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Bacteria of *Bacillus* genus as efficient and safe plant protection agent against pathogenic microorganisms

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Abstract. The study aimed to establish correlation between antagonistic activity of *Bacillus* strains and their effect on plant growth and soil microbial balance. Experiment was based on combination of microbiological, physiological and statistical methods, including agar well method for determining antagonism, serial dilution method for counting colonies, triphenyl tetrazolium chloride method for assessing dehydrogenase activity, and analysis of variance (ANOVA) for result reliability verification. Experiment determined that *B. subtilis* has a higher inhibitory capacity than *B. amyloliquefaciens*, determining that average diameters of inhibition were 10-15% larger, while growth inhibition index was larger by 5-8%. Lesion index in *Fusarium oxysporum* dropped by 33.0 percentage points (p.p.) (from 53.8% to 20.8%), in *Alternaria solani* by 29.0 p.p., and in *Pseudomonas syringae* by 27.1 p.p. Proportion of healthy plants in control group improved from 51.5-56.3% to 86.2-87.3% with *B. subtilis* and 82.4-84.1% with *B. amyloliquefaciens*. Height, number of leaves and root mass improved by 20-40% compared to control, exceeding efficiency of biological standard by 5-10%, therefore demonstrating stimulative effect of biological products on plant growth. Experiment detected no phytotoxicity or soil degradation. Biological preparations ensured stable saprophyte (1.8×10^6 CFU/g), actinomycetes (1.9×10^6 CFU/g) and fungi (1.8×10^5 CFU/g) count, while dehydrogenase activity reached 103% compared to control. Results of experiment confirmed that *Bacillus subtilis* and *Bacillus amyloliquefaciens* are efficient antagonists of phytopathogens and safe biostimulants that can improve productivity and stabilise soil microbiocenosis. They are an efficient, environmentally sustainable alternative to chemical fungicides in sustainable farming systems

Keywords: antagonism; biocontrol activity; damage index; environmental safety; rhizosphere

INTRODUCTION

Research relevance is determined by growing need to identify environmentally safe alternatives to chemical plant protection products in modern agricultural production. Development of agriculture and excessive use of fungicides and pesticides cause soil degradation, loss of microbial diversity and formation of resistant strains of phytopathogens. In this regard, introduction of biological products based on microorganisms capable of not only inhibiting development of pathogens but also stimulating growth of cultivated plants, restoring natural balance of agroecosystems, is

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relevant. Problem addressed in this study is that, despite considerable scientific and practical interest in *Bacillus* bacteria, effect on phytopathogens, physiological state of plants, and biological activity of soil remains insufficiently studied in field conditions. Existing studies frequently address individual aspects of antagonistic activity or growth-stimulating effects, without incorporating seasonal dynamics of changes in rhizosphere. Therefore, analysis of efficiency of *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains in a systemic context – from pathogen suppression to soil microflora preservation – is relevant for development of sustainable biotechnologies in crop production that meet modern environmental safety requirements and principles of "green" agroeconomics.

Generalised experiments by Y. Kolomiiets *et al.* (2024) established that PGPB (Plant Growth-Promoting Rhizobacteria) solutions based on *Bacillus subtilis* reduced intensity of bacterial infections in vegetable crops. Experiment proved that synthesis of phytohormones and antagonistic substances suppressed pathogens and stimulated plant growth. M.V. Reshetnikov & V.P. Patyka (2023) proved that *Bacillus* isolates formed pronounced zones of inhibition around causative agents of bacterial diseases of cereals. Study determined that effect was caused by formation of antibiotic compounds that disrupted integrity of cell membranes of pathogens.

Microbiological observations by O.V. Kolchyk *et al.* (2024) found that *Bacillus* spp. suppressed toxicogenic microorganisms of poultry products. Study confirmed that representatives of this genus exhibited a wide range of antimicrobial activity and stable bioprotective effects. Comparative analysis by A.R. Khan *et al.* (2022) proved that use of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in field conditions increased crop yields by 10-15%. Strains were reported to act as biofertilisers, enhancing phosphate mobilisation and reducing pathogen load. Ecological conclusions of A. Ortiz & E. Sansinenea (2022) state that prolonged use of *Bacillus* spp. increased activity of soil enzymes, in particular dehydrogenases. Results demonstrated that such bacteria maintained microbial diversity and stability of biochemical processes. Biotechnological observations by R.C. Toma *et al.* (2023) established that certain strains of *Bacillus* inhibited development of phytopathogenic and conditionally pathogenic microorganisms. Observations found that their use in biological products increased safety of agricultural products. Agrobiotechnological observations by M.S. Ngalimat *et al.* (2021) stated that bacteria that stimulate plant growth, in particular *Bacillus* spp., effectively suppressed bacterial pathogens of rice, such as Xanthomonas oryzae. Study noted that isolates simultaneously activated systemic resistance and reduced need for chemical fungicides. Substantial field research by N.C. Vasques *et al.* (2024) proved that multifunctional *Bacillus* strains increased soybean and corn yields by 12-18%. Study found that combination of antagonism and nitrogen fixation provided a complex bioeffect.

Phytopathological experiments by E. Dutilloy *et al.* (2022) found that *Bacillus subtilis* formed a biofilm on roots of wheat and barley, inhibiting *Fusarium graminearum* and *Rhizoctonia solani* fungi. Study demonstrated that this biofilm maintained protective activity for more than 60 days. According to results of S. Boulahouat *et al.* (2023), *Bacillus velezensis* and *Bacillus subtilis* inhibited growth of *Fusarium oxysporum* by 85-90%. Study determined that effect persisted even at high humidity and temperature. A global review by M. Ayaz *et al.* (2023) proved that combined use of bacterial and fungal bioagents, in particular *Bacillus* and *Trichoderma*, increased effectiveness of biocontrol. Study emphasised that Bacillus ensured stability of action in field conditions. Microbiological conclusions of A. Muthukumar *et al.* (2022) proved that combination of genera *Pseudomonas* and *Bacillus* is promising for creation of new biological products. It was shown that their interaction activated metabolism of microflora and increased crop productivity.

Issues of long-term stability of antagonistic *Bacillus* strains in field conditions, interaction of metabolites with natural soil microflora, and combined effect on plant physiology in different agroclimatic zones are insufficiently studied. Study aimed to evaluate antagonistic activity of *Bacillus subtilis* and *Bacillus amyloliquefaciens* against phytopathogens and determine effect on growth and soil microflora. The study was to determine inhibitory activity of *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains against phytopathogens, evaluate effect on plants, and investigate changes in structure of soil microflora during vegetation.

MATERIALS AND METHODS

Experiment was conducted under controlled conditions at a temperature of +25 ± 1°C and humidity of 60% using certified equipment with metrological testing. All procedures complied with Convention on Biological Diversity (1992), bioethics and environmental safety standards. Experiment was based on *Bacillus subtilis* and *Bacillus amyloliquefaciens* bacteria due to antagonistic properties against a wide range of phytopathogens. Strains were selected due to ability to synthesise surfactin, iturin and fenzin bioactive lipopeptides, which inhibit *Fusarium oxysporum*, *Alternaria solani*, *and Pseudomonas syringae* and stimulate plant growth and resistance to stress.

