic effectors that targets host defense-associated proteases [7-9]. Oomycete cytoplasmic effectors include the RXLR class, containing the conserved Arg-any amino acid-Leu-Arg (RXLR) peptide motif that is required for delivery of these proteins to plant cells [10]. Plant genetic resistance to *P. infestans* is regulated through recognition of specific RXLR effectors by host NB-LRR resistance proteins within plant cells [10].

Over the past decades, there has been an increase in the aggressiveness of the late blight pathogen *P. infestans* [11, 12]. It is associated with recombination of virulence genes during sexual reproduction and mating leading to the emergence of highly aggressive races of the pathogen [13, 14]. The aggressiveness of *P. infestans* may be due to the predominance of the aggressive clonal line 13_A2 in the population [12].

The gembiotrophic fungus *Rhizoctonia solani* Kьhn, a teleomorph, or sexual stage, of *Thanatephorus cucumeris* (A.B. Frank) Donk is the causative agent of potato rhizoctonia blight. The fungus attacks tubers, stems, stolons and roots of adult plants. The damage is visiblr due to the formation of brown spots and ulcerations on sprouts, stolons and roots of potato plants. Black sclerotia appeare on the tubers. The teleomorph appears as a dirty white felt coating on the lower part of the stems at high humidity and an optimal temperature of 15-21 °C. Sclerotia can remain dormant for many years in soil and dead plant debris [15, 16]. Effectors described for *R. solani* induce necrosis in many plants species [17, 18]. The effector RsRplA is also known which acts as an active protease inhibitor and suppresses the induction of plant hypersensitivity reactions [19].

Currently, the chemical method of protecting potatoes from mycoses is the most effective [20, 21]. Resistance of phytopathogens to fungicides increases due to repeated treatments [22-24]. The development of resistance of pathogens to fungicides is also influenced by the reproduction of the pathogen, the degree of protection by the fungicide, the mechanism of action on the pathogen, and the dynamics of the pathogen population [25]. After the widespread introduction into practice of selective systemic drugs, the frequency of detection of resistant races of pathogens affecting various crops, including potatoes, has increased [26-29]. Resistance to the highly effective systemic fungicide mefenoxam (the R-enantiomer of metaloxyl) in *P. infestans* was observed when previously susceptible isolates were exposed to sublethal doses (5 mg/ml) of the fungicide [27]. Mefenoxam-resistant *P. infestans* isolates showed slow growth compared to susceptible isolates [30].

ABC transporters and detoxifying enzymes (cytochrome P450) are involved in the formation of *P. infestans* resistance to mefenoxam/metalaxyl [31, 32]. Changes occur in the plasmalemma of *P. infestans* that prevent the poison from entering the cells [33]. Thus, when metaloxyl was used in minimal concentrations (0.1 and 1 μg/ml), the growth inhibition of *P. infestan*s from the Priluki population in Belarus exceeded 50.0%. With an increase in the concentration to 10 μg/ml the sensitivity of the pathogen increased to 75.2%, and when using a high concentration (1000 μg/ml), mycelial growth was completely suppressed [34].

As an alternative to chemical fungicides, the widespread introduction of new biological fungicides has been proposed [23, 35]. With the combined use of chemical fungicides and biofungicides, pathogen resistance is reduced and plant immunity is stimulated [23, 36]. In addition, to protect against mycoses in agricultural practice, it is necessary to introduce new potato varieties with resistance to pathogens [14, 37].

Bacteria from the genera *Pseudomonas*, *Bacillus*, *Lysobacter*, *Enterobacter* and *Paenibacillus* are the most effective in suppressing the development of mycoses [38]. The antagonistic activity of bacteria of the genus *Bacillus* against mycoses of various crops has been studied [39-42]. Bacilli are capable of producing metabolites with fungicidal activity [43-45]. Thus, the combination of fengycin B and surfactin, produced by *B. pumilus*, was more effective in protecting potatoes against late blight than each metabolite applied separately [46].

Recombinant endophytic *B. subtilis* 26DCryChS with the *Btcry1Ia* gene encoding Cry1Ia had complex fungicidal and insecticidal effects [47, 48].

The special literature discusses the prospects of using endophytic bacteria of the species B. thuringiensis, which have a complex effect, for plant protection [49-51]. Chitinase-producing *B. thuringiensis* strains can effectively inhibit the pathogenic fungi *Fusarium oxysporum*, *F. graminearum*, *Pyricularia grisea*, and *Physalospora piricola* [49-51]. Chitinases can also enhance the insecticidal activity of *B. thuringiensis* [52].

Endophytic bacteria are promising objects in the biosecurity because they are located inside the host plant and directly interact with it [53-55]. Endophytes are able to reduce the number of phytopathogens due to competition for the ecological niche and the synthesis of biologically active substances (BAS) [56]. In pot and field trials, the effect of endophyte bacteria on the late blight [57-59] and rhizoctonia [60, 61] infection of potato was studied. The effectiveness of these bacteria has been shown to vary under field conditions, with synthetic chemical fungicides being more effective [59].

