

BIOFUNGICIDES BASED ON BACILLUS SUBTILIS AS A SAFE ALTERNATIVE TO CHEMICAL PLANT PROTECTION IN AGRICULTURE

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Statement of the problem. In modern agricultural production, the priority means of increasing yield still remains the use of chemical plant protection products. However, this practice is accompanied by serious environmental and biological risks – development of phytopathogen resistance, upset agrocenosis balance, reduced soil fertility and potential threat to human health. In the context of the search for environmentally safe alternatives, the use of biologics, in particular biofungicides based on bacteria of the genus *Bacillus*, is becoming more and more relevant. Particularly promising is *Bacillus subtilis*, a gram-positive spore-forming bacterium that not only inhibits the development of phytopathogens, but also takes an active part in soil biochemical processes, stimulating the growth and immunity of plants. Due to its high biological activity and environmental safety, biofungicides based on it can not only ensure effective protection of agricultural crops throughout the growing season, but also act as a component of sustainable agriculture.

Analysis of the latest research and publications. The current scientific literature confirms the growing interest in the use of biological plant protection products as an alternative to traditional chemical means. In a number of works of Ukrainian and foreign researchers, the ecological expediency of using biofungicides in agriculture is emphasized, especially in the conditions of strengthening requirements for the environmental safety of agricultural production. In his research, Sergienko V. G. and co-authors proved the high effectiveness of the use of biologics against a wide range of pathogens of agricultural crops. The results of the experiments proved not only a decrease in phytopathogenic load, but also an increase in yield and product quality. The authors emphasize that the use of biological preparations, as well as their combination with fungicides, contributes to the improvement of agrocenoses and ensures the production of more ecologically safe agricultural products [1].

In their research, Honchar A. M. & Patyka M. V. found that *Bacillus subtilis* strains are characterised by a high level of processability: during cultivation, these bacteria form a significant number of spores, the number of which remains stable for at least 60 days. This indicates the feasibility of their use in production and allows for long-term storage of biological products based on them. In addition, scientists have proved the prospects of using these strains to enhance the photochemical activity of winter wheat (*Triticum aestivum* L.) during ontogenesis, which is of great scientific and practical importance in the context of environmental monitoring, assessment of plant resistance and the introduction of biological agents in modern agricultural technologies [2].

In his publications, Serhii Khablak has thoroughly highlighted the prospects for the development of drugs capable of inducing systemic resistance of plants to pathogens. The researcher emphasizes the need to switch to ecologically oriented technologies for the protection of agricultural crops, which are based not only on the suppression of pathogens, but also on the activation of the natural defense mechanisms of the plants themselves, and also points to the importance of creating complex drugs of a new generation that would combine the ability to directly antagonize pathogens and at the same time – to activate natural resistance of plants [3].

Foreign scientists, Guillén-Navarro K. *et al.* have proven that the *Bacillus subtilis* subsp. *spizizenii* strain has a wide range of antimycotic effects. It is able to synthesise biosurfactants and bioemulsifiers that inhibit the growth of various phytopathogenic fungi. This makes this strain an effective biocontrol agent in plant protection and promising for bioremediation of soils contaminated with chemical residues. Scientists emphasize that this microorganism can be used as a natural means of improving the phytosanitary safety of agricultural systems [4].

Hadjouti R. *et al.* have shown that rhizobacteria associated with *Olea europaea* (olive) contribute to improved growth and recovery of crops, including *Phaseolus vulgaris* (bean) and *Cucurbita pepo* (pumpkin). These PGPRs (plant growth-promoting rhizobacteria) have been shown to increase plant resistance to stressful conditions, improve nutrient uptake and stimulate growth. This confirms their effectiveness as environmentally safe stimulants of crop productivity in the post-stress period [5].

Przemieniecki S. W. *et al.* proved that *Bacillus* strain SP-A9 effectively suppresses the development of pathogens in spring wheat (*Triticum aestivum* L.), while improving the morphometric parameters of plants. The biological product based on this strain provided a steady reduction of crop damage by phytopathogens and had a positive effect on yield. Thus, *Bacillus* SP-A9 has considered as a promising component of integrated crop protection [6].

These studies confirm that *Bacillus* and PGPR bacterial strains have significant potential as biocontrol agents and plant growth stimulants. They demonstrate an effective antifungal effect, improve the physiological state of crops, increase yields and can become an ecological alternative to synthetic agrochemicals in modern agriculture.

Research objective. The purpose of the study is to substantiate the feasibility of using biologics based on saprophytic bacteria, in particular *Bacillus subtilis*, in plant protection systems as an effective alternative to chemical fungicides, taking into account their biological activity,

environmental safety, impact on increasing grain sorghum yields, plant resistance to diseases and improving the state of agrophytocenoses.

Presentation of the main research findings. Among the methods aimed at increasing sorghum grain yields, rational soil cultivation and the application of biological products are of great importance, as they improve the soil's water, nutrient and air regime, and facilitate the control of weeds, pests and plant diseases.