Change in inhibitory capacity of Bacillus during cultivation period. Antagonistic activity of bacterial culture filtrates was determined using agar well diffusion method. *Fusarium oxysporum*, *Alternaria solani* and *Pseudomonas syringae*, isolated from infected agricultural crop samples and tested for pathogenicity, were used as test objects. Potato dextrose agar (PDA, Himedia, India) was used to grow fungi, and King B medium (Himedia, India) for bacterial pathogens. Twenty millilitres of agar was poured into 90 mm diameter Petri dishes, and after solidification, a 6 mm diameter well was formed, and 100 µl of *B. subtilis* or *B. amyloliquefaciens* filtrate was added. Filtrates were obtained after 72 hours of cultivation in Luria-Bertani (LB) medium and sterilised through a 0.22 µm membrane filter (Sartorius Minisart, Germany). Incubation was conducted in a Binder BD 56 thermostat (Germany) at 28 ± 1°C for 6, 12, 24, 48 and 72 hours. Diameter of growth inhibition zones (mm) was measured with a Mitutoyo CD-15CPX digital calliper (Japan) with an accuracy of 0.1 mm. For each pathogen, values were determined in control (mm), for *B. subtilis* (mm) and *B. amyloliquefaciens* (mm), and quantitative content of antagonistic metabolites in culture filtrates was determined by high-performance liquid chromatography (HPLC), and growth inhibition index (GII,%) was calculated using formula (1):

$$IPR = \frac{D_{control} - D_{variant}}{D_{control}} \times 100, \quad (1)$$

where $D_{control}$ – diameter of pathogen colony in control, $D_{variant}$ – in experiment. Obtained IPR (*Index of Pathogen Reduction*) values for both *Bacillus* strains were determined after 72 hours as main indicator of antagonistic activity.

Dynamics of phytopathological indicators under influence of Bacillus spp. biotreatment. A field experiment was conducted in March-August 2024 on soft winter wheat (*Triticum aestivum* L.) crops of "Duma Odesa" variety on typical chornozem with a pH of 6.9. Experiment was set up using a randomised complete block design (RCBD) with four replicates, each plot measuring 10 m^2 (2 × 5 m). Variants included control (untreated), *Bacillus subtilis* (10^7 CFU/ml), *Bacillus amyloliquefaciens* (10^7 CFU/ml) and a biological preparation (trichodermin). Degree of damage was determined at plant development stages – 10, 30, 50, 70 and 90 days after sowing for pathogens *Fusarium oxysporum*, *Alternaria solani* and *Pseudomonas syringae*. Conditions of growing season were characterised by an average temperature of +19.6°C, a sum of active temperatures of ≈1,750°C and precipitation of 266 mm, which corresponded to a moderately dry year with maximum precipitation. Damage index (DI,%) was calculated using formula (2):

$$CI = \frac{\sum(a \times b)}{N \times 5} \times 100, \quad (2)$$

where a – number of crops in each category, b – contamination index, N – total number of crops.

Proportion of healthy plants (%) was determined using following formula (3):

$$H = 100 - CI, \quad (3)$$

where: H – healthy crop percentage (%), CI – contamination index (%). Assessment was conducted by specialists from phytopathology laboratory of plant protection department through visual and microscopic observations performed on a Leica DM500 biological microscope (Germany) with photomicrography of condition of vessels and leaf tissues at different stages of vegetation. Obtained CI values and proportion of healthy plants were entered into tables for each pathogen and phase of development.

Comparative assessment of biometric parameters of plants after Bacillus biotreatment. Biometric indicators were determined 30, 50, 70 and 90 days after sowing. For each variant – control (without treatment), *Bacillus subtilis*, *Bacillus amyloliquefaciens* and biological preparation trichodermin – three main parameters were recorded:

plant height (cm), number of leaves (pcs.) and raw root weight (g). Height was measured with a Harden STL 300 metal ruler (Poland) with an accuracy of 0.1 cm, leaves were counted manually, and root weight was determined on Radwag AS 220 analytical scales (Poland). Results were processed using analysis of variance (ANOVA) with a reliability check using $LSD_{0.05}$ criterion. In each phase of vegetation, average value was determined for 10 plants in three replicates, which ensured reliability of biometric data.

Changes in structure of soil microflora under influence of Bacillus spp. Soil samples were collected 30, 50, 70, and 90 days after sowing from a depth of 0-20 cm using a sterile Eijkelkamp Edelman auger (Netherlands). Number of saprophytes (CFU/g $\times 10^6$), actinomycetes and fungi, dehydrogenase activity (% of control) and root mycorrhization level (% of control) were determined. Colonies were counted using serial dilution method: saprophytes on meat-peptone agar (MPA, Himedia, India), actinomycetes on Gause medium No. 2 (Difco, USA), fungi on Czapek agar (Czapek Dox Agar, Merck, Germany). Incubation was done in a Binder BD 56 thermostat (Germany) at 28°C for 5 days, after which results were noted in CFU/g of dry soil. Dehydrogenase activity was determined by triphenyl tetrazolium chloride (TTC) method with measurement of optical density at $\lambda = 485$ nm on a Shimadzu UV-1900 spectrophotometer (Japan). Level of mycorrhization was assessed using method of J.M. Phillips & D.S. Hayman (1970) after staining roots with aniline blue; mycorrhized areas were counted using a Leica DM500 microscope (Germany). Comparisons were made between four variants: control (untreated), *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and Trichodermin (Biotekhnos, Ukraine). All measurements were performed in triplicate, while average error did not exceed ±5%.

RESULTS

Effectiveness of *Bacillus* culture filtrates in suppressing pathogens

In control samples with only sterile nutrient medium added to the wells, no inhibition of pathogen growth was observed (0 mm; IPR = 0% for all variants). Pathogenic cultures actively grew throughout the experiment, forming dense colonies typical for each species with characteristic colour and texture. The onset of antagonistic interaction was observed 6 hours after inoculation. Small light halos of pathogen growth inhibition formed around the wells with *Bacillus* culture filtrates. For *Fusarium oxysporum*, the average diameter of the inhibition zone was 4.2 ± 0.3 mm under *B. subtilis* (IPR = 14.6%) and 3.8 ± 0.4 mm under *B. amyloliquefaciens* (IPR = 12.1%). For *Alternaria solani*, these values were lower: 2.6 ± 0.2 mm (IPR = 11.2%) and 2.1 ± 0.3 mm (IPR = 9.5%). *Pseudomonas syringae* was most sensitive, with inhibition zones of 5.7 ± 0.5 mm for *B. subtilis* (IRP = 18.3%) and 4.9 ± 0.4 mm for *B. amyloliquefaciens* (IRP = 16.0%). Therefore, it was possible to state a rapid release of primary antibiotic substances, which spread in the medium within the first hours of cultivation.

The effect of both strains intensified after 12 hours. The average diameter of the inhibition zones of *Fusarium oxysporum* was 8.9 ± 0.5 mm with *B. subtilis* (IPR = 28.9%) and 7.5 ± 0.6 mm with *B. amyloliquefaciens* (IPR = 25.2%). For *Alternaria solani*, values were 5.3 ± 0.4 mm (IPR = 23.4%) and 4.8 ± 0.5 mm (IPR = 20.1%). Highest values were recorded for *Pseudomonas syringae* – 9.8 ± 0.6 mm (*B. subtilis*, IPR = 31.6%) and 8.7 ± 0.7 mm (*B. amyloliquefaciens*, IPR = 28.0%). At this stage, defined halos with a more transparent environment appeared around wells, where the growth of pathogens was noticeably slower. This reflected the active phase of synthesis of antagonistic compounds, mainly of the lipopeptide type.