The antagonistic activity of four *B. subtilis* strains MTCC-2422 (T-3), KU936344 (T-4), KU936345 (T-5) and KU936341 (T-6) against late blight was studied in field experiments when growing potatoes of the Kafri Jyoti variety. The fungicide mancozeb M 45 (CURZATE®) was a positive control [59]. Treatment with bacteria significantly reduced the incidence of late blight compared to control, but their effectiveness was significantly lower than that of a chemical fungicide. The intensity of disease development was 65% with mancozeb and more than 74% with bacteria [59]. The reduction in the incidence of potato rhizoctoniosis when using the culture filtrate of *B. subtilis* HussainT-AMU was 71% in pot tests and 50% in field conditions, which indicates the high biocontrol activity of this strain against *R. solani* [60]. Treatment of potato tubers with phytosporin (an experimental *Bacillus subtilis* 26-based drug; BashInkom, Russia) in the Kamchatka Territory did not effectively reduce the rhizoctonia disease, the degree of the disease development before harvest decreased by 7.5%, the prevalence of the disease by 8.6%, the yield increased by 4.7 t/ha [61]. However, when tubers were co-treated with the fungicide TMTD (JSC Firm August, Russia) at 1.7 l/t and sprayed with sporobacterin, the development and prevalence of the disease decreased by 11.6 and 48.5%, respectively [61].

Treatment of potato plants with fluopimomide at a minimum dose of 85 g/ha in combination with *B. velezensis* SDTB038 significantly reduced the incidence of late blight and increased yield. Its average efficiency was 69% for 2 years, being comparable to the effect of the maximum dose (170 g/ha) of the fungicide (68.6% average efficiency for 2 years). The *B. velezensis* strain SDTB038 allows fungicide concentrations to be reduced in the field [57]. Currently, the study of new strains of endophyte bacteria to protect potatoes in field conditions is relevant.

In this work, we for the first time established the effectiveness of using experimental samples of preparations based on strains of endophytic bacteria *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 in the conditions of the northwest of the Russian Federation when growing potato varieties that differ in resistance to late blight.

The purpose of the work was to evaluate the effect of experimental samples of preparations based on endophytic bacteria *Bacillus thuringiensis* W65 and *B. amyloliquefaciens* P20 on potato yields and the susceptibility of plants to rhizoctonia and late blight.

Materials and methods. Endophytic *Bacillus* strains were isolated from the internal tissues of potato (*Solanum tuberosum* L.) variety Sudarynya resistant to potato cancer. The bacteria were identified as *B. thuringiensis* (strain W65) and *B. amyloliquefaciens* (strain P20) by the16S rRNA gene sequencing. The nucleotide sequences were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov) under the numbers OP537151 and OP537150, respectively. Strains *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 were also deposited in the Network Bioresource Collection for Genetic Agriculture Technologies (the All-Russian Research Institute of Agriculture).

For endophyte isolation, potato tubers were washed with tap water, sterilized for 5 min in 70% ethanol and 10 min in 15% H_2O_2. After sterilization, they were successively washed in sterile water five times for 2 min. The last water (40 microliters) was surface sown on potato dextrose agar (PDA, Difco, USA), incubated for 2 days at 28 °C, and in the absence of bacterial growth, sterile tubers were used to isolate endophytes. PDA contained (per liter) 4 g of freeze-dried potato decoction obtained from 200 g of potatoes, 20 g of dextrose, 20 g agar, pH 5.6.

Under sterile conditions, the tubers were cut with a scalpel, the internal tissues were cut out and placed in a mortar, 5 ml of distilled water was added, and the sample was ground with pestle until a homogeneous state. Then the tuber tissue homogenates were diluted to 10^{-5}. The lasr dilution was plated on Petri dishes with PDA agar in 3-fold repetition. Petri dishes were placed in a thermostat for 5 days at 28 °C, and indicidual colonies of bacteria were seeded into test tubes with PDA.

Experimental samples of endophytic bacterial preparations based on strains *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 (500×10^6 CFU/ml) were produced using a universal RALF bioreactor (Bioengineering, Switzerland). As a standard, we used the biofungicide BisolbiSan, L (liquid) (Bisolbi-Inter LLC, Russia), registered in the Russian Federation to combat rhizoctonia, late blight and Alternaria potato (State catalog of pesticides and agrochemicals approved for use in the Russian Federation, 2022). Biofungicide BisolbiSan, L is produced on the basis of a strain of rhizosphere bacteria *B. subtilis* Ch-13 (500×10^6 CFU/ml)

A nutrient medium of the following composition was used (in g/dm^3 of distilled water): molasses 25, corn extract 12.5, $MgSO_4 \cdot 7H_2O$ 0.2, $CaCl_2$ 1.0, $MnSO_4$ 0.01.

The counts of bacteria in experimental samples was determined by the limiting dilution method [62] followed by culture on bovine meat fermented (BMF) agar (BMF base 15.0 g, sodium chloride 9.0 g, microbiological agar 13.5 g, NICF, Russia). The fungicidal activity was assessed by the well method on potato dextrose agar PDA [63]. Suspensions of conidia of the studied strains of phytopathogenic fungi (5 ml, 1×10^6 conidia/ml) were added to 250 ml of warm (45 °C) potato dextrose agar. The medium was mixed and poured into Petri dishes. After hardening, wells 8 mm in diameter and a depth of the entire thickness of the agar were made in each dish using a cork drill (4 pieces per dish). The tested bacterial strains were grown stationary in potato dextrose broth PDB (Difco, USA) for 5 days at 28 °C. Then, 100 μl of the bacterial suspension was added to the prepared wells in triplicate. The antifungal activity was assessed by the zone of growth inhibition around the wells after 3-5 days of incubation of inoculated Petri dishes at 28 °C.

To study the growth-stimulating activity, sterile seeds of watercress (*Lepidium sativum* L., 1753) Dukat variety were soaked in the suspensions of bacterial cells with a titer of 5×10^5 CFU/ml for 30 min. Then 30 seeds were placed in sterile Petri dishes on damp filter paper. In the control treatment, the seeds were soaked in a sterile saline solution. The seedlings were grown for 5 days at 28 °C. The experiments were arranged in triplicate.

In field small-plot experiments, new varieties of table potatoes Charoit and Gusar approved for the North-West Russia, differing in resistance to late blight, were grown.

Charoit (originated by the North-Western Research and Production Association for Breeding and Plant Growing Belogorka, Selection Firm Liga LLC, Branch of the Russian Agricultural Center in the Novgorod Province) is an early ripening variety, moderately resistant to late blight, common scab and rhizoctonia. Seed productivity is 20-30 t/ha, commercial prosuctivity 40-60 t/ha. The period from full germination to harvest is 50-60 days.

Gusar (originated by the LLC Selection Firm Liga) is a mid-season variety, moderately susceptible to late blight, moderately resistant to common scab. Productivity for seeds is 25-30 t/ha, commercial productivity is 40-60 t/ha. The growing season from complete germination to harvesting is 75-80 days.

Small-plot experiments were performed at the Leningrad Research Institute of Agriculture Belogorka (Belogorka village, Leningrad Province, Gatchina District). The soil is soddy-podzolic light loamy, medium cultivated, agrochemical parameters of the arable layer: mobile phosphorus P_2O_5 20.2 mg/100 g, exchangeable K_2O 7.7 mg/100 g of soil, organic matter 2.6%, pHsol. 5.1. The granulometric composition of the soil was optimal for cultivating potato plants. The predecessor in field crop rotation was winter rye.

Mineral fertilizers azofoska (NPK 16:16:16) were applied before cutting ridges at the rate of 500 kg/ha (80 kg/ha a.i.). Potatoes were planted on May 8, 2020 and 2021 with two inter-row treatments, the pre-emergence (June 12, 2020 and June 10, 2021) and post-emergence (June 28, 2020 and June 25, 2021) hilling. Before harvesting, chemical desiccation was carried out with Golden Ring (2.0 l/ha, Agro Expert Group, Russia). Spraying of vegetative plants was carried out using a battery-powered backpack sprayer Solo (SOLO Kleinmotoren GmbH, Germany), the flow rate of the working fluid was 500 l/ha.

The test design was randomized with 3-fold repetition. The area of each registration plot was 11.2 m^2, 2.8 m width (4 ridges), 3 m length. The yield was harvested on September 15 in 2020 and September 10 in 2021. The development of diseases was determined in 2021. When assessing the incidence of rhizoctoniosis, the count was carried out on stems and stolons on 24 bushes in each experimental treatment, when assessing the spread of late blight on 45 bushes in each experimental treatment. The degree of development of rhizoctoniosis and late blight was determined according to the guidelines for registration testing of fungicides [64].

The development of late blight was assessed by an 8-point scale with the following gradations: 0 — no signs of damage; 1 — less than 2.5% of the leaf surface affected; 2 — 2.5-5% of leaves affected; 3 — 6-10% of leaves affected; 4 — 11-15% of leaves affected; 5 — almost every leaf affected, 16-25% leaf drying; 6 — 26-50% drying of leaves, beginning of damage to stems; 7 — 51-75% drying of leaves, damage to stems progresses; 8 — the plant died.

The development of rhizoctonia on stolons was assessed on a 3-point scale: 0 — no signs of damage; 1 — up to $^1/_3$ of the stolons are affected; 2 — $^1/_3$-$^2/_3$ stolons are affected; 3 — more than $^2/_3$ of the stolons are affected. On sprouts and stems, a 4-point scale was used to assess the development of rhizoctonia: 0 — no signs of damage; 1 — spots (ulcers) on sprouts or stems are single, superficial, distributed no more than $^1/_4$ of the length of the sprout and the underground part of the stem; 2 — ulcers cover the entire circumference, up to half the sprout and the underground part of the stem; 3 — deep ulcers, covering the entire circumference and more than half of the sprout and the underground part of the stem, the stems have partially withered, the leaves have curled and turned yellow; 4 — complete rotting of the sprout, the lower part of the stem and roots, death of the plant.

Biological effectiveness was calculated by the formula:

$$BE = (C - b)/C \times 100\%,$$

where BE is the biological effectiveness of the drug, C is the intensity of disease in the control (point), b is the intensity of disease in the test (point).

Statistical processing of the obtained results was carried out using the Statistica 10 program (StatSoft, Inc., USA). Arithmetic means (*M*) and standard errors of the means (±SEM) were calculated, as well as the least significant difference (a value indicating the limit of random deviations in the experiment at a significance level of 95%). To assess the statistical significance of differences between the average experimental variants, Duncan's test was used.

Results. Before small-plot field tests, we assessed the growth-stimulating and fungicidal activity of experimental samples of endophytic bacterial strains *B. thuringiensis* W65 and *B. amyloliquefaciens* P20. As shown by the lab tests, the strains had both growth-stimulating (increase in the root length of the test pant vs. control by 12.3 and 18.9%, respectively) ($p < 0.05$) and fungicidal activity (Table 1, Fig. 1).