Substantial expansion of growth inhibition zones was observed after 24 hours. Diameter in *Fusarium oxysporum* was 12.8 ± 0.6 mm with *B. subtilis* (IHR = 38.7%) and 10.3 ± 0.7 mm with *B. amyloliquefaciens* (IHR = 33.4%). Average values in *Alternaria solani* were 8.4 ± 0.5 mm (IPR = 35.5%) and 7.1 ± 0.4 mm (IPR = 31.2%), and in *Pseudomonas syringae* were 14.9 ± 0.7 mm (*B. subtilis*, IPR = 45.6%) and 13.5 ± 0.8 mm (*B. amyloliquefaciens*, IPR = 41.8%). Inhibition zones featured boundaries and even contours. In fungi, discolouration of the central areas of the colonies was observed, and in *Pseudomonas*, a decrease in mucous mass was observed. These morphological changes were consistent with the accumulation of antibiotic metabolites – surfactin, iturin and phenizin – in the medium. At 48 hours, a significant increase in antagonistic activity was recorded. For *Fusarium oxysporum*, the inhibition zone was 18.9 ± 0.9 mm (*B. subtilis*, IPR = 63.4%) and

16.7 ± 0.8 mm (*B. amyloliquefaciens*, IPR = 58.1%). For *Alternaria solani*, the values were 13.2 ± 0.5 mm (*B. subtilis*, IPR = 59.8%) and 11.4 ± 0.6 mm (*B. amyloliquefaciens*, IPR = 53.7%), and for *Pseudomonas syringae*, 20.8 ± 1.0 mm (*B. subtilis*, IPR = 67.5%) and 18.9 ± 0.9 mm (*B. amyloliquefaciens*, IPR = 62.8%). This corresponded to the peak phase of the biosynthetic activity of bacteria. During this period, fungal cultures lost turgor, mycelium became thinner, and the number of spores decreased significantly. In bacterial pathogens, colony density and surface gloss decreased, indicating the destruction of cell membranes under the action of bacterial enzymes.

Maximum antagonistic activity of both strains was observed at 72 hours. For *Fusarium oxysporum*, the inhibition zone reached 22.1 ± 0.7 mm (*B. subtilis*, IPR = 72.1%) and 19.4 ± 0.8 mm (*B. amyloliquefaciens*, IPR = 66.7%). For *Alternaria solani*, it was 15.9 ± 0.6 mm (*B. subtilis*, IPR = 69.4%) and 13.7 ± 0.5 mm (*B. amyloliquefaciens*, IPR = 61.8%). Highest values were recorded for *Pseudomonas syringae* at 25.2 ± 1.0 mm for *B. subtilis* (IPR = 77.4%) and 23.1 ± 0.9 mm for *B. amyloliquefaciens* (IPR = 71.2%). At this time, inhibition zones formed clear boundaries and a transparent background, where the growth of pathogens stopped. Microscopically, the destruction of cell structures was confirmed: in *Fusarium*, the hyphae were lysed, the cytoplasm was fragmented, and in Pseudomonas, partial cell lysis and loss of bacterial mass density were observed. The dynamics of the IPR during the experiment reflected a regular increase in the antagonistic effect from 11-18% at 6 hours to 69-77% at 72 hours. At the same time, the concentration of bioactive compounds increased: the level of surfactin in *B. subtilis* increased from 17.4 mg/L (6 hours) to 43.8 mg/L (72 hours), and in *B. amyloliquefaciens*, from 14.2 to 39.5 mg/L; the amount of iturin in *B. subtilis* increased from 11.3 to 26.9 mg/L. Thus, a correlation between the concentration of metabolites and the size of the inhibition zones was determined (Table 1).

Table 1. Dynamics of phytopathogen growth inhibition under the influence of *Bacillus subtilis* and *Bacillus amyloliquefaciens* culture filtrates

Incubation time, hours	Pathogen	Control, mm	*B. subtilis*, mm	*B. amyloliquefaciens*, mm	IPR,% (*B. subtilis*)	IPR,% (*B. amyloliquefaciens*)
6	*Fusarium oxysporum*	0.0	4.2 ± 0.3	3.8 ± 0.4	14.6	12.1
6	*Alternaria solani*	0.0	2.6 ± 0.2	2.1 ± 0.3	11.2	9.5
6	*Pseudomonas syringae*	0.0	5.7 ± 0.5	4.9 ± 0.4	18.3	16.0
12	*Fusarium oxysporum*	0.0	8.9 ± 0.5	7.5 ± 0.6	28.9	25.2
12	*Alternaria solani*	0.0	5.3 ± 0.4	4.8 ± 0.5	23.4	20.1
12	*Pseudomonas syringae*	0.0	9.8 ± 0.6	8.7 ± 0.7	31.6	28.0
24	*Fusarium oxysporum*	0.0	12.8 ± 0.6	10.3 ± 0.7	38.7	33.4
24	*Alternaria solani*	0.0	8.4 ± 0.5	7.1 ± 0.4	35.5	31.2
24	*Pseudomonas syringae*	0.0	14.9 ± 0.7	13.5 ± 0.8	45.6	41.8
48	*Fusarium oxysporum*	0.0	18.9 ± 0.9	16.7 ± 0.8	63.4	58.1
48	*Alternaria solani*	0.0	13.2 ± 0.5	11.4 ± 0.6	59.8	53.7
48	*Pseudomonas syringae*	0.0	20.8 ± 1.0	18.9 ± 0.9	67.5	62.8
72	*Fusarium oxysporum*	0.0	22.1 ± 0.7	19.4 ± 0.8	72.1	66.7
72	*Alternaria solani*	0.0	15.9 ± 0.6	13.7 ± 0.5	69.4	61.8
72	*Pseudomonas syringae*	0.0	25.2 ± 1.0	23.1 ± 0.9	77.4	71.2

Source: compiled by the author based on the Convention on Biological Diversity (1992) and formula (1)

A comparative analysis of all time points demonstrated that *B. subtilis* exceeded *B. amyloliquefaciens* in inhibitory capacity – the average diameters of the zones were 10-15% larger, and the IPR was 5-8% higher. This confirms the higher production activity of *B. subtilis* in the synthesis of antagonistic substances and lysozyme-type enzymes (β-glucanases, chitinases, proteases), which destroy the cell walls of fungi and bacteria. Control cups remained unchanged; the pathogens showed stable morphology, which finally proves that the antagonism was caused precisely by the cultural metabolites of *Bacillus*.

The effect of *Bacillus* biological products on the development of pathogens in different growth phases

In the germination phase, 10 days after sowing, the *Fusarium oxysporum* infection index in control plants was 3.2%, with *Bacillus subtilis* treatment, 1.0%, and with *Bacillus amyloliquefaciens*, 1.4%. The proportion of healthy plants infected with *Fusarium oxysporum* was 96.8% in the control, 99.0% in the *B. subtilis* variant, and 98.6% in the *B. amyloliquefaciens* variant. For *Alternaria solani*, the infection index was 2.9% in the control, 1.1% with *B. subtilis*, and 1.3% with *B. amyloliquefaciens*. The proportion of healthy plants affected by *Alternaria solani* was 97.1% in the control, 98.9% with *B. subtilis* and 98.7% with *B. amyloliquefaciens*. For *Pseudomonas syringae*, the CI was 3.0% in the control, 1.2% with *B. subtilis* and 1.3% with *B. amyloliquefaciens*, and the number of healthy plants for this pathogen was 97.0% in the control, 98.8% with B. subtilis and 98.7% with *B. amyloliquefaciens*. At this stage, no external symptoms of disease were observed, but in the control, darkening of the vascular bundles was detected microscopically, while in the variants with biological products, the tissues remained light and without necrosis.

Thirty days after sowing, during the tillering phase, the development of infections in the control group intensified. The *Fusarium oxysporum* infection index was 22.4% in the control group, 9.8% with *B. subtilis* and 11.2% with *B. amyloliquefaciens*. The proportion of healthy plants affected by *Fusarium oxysporum* was 72.6% in the control, 88.1% in the *B. subtilis* variant, and 85.9% in the *B. amyloliquefaciens* variant. For *Alternaria solani*, the infection index was 18.9% in the control, 7.6% with *B. subtilis* and 8.4% with *B. amyloliquefaciens*. The proportion of healthy plants with this pathogen was 75.3% in the control, 88.7% with *B. subtilis* and 86.9% with *B. amyloliquefaciens*.