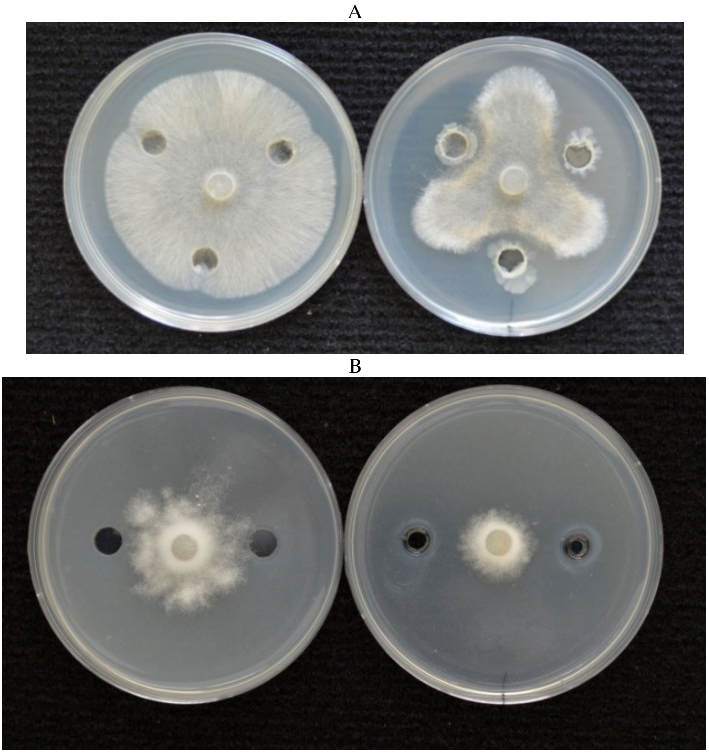

Fig. 1. Fungicidal activity of the *Bacillu amyloliquefaciens* P20 against the micromycetes *Rhizoctonia solani* Kühn (A) **and *Phytophtora infestans* (Mont.) de Bary** (B)**:** on the left — control (sterile water), on the right — bacterial suspension of B. amyloliquefaciens P20.

1. Growth-stimulating and fungicidal activity of experimental preparations of the genus *Bacillus* strains of endophytic bacteria isolated from the tissues of potatoes (*Solanum tuberosum* L.) variety Sudarynya (*M*±SEM)

Treatment	Root length increase, % from cotrol ($n = 100$)	Diameter of fungal growth inhibition zones, mm ($n = 3$)			
		Phytophtora infestans (Mont.) de Bary	*Rhizoctonia solani* Kühn	*Fusarium solani* (Mart.) Sacc.	*Fusarium coeruleum* (Lib. ex Sacc.)
B. amyloliquefaciens P20	18.9±1.3	17.5±1.0	25.1±1.9	19.9±1.5	21.5±1.7
B. thuringiensis W65	12.3±0.9	13.5±0.3	18.7±0.5	8.7±0.7	13.1±1.1

Note. Growth-stimulating activity was assessed using watercress (*Lepidium sativum* L., 1753) cv. Dukat.

Thus, samples of endophytic bacteria *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 were selected for field small-plot experiments.

Main meteorological indicators of the growing season 2020 and 2021. according to the United Hydrometeorological Station Belogorka are presented in Figure 2. The scheme of field small-plot experiments with two potato varieties is presented in Table 2.

The 2021 season was characterized by severe soil drought because of which the seedlings were very uneven with sections without growth and those stunted in growth. From the beginning of June to the end of the first ten days of July, no precipitation occurred. Under these conditions, the Gusar variety showed resistance to drought, the yield in the control was 27% higher compared to 2020

(Table 3). The yield of the Charoit decreased by 4.7%. In 2021, due to dry conditions during the potato flowering period, flowering of both varieties was poor with massive fall of buds in the Gusar variety. Precipitation in August 2021 led to an increase in the aboveground mass of plants and increased branching in both potato varieties. The trend of increasing flowering duration with the use of microbiological preparations, noted in 2020, continued in 2021.

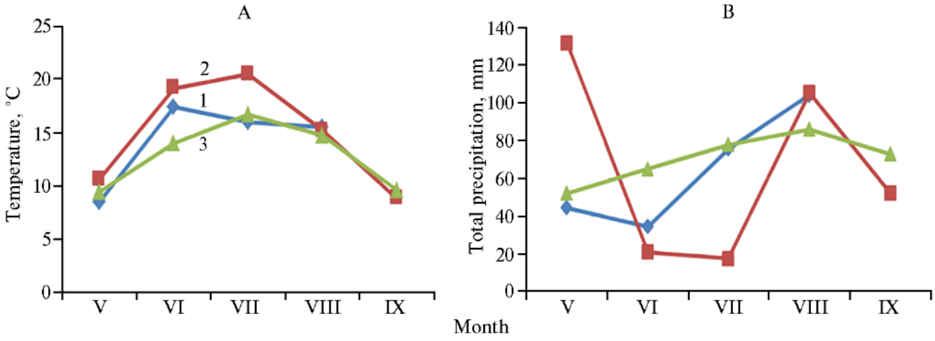

Fig. 2. Average air temperature (A) **and total precipitation** (B) **by month in the years of observation:** 1 — 2020, 2 — 2021, 3 — average long-term data for 1990-2020 (the United Hydrometeorological Station OGMS Belogorka, village Belogorka, Leningrad Perovince, Gatchina District).

2. Scheme of application of chemical and microbiological plant protection products in small-plot field trials on potato (*Solanum tuberosum* L.) varieties Charoit and Gusar (experimental field, the Leningrad Research Institute of Agriculture Belogorka, village Belogorka. Leningrad Province, Gatchina District, 2020-2021)

Treatment	Drugs	Rate	Date
Pure control	Treatment of tubers and spraying with water		
Chemical control	CELEST® Top, SC (Syngenta, Russia)	0.4 l/t	May 28
	Buzzer, SC (CROPEX LLC, Russia)	0.3 l/ha	July 05
	Infinito, SC (Bayer Crop Science, Germany)	1.2 l/ha	July 13
	Rapid Duo, WP (AgroRus and Co., Russia)	2.0 kg/ra	July 22
	Infinito, SC (Bayer Crop Science, Germany)	1.4 l/ha	August 02
	Mankoceb, WP (AgroRus and Co., Russia)	1.2 kg/ra	August 10
	Golden Ring, WS (Agro Expert Group, Russia) + Buzzer, SC (CROPEX LLC, Russia)	2.0 + 0.3 l/ha	August 19
Biological standard	BisolbiSan, L (Bisolbi-Inter LLC, Russia)	4.0 l/t	May 28
		10 l/ha	July 01. July 05. 13 July. 22 July. 02 August. 10 August
	Golden Ring, WS (Agro Expert Group. Russia) + Buzzer, SC (CROPEX LLC, Russia)	2.0 + 0.3 l/ha	August 19
B. thuringiensis W65	An experimental drug	–	–
Bacillus sp. X20	An experimental drug	–	–

Note. Dashes mean that the application scheme is similar to that for the biological standard.

3. Plant biometric parameters of potato (*Solanum tuberosum* L.) varieties in small-plot field trials with chemical fungicides and experimental preparations of endophytic *Bacillus* bacteria isolated from potato cv. Sudarynya ($n = 45$. $M \pm$SEM; experimental field, the Leningrad Research Institute of Agriculture Belogorka, village Belogorka. Leningrad Province, Gatchina District, 2020-2021)

Treatment	Plant height, cm		Average tuber weight, g		Tuber number per plant		Productivity, g per plant	
	2020	2021	2020	2021	2020	2021	2020	2021
Charoit (resistant variety)								
Pure control	51.2±4.8^{a}	50.1±4.6^{a}	79.8±5.4^{ab}	70.0±4.7^{b}	8.1±0.4^{b}	8.8±0.4^{b}	647±28^{b}	617±32^{c}
Chemical control	54.3±4.6^{a}	52.4±4.8^{a}	78.6±5.2^{b}	83.7±6.2^{a}	9.0±0.5^{a}	8.8±0.4^{b}	708±36^{ab}	732±51^{a}
BisolbiSan L	56.1±7.0^{a}	53.6±4.4^{a}	85.5±5.8^{a}	79.5±5.4^{a}	9.2±0.7^{a}	9.2±0.4^{ab}	787±54^{a}	727±48^{a}
B. amyloliquefaciens P20	58.4±6.6^{a}	53.7±4.6^{a}	79.1±5.0^{a}	75.6±5.0^{ab}	8.6±0.4^{ab}	9.0±0.3^{ab}	625±30^{c}	681±34^{b}
B. thuringiensis W65	55.2±5.2^{a}	51.1±4.0^{a}	85.5±5.4^{a}	70.3±4.0^{b}	7.9±0.3^{b}	9.8±0.6^{a}	736±50^{a}	686±36^{b}
LSD05	7.3	4.8	3.9	4.3	0.8	0.6	32.3	40.6

Continued Table 3

				Gusar (susceptible variety)				
Pure control	52.3±4.2^{a}	44.5±4.0^{b}	42.3±3.4^{a}	45.4±3.8^{ab}	12.0±0.7^{b}	14.2±0.9^{b}	508±31^{b}	645±32^{b}
Chemical control	53.3±4.0^{a}	50.9±4.6^{a}	40.6±3.6^{a}	44.5±3.6^{b}	12.2±0.7^{b}	15.5±0.8^{a}	496±28^{b}	689±30^{ab}
BisolbiSan L	56.3±6.8^{a}	49.9±4.4^{a}	40.3±4.2^{a}	49.6±4.0^{a}	13.0±0.8^{a}	14.3±1.0^{b}	524±32^{b}	709±34^{a}
B. amyloliquefaciens P20	56.0±6.2^{a}	48.2±4.2^{a}	40.0±2.8^{a}	45.0±3.8^{ab}	14.1±0.9^{a}	16.1±1.1^{a}	564±30^{ab}	702±40^{a}
B. thuringiensis W65	55.4±4.6^{a}	47.1±4.0^{a}	39.5±3.0^{b}	43.2±2.8^{b}	14.7±0.9^{a}	15.6±0.6^{b}	581±25^{a}	695±36^{a}
LSD$_{05}$	6.4	4.2	2.4	2.8	0.6	0.4	25.4	34.2

$^{a.\ b.\ c}$ Different letters mean that the average values of the indicator for the options in the column are statistically significantly different according to the Duncan's test at $p < 0.05$.

The results of 2-year field small-plot experiments showed that there was no significant increase in plant height upon inoculations with experimental preparations (see Table 3). These data are consistent with the results of other studies [65].