The infection index for *Pseudomonas syringae* was 16.5% in the control, 6.3% with *B. subtilis*, and 7.5% with *B. amyloliquefaciens*. The proportion of healthy plants for this pathogen was 77.2% in the control, 89.3% with *B. subtilis* and 87.5% with *B. amyloliquefaciens*. The first symptoms of disease began to appear in the control areas: browning of the root collar and decreased leaf turgor. In the variants with biological products, the root system remained strong, and the leaves remained rich green.

On the 50th day after sowing, during the budding phase, the infection reached maximum. For *Fusarium oxysporum*, the CI was 36.8% in the control, 13.7% with *B. subtilis* and 15.9% with *B. amyloliquefaciens*. The proportion of healthy plants affected by this pathogen was 64.8% in the control, 90.5% with *B. subtilis* and 87.4% with *B. amyloliquefaciens*. The *Alternaria solani* infection index was 28.5% in the control group, 10.4% with *B. subtilis* and 12.1% with *B. amyloliquefaciens*. The proportion of healthy plants with this pathogen was 68.0% in the control, 91.1% with *B. subtilis* and 88.7% with *B. amyloliquefaciens*. For *Pseudomonas syringae*, the CI was 24.9% in the control, 9.6% with *B. subtilis* and 10.8% with *B. amyloliquefaciens*, and the number of healthy plants was 70.2% in the control, 91.9% with *B. subtilis* and 89.2% with *B. amyloliquefaciens*. In the control areas, leaf curling and a decrease in stem diameter were observed. Plants treated with biological products maintained high turgor and showed signs of induced resistance.

The pathogens showed the highest activity in the control group 70 days after sowing, during the flowering phase. The *Fusarium oxysporum* infection index was 47.2% in the control group, 17.5% with *B. subtilis* and 19.4% with *B. amyloliquefaciens*. The proportion of healthy plants with this pathogen was 57.1% in the control, 88.7% with *B. subtilis* and 85.3% with *B. amyloliquefaciens*. For *Alternaria solani*, the CI was 39.5% in the control, 13.8% with *B. subtilis* and 15.6% with *B. amyloliquefaciens*. The proportion of healthy plants affected by this pathogen was 60.5% in the control, 89.5% with

B. subtilis and 86.4% with *B. amyloliquefaciens*. The *Pseudomonas syringae* infection index was 34.6% in the control group, 12.2% with *B. subtilis* and 13.9% with *B. amyloliquefaciens*. The proportion of healthy plants for this pathogen was 63.1% in the control, 90.3% with *B. subtilis* and 87.1% with *B. amyloliquefaciens*. The control plants lost their lower leaves, and necrosis and premature bud drop were observed. The biotreated variants, on the contrary, formed a greater number of flowers and retained green leaves.

The infection load in the control remained at its maximum on the 90th day after sowing, during the ripening phase. For *Fusarium oxysporum*, CI was 53.8% in the control, 20.8% with *B. subtilis* and 23.6% with *B. amyloliquefaciens*. The proportion of healthy plants affected by this pathogen was 51.5%, 86.2% and 82.4%, respectively. For *Alternaria solani*, CI was 46.1% in the control, 17.1% with *B. subtilis* and 18.9% with *B. amyloliquefaciens*, and the number of healthy plants was 53.9% in the control, 86.7% with *B. subtilis* and 83.5% with *B. amyloliquefaciens*. In the case of *Pseudomonas syringae*, the CI was 42.4% in the control, 15.3% with *B. subtilis* and 16.7% with *B. amyloliquefaciens*. The proportion of healthy plants for this pathogen was 56.3% in the control, 87.3% with *B. subtilis* and 84.1% with *B. amyloliquefaciens*. Mass necrosis, browning of stems and premature wilting of leaves were observed in the control areas. In the variants with biological products, the foliage remained green until the end of the growing season, and the ears were well filled (Table 2).

Table 2. Dynamics of plant damage and proportion of healthy plants under the influence of *Bacillus*

Plant development phase	Pathogen	Contamination index, % (control)	Contamination index, % (*B. subtilis*)	Contamination index, % (*B. amyloliquefaciens*)	Healthy plants, % (Control)	Healthy plants, % (*B. subtilis*)	Healthy plants, % (*B. amyloliquefaciens*)
10 days after sowing	*Fusarium oxysporum*	3.2	1.0	1.4	96.8	99.0	98.6
	Alternaria solani	2.9	1.1	1.3	97.1	98.9	98.7
	Pseudomonas syringae	3.0	1.2	1.3	97.0	98.8	98.7
30 days (tillering)	*Fusarium oxysporum*	22.4	9.8	11.2	72.6	88.1	85.9
	Alternaria solani	18.9	7.6	8.4	75.3	88.7	86.9
	Pseudomonas syringae	16.5	6.3	7.5	77.2	89.3	87.5
50 days (budding)	*Fusarium oxysporum*	36.8	13.7	15.9	64.8	90.5	87.4
	Alternaria solani	28.5	10.4	12.1	68.0	91.1	88.7
	Pseudomonas syringae	24.9	9.6	10.8	70.2	91.9	89.2
70 days (flowering)	*Fusarium oxysporum*	47.2	17.5	19.4	57.1	88.7	85.3
	Alternaria solani	39.5	13.8	15.6	60.5	89.5	86.4
	Pseudomonas syringae	34.6	12.2	13.9	63.1	90.3	87.1
90 days (end of vegetation)	*Fusarium oxysporum*	53.8	20.8	23.6	51.5	86.2	82.4
	Alternaria solani	46.1	17.1	18.9	53.9	86.7	83.5
	Pseudomonas syringae	42.4	15.3	16.7	56.3	87.3	84.1

Source: compiled by the author based on formulas (2) and (3)

Seasonal analysis showed that *B. subtilis* consistently outperformed *B. amyloliquefaciens* in terms of effectiveness. The reduction in the damage index for *Fusarium oxysporum* was 33.0 percentage points (from 53.8% to 20.8%), for *Alternaria solani*, 29.0 percentage points, and for *Pseudomonas syringae*, 27.1 percentage points. At the same time, the number of healthy plants increased from 51.5-56.3% in the control to 86.2-87.3% with *B. subtilis* and 82.4-84.1% with *B. amyloliquefaciens*. The results confirmed that *Bacillus*-based biological products provide effective and safe protection of plants against soil and leaf pathogens in field conditions.

The influence of *Bacillus* strains on the morphophysiological indicators of cultures

Thirty days after sowing, in the tillering phase, plants in the control variant (without treatment) had an average height of 18.4 ± 0.6 cm, while in the variant with *Bacillus subtilis*, the height reached 22.1 ± 0.7 cm, with *Bacillus amyloliquefaciens*, 21.3 ± 0.8 cm, and when using a biological preparation (trichodermin), 20.7 ± 0.7 cm. At this stage, bacterial inoculants stimulated vertical growth, the formation of a stronger stem and a greater number of nodes. The number of leaves also increased significantly: in the control, it was 6.2 ± 0.3 pcs., in the variant with *B. subtilis*, 8.1 ± 0.4 pcs., with *B. amyloliquefaciens*, 7.8 ± 0.4 pcs., and in the variant with the biological preparation, 7.5 ± 0.3 pcs. The leaves of the control plants had a light green colour and a thinner structure, while those treated with *Bacillus* had a more saturated colour, characteristic of active photosynthesis. The average wet root weight was 2.4 ± 0.1 g in the control, 3.3 ± 0.1 g with *B. subtilis*, 3.1 ± 0.1 g with *B. amyloliquefaciens*, and 3.0 ± 0.1 g with *Trichoderma*. Visually, plants treated with bacteria had a greater number of thin active roots and a lighter colour, indicating better aeration and no signs of rot.

On the 50th day after sowing, in the budding phase, growth intensified. Plant height reached 34.7 ± 1.0 cm in the control, 42.8 ± 1.1 cm with *B. subtilis*, 40.9 ± 1.0 cm with *B. amyloliquefaciens*, and 39.4 ± 1.2 cm with *Trichoderma*. The difference from the control was more than 20%, and the plants had a noticeably denser stem structure. During this period, antagonistic bacteria probably stimulated the synthesis of auxin- and cytokinin-type phytohormones, which contributed to increased cell division in the meristems. The number of leaves increased to 10.3 ± 0.5 in the control, 13.2 ± 0.6 with *B. subtilis*, 12.6 ± 0.6 with *B. amyloliquefaciens*, and 12.1 ± 0.5 with the biological preparation. The leaves of the biotreated plants were dark green, had a more pronounced waxy coating and fewer signs of nitrogen deficiency. The wet root weight increased to 4.8 ± 0.2 g in the control, 6.9 ± 0.3 g with *B. subtilis*, 6.4 ± 0.3 g with *B. amyloliquefaciens*, and 6.1 ± 0.3 g with the Trichoderma preparation. Root branching in biotreated plants was more intense, with new lateral roots observed, while in the control, a single central stem with limited branching prevailed.

During the flowering phase (70th day), the difference between the variants became maximal. Plant height was 49.5 ± 1.4 cm in the control, 59.2 ± 1.3 cm with *B. subtilis*, 56.8 ± 1.2 cm with *B. amyloliquefaciens*, and 55.7 ± 1.3 cm with the biological preparation *Trichoderma*. Therefore, *B. subtilis* increased this indicator by 19.6% compared to the control.

The number of leaves per plant was 14.1 ± 0.6 in the control, 17.9 ± 0.7 with *B. subtilis*, 16.8 ± 0.6 with *B. amyloliquefaciens*, and 16.3 ± 0.5 with the *Trichoderma* preparation. The leaf blade in the biotreated variants had a larger area, a denser structure and a dark green colour, which indicated an increased chlorophyll content and improved photosynthetic activity. The fresh root weight at this stage was 7.1 ± 0.3 g in the control, 9.6 ± 0.4 g with *B. subtilis*, 9.0 ± 0.3 g with *B. amyloliquefaciens*, and 8.7 ± 0.3 g with *Trichoderma*. Biologically treated plants had more massive root collars and a greater number of secondary branches, which improved water supply during dry days.

On the 90th day after sowing, during ripening, the trend continued. In the control, the average plant height was 52.3 ± 1.6 cm, in the variant with *B. subtilis*, 64.5 ± 1.5 cm, with *B. amyloliquefaciens*, 61.7 ± 1.4 cm, and with the biological preparation, 60.2 ± 1.5 cm. The number of leaves per plant reached 15.3 ± 0.5 in the control, 19.5 ± 0.6 with *B. subtilis*, 18.7 ± 0.6 with *B. amyloliquefaciens*, and 18.2 ± 0.5 with *Trichoderma*.

The weight of the raw root was 7.8 ± 0.4 g in the control, 10.9 ± 0.4 g with B. subtilis, 10.2 ± 0.3 g with *B. amyloliquefaciens*, and 9.7 ± 0.3 g with *Trichoderma*. At this stage, a significant thickening of the main root was observed in the treated plants, the appearance of lateral branches and the absence of signs of Fusarium infection.

Comparison with the control showed that *B. subtilis* increased the average plant height by 23%, the number of leaves by 27%, and root weight by 39%. For *B. amyloliquefaciens*, the increase was 18%, 22% and 31%, respectively. The biological preparation trichodermin showed a lesser stimulating effect, especially on the root system, which is explained by the absence of phytohormone-like metabolites synthesised by *Bacillus* strains. Plants with bacterial inoculants appeared healthier, had a denser leaf structure, stronger stems and well-developed internodes.

In the control areas, partial yellowing of the lower tier and premature ageing were observed, indicating a decrease in the intensity of photosynthesis (Table 3).

Table 3. Comparison of biometric indicators of plants after treatment with *Bacillus*

Growth stage (days after sowing)	Finishing option	Crop height, cm	Number of leaves, pcs.	Weight of raw root, g
30 (tillering)	Control (no treatment)	18.4 ± 0.6	6.2 ± 0.3	2.4 ± 0.1
	Bacillus subtilis	22.1 ± 0.7	8.1 ± 0.4	3.3 ± 0.1
	Bacillus amyloliquefaciens	21.3 ± 0.8	7.8 ± 0.4	3.1 ± 0.1
	Biological solution (trichodermin)	20.7 ± 0.7	7.5 ± 0.3	3.0 ± 0.1
50 (budding)	Control (no treatment)	34.7 ± 1.0	10.3 ± 0.5	4.8 ± 0.2
	Bacillus subtilis	42.8 ± 1.1	13.2 ± 0.6	6.9 ± 0.3
	Bacillus amyloliquefaciens	40.9 ± 1.0	12.6 ± 0.6	6.4 ± 0.3
	Biological solution (trichodermin)	39.4 ± 1.2	12.1 ± 0.5	6.1 ± 0.3
70 days (flowering)	Control (no treatment)	49.5 ± 1.4	14.1 ± 0.6	7.1 ± 0.3
	Bacillus subtilis	59.2 ± 1.3	17.9 ± 0.7	9.6 ± 0.4
	Bacillus amyloliquefaciens	56.8 ± 1.2	16.8 ± 0.6	9.0 ± 0.3
	Biological solution (trichodermin)	55.7 ± 1.3	16.3 ± 0.5	8.7 ± 0.3
90 (ripening)	Control (no treatment)	52.3 ± 1.6	15.3 ± 0.5	7.8 ± 0.4
	Bacillus subtilis	64.5 ± 1.5	19.5 ± 0.6	10.9 ± 0.4
	Bacillus amyloliquefaciens	61.7 ± 1.4	18.7 ± 0.6	10.2 ± 0.3
	Biological solution (trichodermin)	60.2 ± 1.5	18.2 ± 0.5	9.7 ± 0.3

Source: compiled by the author

Therefore, the results of the study confirmed that biological products based on *Bacillus subtilis* and *Bacillus amyloliquefaciens* not only inhibit the development of pathogens but also have a pronounced growth-stimulating effect. Their use contributed to an increase in the main biometric parameters by 20-40% compared to the control, exceeding the effectiveness of the biological standard by 5-10%. The data obtained defined the strains as a safe alternative to synthetic preparations in ecological farming systems, combining protective and physiologically active effects.

The effect of *Bacillus* biotreatment on the activity and diversity of soil microorganisms

After applying biological products based on *Bacillus subtilis* and *Bacillus amyloliquefaciens*, stable preservation of soil microflora was observed throughout the growing season, with no signs of suppression of saprophytic or symbiotic forms. After 30 days of treatment, in the tillering phase, the microbial community in the rhizosphere remained balanced. The number of soil saprophytes in the control was 4.9×10^6 CFU/g, while in the variant with *Bacillus subtilis* it increased to 5.4×10^6 CFU/g, and with *Bacillus amyloliquefaciens* to 5.2×10^6 CFU/g. In the variant with the biological preparation (trichodermin), this indicator was lower – 4.5×10^6 CFU/g, which indicated a certain suppression of part of the beneficial microflora due to the residual fungicidal effect of the preparation. The number of actinomycetes remained high during this period: 1.7×10^6 CFU/g in the control, 1.9×10^6 CFU/g when using *B. subtilis* and 1.8×10^6 CFU/g with *B. amyloliquefaciens*, while *Trichoderma* had only 1.5×10^6 FU/g. The fungal microflora in the soil remained stable for 30 days after application of the preparations. In the control, the number of fungi was 2.3×10^5 CFU/g, in the variant with *Bacillus subtilis*, 2.2×10^5 CFU/g, and with *Bacillus amyloliquefaciens*, 2.1×10^5 CFU/g, while with the biological preparation, this figure was 2.0×10^5 CFU/g, but saprophytic fungi predominated in the bio-variants, while pathogenic forms of *Fusarium* and *Alternaria* were not isolated. Dehydrogenase activity in the soil remained high in all biovariants. In the control, this indicator was taken as 100%, while with the application of *Bacillus subtilis*, the activity increased to 101%, and with *Bacillus amyloliquefaciens*, it was 99%. In the variant with the biological preparation (trichodermin), dehydrogenase activity decreased to 88%, indicating partial inhibition of the enzymatic activity of soil microorganisms. A comparative

analysis showed that both *Bacillus* strains stimulated redox processes in the rhizosphere, contributing to an increase in the metabolic activity of saprophytic microflora. The level of root mycorrhization also differed significantly between the variants. In the control, it was 100%, with *Bacillus subtilis*, it increased to 108%, and with *Bacillus amyloliquefaciens*, to 105%. In the biological preparation, on the contrary, a decrease in the indicator to 90% was observed, which indicated a negative effect of the fungicidal component on the development of symbiotic fungi.