On average, with a drug based on *B. amyloliquefaciens* P20, potato yields in the 2020 growing season decreased by 3% compared to the control, while in 2021 it increased by 10.3% for the late blight-resistant variety Charoit. The yield of the susceptible variety Gusar increased with the use of a *B. amyloliquefaciens* P20 preparation by 11 and 8.8% in 2020 and 2021, respectively. With a *B. thuringiensis* W65 preparation, the potato yield of both studied varieties increased significantly ($p < 0.05$) from 7.7 to 14.4%. The greatest increase in yield with the *B. thuringiensis* W65-based drug was in 2020 and amounted to 13.7% for the Charoit variety and 14.4% for the Gusar variety.

The increase in potato tuber yield of the Charoit variety treated with microbiological preparations was mainly due to an increase in the average tuber weight, of the Gusar variety due to an increase in the number of tubers per plant.

4. Fractions of tubers of potato (*Solanum tuberosum* L.) varieties in small-plot field trials with chemical fungicides and experimental preparations of endophytic *Bacillus* bacteria isolated from potato cv. Sudarynya (*n* = 30. *M*±SEM; experimental field, the Leningrad Research Institute of Agriculture Belogorka, village Belogorka. Leningrad Province, Gatchina District, 2020-2021)

Treatment	Tuber yield by fractions, %							
	< 35 mm		35-45 mm		46-55 mm		> 55 mm	
	2020	2021	2020	2021	2020	2021	2020	2021
				Charoit (resistant variety)				
Pure control	22.2±1.1^{b}	20.5±0.8^{b}	37.0±1.4^{a}	38.6±1.4^{ab}	34.6±1.6^{b}	38.6±1.2^{a}	6.2±0.4^{c}	2.3±0.1^{c}
Chemical control	21.6±0.8^{ab}	14.8±0.4^{d}	35.5±1.2^{ab}	39.8±1.3^{a}	36.7±1.8^{a}	37.5±1.6^{ab}	6.7±0.3^{c}	8.0±0.3^{b}
BisolbiSan L	19.4±0.6^{c}	17.4±0.4^{c}	32.6±0.8^{c}	34.8±1.8^{b}	38.0±2.7^{a}	39.1±1.6^{a}	10.0±0.6^{a}	8.7±0.2^{a}
B. amyloliquefaciens P20	24.1±1.0^{a}	23.5±0.9^{a}	32.9±0.6^{c}	34.7±0.8^{b}	35.4±1.4^{ab}	36.7±1.8^{b}	7.6±0.5^{b}	5.1±0.1^{b}
B. thuringiensis W65	19.8±0.4^{c}	20.0±0.4^{b}	34.9±0.5^{b}	41.1±1.6^{a}	37.2±2.0^{a}	33.3±1.4^{c}	8.1±0.6^{b}	5.6±0.1^{b}
LSD$_{05}$	1.2	0.9	1.1	1.4	1.9	1.8	0.8	0.3
				Gusar (susceptible variety)				
Pure control	41.6±2.7^{b}	29.6±0.8^{b}	43.3±2.2^{a}	45.8±2.7^{ab}	15.0±0.6^{a}	22.6±1.2^{b}	0.08±0.00^{d}	0
Chemical control	46.4±2.8^{a}	36.8±1.8^{a}	38.5±1.4^{ab}	47.2±3.1^{a}	15.0±0.8^{a}	15.5±0.6^{c}	0.13±0.01^{c}	0
BisolbiSan L	44.5±2.4^{ab}	27.3±1.1^{c}	38.5±1.6^{ab}	46.8±3.0^{ab}	15.0±0.4^{a}	25.2±1.4^{a}	0.13±0.01^{c}	0.7±0.0
B. amyloliquefaciens P20	47.3±2.4^{a}	38.5±2.0^{a}	39.8±1.4^{b}	44.8±2.4^{b}	11.1±0.4^{c}	16.7±0.8^{c}	1.80±0.12^{a}	0
B. thuringiensis W65	46.7±3.0^{a}	34.8±0.9^{b}	38.1±1.5^{b}	50.3±3.2^{a}	13.6±0.7^{b}	14.9±0.4^{d}	1.60±0.10^{b}	0
LSD$_{05}$	2.7	1.3	1.5	3.1	0.7	1.3	0.02	0

$^{a.\ b.\ c.\ d}$ Different letters mean that the average values of the indicator for the options in the column are statistically significantly different according to the Duncan's test at $p < 0.05$.

Treatments with chemical fungicides did not have a statistically significant effect on the average tuber weight in both studied varieties, with the exception of the Charoit in 2021. Our results are consistent with the data of other researchers. It is known that endophytic strains can stimulate plant growth due to phytohormones they produced, increasing the availability of nutrients and stress resistance [53, 66]. Bacteria of the genus *Bacillus*, producing various metabolites, exhibit plant protection in arid conditions [67]. The effectiveness of the *B. velezensis* AFB2-2 against potato late blight under greenhouse conditions was 85.7% due to the biosynthesis of bacillomycin D, iturin and surfactin [58]. Another strain, *B. velezensis* SDTB038 provided effective control of potato late blight in greenhouses and fields and promoted the growth of potato plants [57].

When we used experimental *B. amyloliquefaciens* P20 and *B. thuringiensis* W65 preparations, the yield of the tuber fraction larger than 55 mm in size increased in the Charoit potato variety by 22.5-30.6% ($p < 0.05$) (Table 4). The use of the biofungicide BisolbiSan had the same effect on the tuber yield structure of this variety. However, no such changes were noted in the Gusar variety (Table 4).