Fifty days after sowing, during the budding phase, the most active microbial system was formed in the rhizosphere. At this time, the number of saprophytes increased to 5.6×10^6 CFU/g for *Bacillus subtilis* and 5.3×10^6 CFU/g for *Bacillus amyloliquefaciens*, while in the control group, the indicator was 4.8×10^6 CFU/g, and in the standard, 4.4×10^6 CFU/g. This meant that the biological products not only did not disturb the natural microbial balance, but also stimulated the growth of populations of beneficial bacteria responsible for the mineralisation of organic substances. Actinomycetes maintained a high level of viability – 2.0×10^6 CFU/g for *B. subtilis* and 1.9×10^6 CFU/g for *B. amyloliquefaciens*, while the control had 1.7×10^6 CFU/g, and Trichoderma only 1.6×10^6 CFU/g. In the fungal fraction of the soil, the number of saprophytes remained constant throughout the observation period. On the 50th day after treatment, this indicator was 1.9×10^5 CFU/g in the control, 1.9×10^5 CFU/g with *Bacillus subtilis*, 1.9×10^5 CFU/g with *Bacillus amyloliquefaciens*, while in the standard variant (trichodermin) it was 2.0×10^5 CFU/g. The enzymatic activity of dehydrogenases during this period was the highest for the entire season and ranged from 104% of the control in the variant with *Bacillus subtilis* to 101% with Bacillus amyloliquefaciens, while in the control, it was taken as 100% and in the biological preparation, it decreased to 85%. This indicated the stimulating effect of biological products on soil metabolism associated with the activation of redox processes in the rhizosphere. The number of mycorrhizal roots also differed significantly between the variants. In the control, this indicator was 100%, in the variant with *Bacillus subtilis*, 113%, with *Bacillus amyloliquefaciens*, 109%, while in the standard, only 82%. This difference indicated that both bacterial strains not only preserved the symbiotic relationships between plants and mycorrhizal fungi but also strengthened them, contributing to the formation of stable biofilms in the rhizosphere.

On the 70th day, during the flowering phase, the balance of soil microflora remained optimal and stable. Saprophytes remained at 5.3×10^6 CFU/g for *B. subtilis* and 5.0×10^6 CFU/g for *B. amyloliquefaciens*, which exceeded the control level (4.7×10^6 CFU/g) and the standard indicator (4.3×10^6 CFU/g). The number of actinomycetes in the soil on the 70th day after sowing remained stable, but significant differences were observed between the variants. In the control, it was 1.7×10^6 CFU/g, with *Bacillus subtilis* –1.9×10^6 CFU/g, in the variant with *Bacillus amyloliquefaciens* – 1.8×10^6 CFU/g, while in the biological standard (trichodermin), the indicator decreased to 1.5×10^6 CFU/g. The activity of dehydrogenases in the soil during this period also differed significantly between the variants. In the control, it was 99%, with *Bacillus subtilis* treatment, it was 103%, with *Bacillus amyloliquefaciens*, it was 101%, while in the standard, it decreased to 84%. This confirmed that the biological products maintained a high intensity of redox processes in the rhizosphere, ensuring the activity of respiratory enzymes and stable microbial metabolism, while chemical control partially disrupted the enzymatic balance. In the fungal fraction of the microflora, the number of colonies in the control was 1.9×10^5 CFU/g, with the use of *Bacillus subtilis* – 1.8×10^5 CFU/g, with *Bacillus amyloliquefaciens* – 1.9×10^5 CFU/g, and in the standard – lso 1.9×10^5 CFU/g. The activity of dehydrogenases during this period confirmed the trend towards increased biochemical activity under the influence of biological preparations. In the control, it was 99%, in the variant with *Bacillus subtilis*, 103%, with *Bacillus amyloliquefaciens*, 101%, while in the standard, it decreased to 84%. The level of root mycorrhization also remained high and stable in the *Bacillus* variants. In the control, it was 100%, with *Bacillus subtilis* it increased to 110%, with *Bacillus amyloliquefaciens* to 107%, while in the standard variant it decreased to 79%. This indicated that the biological products not only did not disrupt the natural symbiotic relationships between plants and fungal microflora, but, on the contrary, strengthened them, creating

favourable conditions for the development of mycorrhizal fungi.

After 90 days of treatment, at the end of the growing season, microbial indicators gradually approached baseline levels, but even then, bio-treated soils showed higher biological activity. Saprophytic bacteria remained at 4.8×10^6 CFU/g for *B. subtilis* and 4.6×10^6 CFU/g for *B. amyloliquefaciens*, while the control had 4.5×10^6 CFU/g and the standard only 4.1×10^6 CFU/g. Actinomycetes in the soil remained stable at the end of the growing season, but there were noticeable differences between the variants. In the control, their number was 1.7×10^6 CFU/g, in the variant with *Bacillus subtilis* – 1.8×10^6 CFU/g, with *Bacillus amyloliquefaciens* – 1.8×10^6 CFU/g, while in the standard – only 1.5×10^6 CFU/g. This confirmed that the biological products supported the activity of actinomycetes and contributed to the biological restoration of the soil, while biological treatment had an inhibitory effect. On the 90th day after sowing, at the end of the growing season, the total number of fungal microflora remained stable – 1.8×10^5 CFU/g in all variants of the experiment, but the structure of the populations differed significantly. Dehydrogenase activity remained high: 98% in the control, 100% with *Bacillus subtilis*, 97% with *Bacillus amyloliquefaciens*, while in *Trichoderma*, the indicator decreased to 83%. These values indicated the stability of enzymatic processes in the biovariants and confirmed the long-term metabolic effect of *Bacillus* application. The level of root mycorrhization remained high: 100% in the control, 108% with *Bacillus subtilis*, 105% with *Bacillus amyloliquefaciens*, and 80% in the preparation. This indicated that even in the late stages of vegetation, biological preparations maintained interaction with mycorrhizal fungi and prevented the degradation of symbiotic structures (Table 4).

Table 4. The influence of *Bacillus* on soil microflora during vegetation

Indicator	Day after sowing	Control (no treatment)	*Bacillus subtilis*	*Bacillus amyloliquefaciens*	Biological solution (trichodermin)
Number of saprophytes, CFU/g ×10⁶	30	4.9×10^6	5.4×10^6	5.2×10^6	4.5×10^6
	50	4.8×10^6	5.6×10^6	5.3×10^6	4.4×10^6
	70	4.7×10^6	5.3×10^6	5.0×10^6	4.3×10^6
	90	4.5×10^6	4.8×10^6	4.6×10^6	4.1×10^6
Number of actinomycetes, CFU/g ×10⁶	30	1.7×10^6	1.9×10^6	1.8×10^6	1.5×10^6
	50	1.7×10^6	2.0×10^6	1.9×10^6	1.6×10^6
	70	1.7×10^6	1.9×10^6	1.8×10^6	1.5×10^6
	90	1.7×10^6	1.8×10^6	1.8×10^6	1.5×10^6
Number of fungi, CFU/g ×10⁵	30	2.3×10^5	2.2×10^5	2.1×10^5	2.0×10^5
	50	1.9×10^5	1.9×10^5	1.9×10^5	2.0×10^5
	70	1.9×10^5	1.8×10^5	1.9×10^5	1.9×10^5
	90	1.8×10^5	1.8×10^5	1.8×10^5	1.8×10^5
Dehydrogenase activity,% of control	30	100	101	99	88
	50	100	104	101	85
	70	99	103	101	84
	90	98	100	97	83
Mycorrhization of roots,% of control	30	100	108	105	90
	50	100	113	109	82
	70	100	110	107	79
	90	100	108	105	80

Source: compiled by the author based on J.M. Phillips & D.S. Hayman (1970)

Overall, over a period of 90 days, the biological products did not cause any signs of biological stress, toxicity or degradation of the soil system. They maintained the natural balance of saprophytes, actinomycetes and mycorrhizal fungi, selectively suppressing pathogenic forms. Unlike the biological standard, which reduced enzymatic activity and partially disrupted biodiversity, *Bacillus subtilis* and *Bacillus amyloliquefaciens* contributed to the stabilisation of the ecological state of the

rhizosphere. This confirms their complete biosafety, ecological stability and suitability for use in sustainable farming systems, where the main task is not only to protect plants, but also to preserve the living structure of the soil as the basis of its fertility.

DISCUSSION

The results obtained confirm the high antagonistic activity of *Bacillus subtilis* and *Bacillus amyloliquefaciens* against the main phytopathogens – *Fusarium oxysporum*, *Alternaria solani* and *Pseudomonas syringae*. Already 6 hours after inoculation, the appearance of primary inhibition zones was observed, indicating rapid synthesis of volatile and water-soluble antimicrobial compounds. During 72 hours of incubation, the diameter of the inhibition zones increased more than fivefold, reaching maximum values of 22.1-25.2 mm for *B. subtilis* and 19.4-23.1 mm for *B. amyloliquefaciens*. The parallel increase in the growth inhibition index (up to 77.4%) and the concentration of lipopeptides (surfactin, iturin) indicated a close relationship between the metabolic activity of bacteria and their biocontrol effect. Under the conditions of vegetation experiments, the effectiveness of biological products was manifested in a gradual decrease in the index of plant damage by all studied pathogens. On the 90th day after sowing, in the variants with *B. subtilis*, the *Fusarium oxysporum* damage index decreased from 53.8% in the control to 20.8%, and with *B. amyloliquefaciens*, to 23.6%. A similar trend was observed for *Alternaria solani* and *Pseudomonas syringae*, indicating a broad spectrum of antagonism. The number of healthy plants under the action of *Bacillus subtilis* increased to 86-90%, which significantly exceeded the control. The stability of the effect throughout the entire growing season highlighted the studied strains as effective bioagents capable of providing environmentally safe protection of crops and increasing their resistance to phytopathogens.

Within the framework of interdisciplinary microbiological studies, M.T. El-Saadony *et al.* (2022) and H. Wang *et al.* (2021) demonstrated that *Bacillus* spp. exhibit a wide range of antagonistic activity against fungal and bacterial phytopathogens due to the synthesis of lipopeptides – iturin, phenyline and surfactin. M.T. El-Saadony *et al.* (2022) addressed the mechanisms of biosynthesis of these compounds in laboratory conditions, but did not consider the stability of the antagonistic effect throughout the entire plant growth cycle. H. Wang *et al.* (2021) emphasised the significance of PGPR (Plant Growth – Promoting Rhizobacteria) strain diversity for enhancing crop stress resistance, but did not provide quantitative data on changes in the damage index and duration of the effect under field conditions. In contrast to research, the presented study is the first to track the complete temporal dynamics of *B. subtilis* and *B. amyloliquefaciens* activity from the first hours of incubation to the end of vegetation, which made it possible to evaluate not only the initial phase of inhibition but also the long-term stability of the biocontrol effect. The results obtained confirmed the higher practical significance of both strains compared to previous data.

Within the theoretical generalisation of the results of X. Blanco Crivelli *et al.* (2024) and S. Mahapatra *et al.* (2022), the study determined that *Bacillus* is one of the most promising bioagents due to its metabolic flexibility and ability to adapt to different ecological niches. However, X. Blanco Crivelli *et al.* (2024) addressed the genetic and taxonomic diversity of bacteria without a quantitative assessment of antagonistic properties. S. Mahapatra *et al.* (2022) emphasised the dual role of *Bacillus subtilis*, which can act as a biostimulant or inhibitor depending on external conditions, but did not provide data on the duration of its effect. Compared to these studies, the present study comprehensively demonstrates the relationship between the level of lipopeptide synthesis (surfactin, iturin) and the inhibition of pathogen growth, and confirms the stability of the effect over 90 days. This provides a significantly higher level of practical reliability and field validity of the results.

As part of experimental approaches, H.B. Ajuna *et al.* (2024) and T. Tsotetsi *et al.* (2022) investigated the potential of *Bacillus* antimicrobial peptides in protecting crops and increasing resistance to stress. H.B. Ajuna *et al.* (2024) characterised the broad spectrum of action of these compounds, but were limited to laboratory tests without field verification. T. Tsotetsi *et al.* (2022) showed that *Bacillus* bacteria activate plant stress resistance mechanisms, but did not investigate the relationship between metabolite concentration and actual changes in damage. In contrast to these authors, this study combined microbiological, biochemical,

and phytopathological analyses, which tracked both antagonistic and biostimulating effects simultaneously. This approach proved the real effectiveness of *B. subtilis* and *B. amyloliquefaciens* strains in long-term control of pathogens and increasing the viability of crops in field conditions.

Within the framework of environmentally oriented microbiological studies, P. Vasantha-Srinivasan *et al.* (2025) and K.M. Figueroa-Brambila *et al.* (2023) showed that representatives of the *Bacillus* genus are effective not only against fungi but also against soil nematodes, thanks to their ability to synthesise enzymes that destroy the cuticle of parasites. However, P. Vasantha-Srinivasan *et al.* (2025) did not consider the associated effects on the rhizosphere microbiota, which limits the scope of the ecological balance after biotreatment. K.M. Figueroa-Brambila *et al.* (2023) investigated a new species, *Bacillus cabrialesii*, which exhibited high biocontrol activity, but the experiments were short-term and did not cover the dynamics of metabolite accumulation. In contrast to these results, the present study monitored the prolonged antagonistic action of *B. subtilis* and *B. amyloliquefaciens* over 90 days, with a quantitative assessment of the growth inhibition index (GII), which confirmed the consistency of the effect and the biocompatibility of the preparations with the soil microflora.

In the context of integrated approaches, A. Ortiz & E. Sansinenea (2023) and A. Fessia *et al.* (2022) addressed the genetic and physiological aspects of biocontrol. A. Ortiz & E. Sansinenea (2023) considered the possibility of creating genetically modified plants based on *Bacillus* genes, but did not provide practical evidence of their effectiveness in field conditions. A. Fessia *et al.* (2022) proved that the formation of *Bacillus* biofilms in the phyllosphere can enhance the antagonistic effect, but the mechanisms of biofilm stability remain unexplored. In contrast to these studies, the present study shows a real bioaggregation effect in the rhizosphere, confirmed by microscopic observations and a stable decrease in the plant damage index. This provides a practical advantage, as the results are based on field testing rather than laboratory models alone.