The maximum yield (t/ha) of potato variety Charoit was harvested in 2020 with the biological standard BisolbiSan (Table 5). In 2020, using experimental samples of preparations *B. amyloliquefaciens* P20 and *B. thuringiensis* W65, significant differences ($p < 0.05$) occurred for the yield of the Gusar variety compared to the chemical control, however, these differences were not revealed in 2021. It should be noted that the increase in yield upon treatment with the experimental samples was comparable or higher than for chemical fungicides (see Table 5).

5. Productivity of potato (*Solanum tuberosum* L.) varieties in small-plot field trials with chemical fungicides and experimental preparations of endophytic *Bacillus* bacteria isolated from potato cv. Sudarynya ($n = 45$, $M \pm SEM$; experimental field, the Leningrad Research Institute of Agriculture Belogorka, village Belogorka. Leningrad Province, Gatchina District, 2020-2021)

Treatment	2020			2021		
	yield, t/ha	Δ, %		yield, t/ha	Δ, %	
		to control	to chemical control		to control	to chemical control
		Charoit (resistant variety)				
Pure control	39.3 ± 1.1^{b}	–	–	35.2 ± 1.7^{c}	–	–
Chemical control	43.0 ± 4.1^{b}	9.4	–	41.7 ± 1.6^{a}	18.4	–
BisolbiSan L	47.8 ± 2.0^{a}	21.6	11.2	41.4 ± 1.4^{a}	17.6	–
B. amyloliquefaciens P20	38.0 ± 1.0^{bc}	–	–	38.8 ± 1.5^{bc}	10.2	–
B. thuringiensis W65	44.7 ± 2.2^{ab}	13.7	4.0	39.1 ± 1.2^{b}	11.1	–
LSD$_{05}$	1.9			1.8		
		Gusar (susceptible variety)				
Pure control	30.8 ± 1.5^{b}	–	–	36.7 ± 0.3^{b}	–	–
Chemical control	30.2 ± 3.0^{b}	–		39.2 ± 1.2^{ab}	6.8	–
BisolbiSan L	31.9 ± 1.5^{ab}	3.5	5.6	40.4 ± 1.7^{a}	10.1	3.1
B. amyloliquefaciens P20	34.3 ± 2.1^{a}	11.4	13.6	40.0 ± 1.4^{a}	9.0	2.1
B. thuringiensis W65	35.3 ± 1.5^{a}	14.6	16.9	39.6 ± 1.5^{a}	7.9	1.0
LSD$_{05}$	1.5			1.7		

Note. Dashes mean that the application scheme is similar to that for the biological standard.

a. b. c Different letters mean that the average values of the indicator for the options in the column are statistically significantly different according to the Duncan's test at $p < 0.05$.

6. Incidence (I) **and severity** (S) **of rhizoctonia and late blight infections of potato (*Solanum tuberosum* L.) plants in small-plot field trials with chemical fungicides and experimental preparations of endophytic *Bacillus* bacteria isolated from potato cv. Sudarynya** (budding stage, the scale of the All-Russian Research Institute of Plant Protection; $M \pm SEM$; experimental field, the Leningrad Research Institute of Agriculture Belogorka, village Belogorka. Leningrad Province, Gatchina District, 2021)

Treatment	Rhizoctonia					Late blight		
	stems		stolones		BE, %	I, %	S, points	BE, %
	I, %	S, points	I. %	S, points				
			Charoit (resistant variety)					
Pure control	100	1.0	100	2.6 ± 0.3^{a}		100	2.1 ± 0.30^{a}	
Chemical control	100	1.0	100	2.5 ± 0.3^{a}	3.8	6.7	0.1 ± 0.01^{c}	95.2
BisolbiSan L	100	1.0	100	2.5 ± 0.3^{a}	3.8	88.9	0.9 ± 0.1^{b}	57.2
B. amyloliquefaciens P20	100	1.0	100	2.3 ± 0.3^{a}	11.5	90.5	1.2 ± 0.07^{b}	42.9
B. thuringiensis W65	100	1.0	100	2.6 ± 0.4^{a}	–	100	2.1 ± 0.2^{a}	–
LSD$_{05}$				0.6			0.8	
			Gusar (susceptible variety)					
Pure control	100	1.0	100	2.9 ± 0.1^{a}		100	7.2 ± 0.2^{a}	
Chemical control	100	1.0	100	2.4 ± 0.2^{b}	17.2	28.9	0.7 ± 0.04^{c}	90.3

Continued Table 6

BisolbiSan L	100	1.0	100	2.6±0.2^{ab}	10.3	100	6.9±0.2^{a}	4.2
B. amyloliquefaciens P20	100	1.0	100	2.5±0.1^{ab}	13.7	98.0	6.2±0.1^{b}	13.8
B. thuringiensis W65	100	1.0	100	2.9±0.3^{a}	–	100	7.1±0.5^{a}	1.4
LSD$_{05}$				0.5			0.8	

Note. BE — biological effectiveness of the drug. The number of plants when assessing the incidence of rhizoctonia in each variant was 24, for the incidence of late blight 45. Dashes mean the absence of a statistically significant increase compared to the control.