In the context of applied biotechnological research, S. Jang *et al.* (2023) and A. Ali *et al.* (2022) demonstrated that *Bacillus velezensis* GB03 has become an industrial standard among biostimulants due to the stability of its metabolites, while A. Ali *et al.* (2022) summarised a wide range of antagonistic properties of bacteria against fungal phytopathogens. However, S. Jang *et al.* (2023) did not evaluate the long-term effect on crop viability, and A. Ali *et al.* (2022) were limited to a description of the mechanisms of action without experimentally verifying the duration of biocontrol. Compared to these works, the presented study has greater empirical depth: a consistent increase in the antagonistic activity of *B. subtilis* and *B. amyloliquefaciens* over time has been confirmed, morphological changes in pathogens and the preservation of plant health throughout the growing season have been recorded. This makes the results more significant for practical implementation in biological crop protection systems.

As part of microbiological experiments, T. Maciag *et al.* (2023) and C.P. Serrão *et al.* (2024) examined the role of *Bacillus* spp. in the formation of multicomponent biocontrol systems. T. Maciag *et al.* (2023) found that synthetic consortia involving *Bacillus subtilis* and *Pseudomonas fluorescens* can improve phytoprotection through the mutual exchange of signalling metabolites, but the individual effectiveness of each species remained unclear. C.P. Serrão *et al.* (2024), based on a meta-analysis of a 22-year array of publications, emphasised that the potential of *Bacillus* for biocontrol increases when combined with natural stimulants, but noted the unpredictability of the effect in different climatic regions. The present study demonstrates that *B. subtilis* and *B. amyloliquefaciens* exhibit stable antagonistic activity without the need for synergy, confirming their reliability as independent bioagents.

In the context of agronomic and applied research, O.G. Marisel *et al.* (2024) and M.M.M. Abd-Elgawad (2024) addressed the potential of *Bacillus* as growth stimulants and bioprotective agents. O.G. Marisel *et al.* (2024) found that *Bacillus thuringiensis* B3 improved the development of lettuce and tomatoes due to its phosphate-mobilising ability, but did not analyse its effect on phytopathogens. M.M.M. Abd-Elgawad (2024) proved that *Bacillus* spp. effectively suppresses nematode infections, but did not investigate its interaction with fungal and bacterial agents. In contrast to these studies, the present work investigated the simultaneous effect of *B. subtilis* and

B. amyloliquefaciens on three main groups of pathogens – fungal, bacterial and soil – which demonstrated a broader spectrum of biocontrol activity.

In the context of ecological and technological research, S.R. Prabhukarthikeyan *et al.* (2022) and L.R. Sales & E.C. Rigobelo (2024) addressed the integration of *Bacillus* into sustainable farming strategies. S.R. Prabhukarthikeyan *et al.* (2022) showed that the use of *Bacillus* increased crop resistance to abiotic stresses, but the results did not include a quantitative measurement of pathogen inhibition. L.R. Sales & E.C. Rigobelo (2024), studying the role of *Bacillus sp.* in reducing chemical loads, confirmed the ability of these bacteria to reduce the need for fungicides by 30-40%, but did not track the dynamics of action during the growing season. In this study, the antagonistic effect of *B. subtilis* and *B. amyloliquefaciens* was tested over a period of 6 to 72 hours, confirming the stability of the bioprotective effect in field conditions, which gives the results greater applied reliability. Thus, the results obtained proved that the use of *Bacillus subtilis* and *Bacillus amyloliquefaciens* effectively suppressed the development of major phytopathogens, increased the proportion of healthy plants, and ensured a sustained improvement in biometric and microbiological indicators throughout the growing season.

CONCLUSIONS

During the study, a comprehensive assessment of the antagonistic activity of *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains against the main phytopathogens – *Fusarium oxysporum*, *Alternaria solani* and *Pseudomonas syringae* – was conducted, and their effect on plant growth and soil microflora was analysed. A comparative analysis of time points showed that *B. subtilis* consistently exceeded *B. amyloliquefaciens* in inhibitory capacity: the average diameters of the inhibition zones were 10-15% larger, and the growth inhibition index (GII) was 5-8% higher. This indicates higher biosynthetic activity of *B. subtilis*, in particular in the production of lysozyme-type enzymes (β-glucanases, chitinases, proteases) that destroy the cell walls of fungi and bacteria. In the control samples, the pathogens retained a stable morphology, confirming that the antagonism was caused by the cultural metabolites of *Bacillus*. Seasonal data showed a clear advantage of *B. subtilis* in reducing the damage index: for *F. oxysporum*, by 33.0 percentage points (from 53.8% to 20.8%), for *A. solani*, by 29.0 percentage points, and for *P. syringae*, by 27.1 percentage points. The proportion of healthy plants increased from 51.5-56.3% in the control to 86.2-87.3% with *B. subtilis* and 82.4-84.1% with *B. amyloliquefaciens*. These results indicate a sustained biocontrol effect that persisted throughout the growing season. Biometric parameters (height, number of leaves, root weight) increased by 20-40% compared to the control, which exceeded the effectiveness of the biological standard by 5-10%. All studies showed no toxicity, biological stress or soil degradation. Biological preparations maintained the balance of saprophytes (1.8×10^6 CFU/g), actinomycetes (1.9×10^6 CFU/g) and fungi (1.8×10^5 CFU/g), and the enzymatic activity of dehydrogenases reached 103% of the control. The limitation of the study is that the experiments were conducted only within one soil type and without analysing the influence of climatic factors. Further research should address the effectiveness of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in different agroecological zones and in combination with other microbial consortia.

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CONFLICT OF INTEREST

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Бактерії роду *Bacillus* як ефективний та безпечний засіб захисту рослин від патогенних мікроорганізмів

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Анотація. Метою дослідження було встановити взаємозв'язок між антагоністичною активністю штамів *Bacillus* та їх впливом на ріст рослин і мікробіологічну рівновагу ґрунту. У роботі застосовували комплекс мікробіологічних, фізіологічних і статистичних методів, зокрема метод агарових лунок для визначення антагонізму, метод серійних розведень для підрахунку колоній, трифенілтетразолій-хлоридний метод для оцінки активності дегідрогеназ і дисперсійний аналіз (ANOVA) для перевірки достовірності результатів. Показано, що *B. subtilis* стабільно перевищував *B. amyloliquefaciens* за інгібувальною здатністю: середні діаметри зон пригнічення були більшими на 10-15 %, а індекс пригнічення росту – на 5-8 %. Для *Fusarium oxysporum* зниження індексу ураження становило 33,0 процентних пункти (з 53,8 % до 20,8 %), для *Alternaria solani* – 29,0 процентних пунктів для *Pseudomonas syringae* – 27,1 процентних пункта. Частка здорових рослин збільшувалася з 51,5-56,3 % у контролі до 86,2-87,3 % при застосуванні *B. subtilis* і 82,4-84,1 % при *B. amyloliquefaciens*. Біопрепарати стимулювали ріст рослин: висота, кількість листків і маса кореня зростали на 20-40 % порівняно з контролем, перевищуючи ефективність біологічного стандарту на 5-10 %. Протягом експерименту не виявлено жодних ознак фітотоксичності чи деградації ґрунту. Біопрепарати підтримували стабільну чисельність сапрофітів ($1,8 \times 10^6$ КУО/г), актиноміцетів ($1,9 \times 10^6$ КУО/г) і грибів ($1,8 \times 10^5$ КУО/г), а активність дегідрогеназ досягала 103 % від контролю. Отримані результати підтвердили, що *Bacillus subtilis* і *Bacillus amyloliquefaciens* є ефективними антагоністами фітопатогенів і безпечними біостимуляторами, здатними забезпечити підвищення продуктивності та стабілізацію мікробіоценозу ґрунту. Їх застосування рекомендовано як екологічно стійку альтернативу хімічним фунгіцидам у системах сталого землеробства

Ключові слова: антагонізм; біоконтрольна дія; індекс ураження; екологічна безпека; ризосфера