$^{a, b, c}$ Different letters mean that the average values of the indicator for the options in the column are statistically significantly different according to the Duncan test at $p < 0.05$

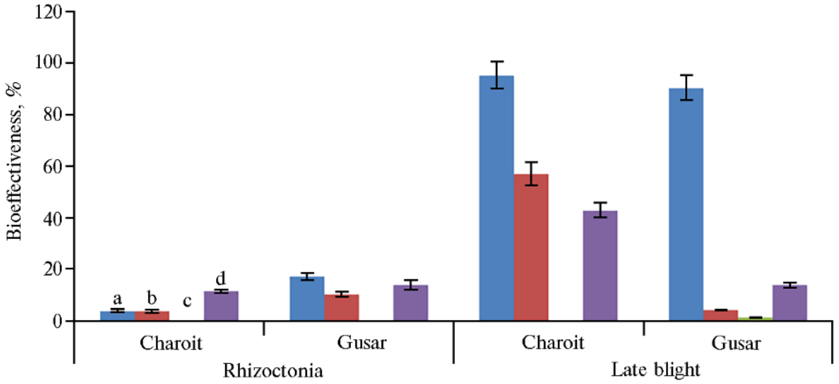

Fig. 3. Biological effectiveness of chemical fungicides and experimental samples of preparations based on strains of endophytic *Bacillus* bacteria isolated from the Sudarynya variety against rhizoctonia and late blight for two potato (*Solanum tuberosum* L.) varieties in small-plot field trials: a — chemical control, b — BisolbiSan, c — *B. thuringiensis* W65, d — *B. amyloliquefaciens* P20 ($n = 24$. *M*±SEM; the Leningrad Research Institute of Agriculture Belogorka, village Belogorka, Leningrad Province, Gatchina District, 2021).

The weather conditions in 2020-2021 were not favourable for the development of rhizoctoniosis. In all treatments the number of affected plants (stems and stolons) during the budding phase for both potato varieties was 100%, however, the lesion was very weak, 1 point on the rhizoctonia development scale (Table 6). Despite the fungicidal activity of the studied preparations based on *B. thuringiensis* W65 and *B. amyloliquefaciens* P20 identified in lab tetst they did not have an effective influence on the development of rhizoctonia in field conditions (Table 6). However, for the Charoit variety, a drug based on the *B. amyloliquefaciens* P20 was more effective against rhizoctonia than chemicals and for the Gusar variety, the effect was comparable (see Table 6, Fig. 3).

Thus, the weak development of rhizoctonia and late blight in the field conditions of 2021 did not allow us to fully evaluate the fungicidal effects. Obviously, the fungicidal effect of *B. thuringiens*is W65 and *B. amyloliquefaciens* P20 preparations must be assessed in model experiments upon artificial infestation under phytotron conditions.

To date, not a single potato variety with immunity to rhizoctonia blight has been found in the Russian Federation [24]. Analysis of special publications showed that the *B. subtilis* SR22 strain exhibited high antagonistic activity against *Rhizoctonia solani* and reduced the development of the disease by 53.8% on tomato plants. Treatment with this strain increased the total phenolic content (by 76.8%) and the activity of the antioxidant enzymes peroxidase (by 56%) and polyphenoloxidase (by 29.2%) in tomato roots [40]. *Bacillus subtilis* HussainT-AMU isolated from potato tubers produced surfactin and reduced the development of potato rhizoctonia blight by 50% in field conditions [60].

An analysis of the incidence of late blight in potato plants showed that chemical fungicides minimized the development and prevalence of the disease.

whereas preparations based on strains *B. amyloliquefacien*s P20 and *B. thuringiensis* W65 had little effect on its spread (Table 6). Under the influence of weather conditions in the second half of August causing soil drought, protection with microbiological preparations was not effective enough. The prevalence of the disease when using the BisolbiSan standard and the *B. amyloliquefaciens* P20 preparation was 89 and 90%, respectively, on the Charoit variety. However, the biologicalscals reduced the late blight development on the Charoit variety by 57.1 for BisolbiSan and 42.9% for *B. amyloliquefaciens* P20 (see Fig. 3, 4). Our data confirm the results of other researchers. The use of a microbiological preparation based on *Bacillus velezensis* SDTB038 made it possible to reduce the incidence of late blight by 40.79 and 37.67% in 2018-2019 [57]. As some researchers believe, progress in the use of endophytes against late blight can be achieved with an integrated protection scheme for joint use of biologicals with small doses of fungicides and inducers of systemic plant resistance [57, 68-70].

Fig. 5. Development of late blight on the potato (*Solanum tuberosum* L.) variety Charoite on the date of phytopathological registration: left — pure control, right — treatment with a *Bacillus amyloliquefaciens* P20 preparation (the Leningrad Research Institute of Agriculture Belogorka, village Belogorka, Leningrad Province, Gatchina District, August 29, 2021).

So, in small-plot field experiments upon inoculation of Charoit and Gusar potato plants with experimental preparations of endophytic bacteria *Bacillus amyloliquefaciens* P20 and *B. thuringiensis* W65, the flowering stage in both varieties was by 8-13 days longer compared to the control and tuber yield was higher by 7.9-14.6%. The yield structure changed. In the Charoit variety, the yield of the maximum-sized fraction increased by 22.5-30.6%. The experimental preparations do not have a significant effect on the development of rhizoctoniasis, and the *B. amyloliquefaciens* P20 (42.8%) reduced the late blight famage to the Charoit variety. The drug based on the *B. amyloliquefaciens* P20 strain can be recommended for further in commercial testing as a biofungicide and the drug based on the *B. thuringiensis* W65 strain as a growth stimulant.

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