Sorghum is a valuable cereal crop that combines unique biological properties and high economic performance. Among the key advantages of sorghum are its exceptional drought tolerance, tolerance to soil salinity and high productivity. Under conditions of prolonged moisture deficit, a protective silicon barrier is formed in the root system, which reduces evaporation and prevents drying out. During periods of soil or air drought, the plant can enter a state of anabiosis, a temporary slowdown in life processes with the possibility of their rapid activation after external conditions improve. Thanks to these adaptation mechanisms, sorghum is becoming a promising crop for growing in the face of global climate warming and limited water resources, while providing consistently high yields of marketable grain [7-11].

Studies on the feasibility of using biologics on grain sorghum crops were conducted in 2017-2020 on non-irrigated lands of the southern Steppe of Ukraine under conditions of natural moisture. The soils of the experimental plot are dark chestnut, medium loamy, slightly saline. The climate of the region is characterized as moderately hot and very arid, with insufficient rainfall in summer and its uneven annual distribution. To achieve the aim of the study, a two-factor field experiment was designed in a randomized split-plot design with four replications.

Factor A – grain sorghum hybrids: Sontsedar, Prime, Burggo, Sprint W, Dash E, Targa. Factor B – plant treatment options: pure water (control) and biological products.

The area of one plot was 56.0 m², the accounting area was 33.6 m².

Agronomic measures were carried out according to the recommendations for the Steppe zone, with adjustments to certain elements of the technology (hybrid, plant density, use of biological products).

Biologics used as biofungicides are based on selected natural strains of saprophytic bacteria. They are characterized by high biological activity and environmental safety for all components of the ecosystem – soil, plants, insects, animals and humans. Once in the natural environment, these microorganisms synthesize a wide range of antibiotic substances, enzymes and biologically active compounds that effectively inhibit the development of phytopathogenic bacteria and fungi. Due to their high reproductive capacity and activity, antagonist bacteria quickly colonize the soil substrate, participating in the transformation of organic matter, ammonification and nitrification processes. They also activate the mobilization of phosphorus and potassium, enriching the soil with nutrients available to plants. Biologically active metabolites produced by microorganisms in certain concentrations stimulate the growth and development of cultivated plants, increasing their immune resistance to pathogens.

Bacillus subtilis is a Gram-positive, spore-forming, aerobic soil bacterium. It was originally described in 1835 by Ehrenberg as *Vibrio subtilis*, and in 1872 was renamed *Bacillus subtilis* by Kohn. The name 'hay bacillus' was given to it because the accumulative cultures of this microorganism are obtained from hay extract. It is a producer of some polypeptide antibiotics and industrially produced enzymes (amylase, protease). It is a rod-shaped bacterium, 3-5 × 0.6 µm in size. Spores are oval, not exceeding the size of a cell, centrally located. Peritrichial arrangement of flagella, mobile. Colonies are dry, finely wrinkled, velvety, colorless or pinkish. The edge of the colony is wavy. It grows on MPA, MPB, as well as on media containing plant residues, simple synthetic nutrient media for heterotrophs. It is an organo-chemorhizal heterotroph, ammonifies proteins, breaks down starch and glycogen [12].

Biofungicidal preparations based on saprophytic bacteria are made from selected natural strains characterised by high biological activity and environmental safety for all environmental components – soil, plants, insects, animals and humans. Once in the agro-ecosystem, these microorganisms produce a wide range of biologically active compounds, including antibiotic substances and enzymes that inhibit the development of pathogenic bacteria and fungi.

Due to their rapid reproduction and high adaptability, microbial agents effectively colonise the soil environment, activating the processes of organic matter mineralisation, ammonification, nitrification, and mobilisation of phosphorus and potassium into readily available forms. As a result, soil fertility is improved and balanced nutrition of cultivated plants is ensured. In addition, biologically active metabolites synthesized by bacteria in certain concentrations help to intensify growth processes and build plant resistance to infectious diseases.

Among the wide range of biological plant protection products in agriculture, a special place is occupied by biofungicides based on strains of *Bacillus subtilis* (hay bacillus). The most well-known products of this type include: Fitosporin, Baxis, Alirin, Bactofit, Gamair and others. The use of such biological products provides a long-lasting protective effect throughout the entire growing season.

The treatment of seeds and vegetative organs of crops with *Bacillus*-containing preparations promotes the accumulation of active bacteria in plant tissues, which creates a stable protective barrier against pathogens. An important advantage of these products is their resistance to ultraviolet radiation, which ensures that they remain active even after prolonged exposure to the plant surface. To maintain the effectiveness of the product during storage, oxygen is required, as bacteria are aerobes.

Bacillus-based biofungicides have a dual function: on the one hand, they provide biological control of bacterial, fungal and viral pathogens, and on the other hand, they stimulate plant growth and boost plant immunity. Thanks to the combination of *Bacillus subtilis* and *Bacillus stearothermophilus* strains, these products demonstrate a synergistic effect that significantly increases their biological effectiveness and surpasses both domestic and foreign analogues, including traditional chemical fungicides, in a number of respects.

In the technological cycle of grain sorghum cultivation, a key role is played by the phased application of biological products in combination with nutrient medium and microelements, which ensures increased plant resistance, optimized nutrition and activation of soil microflora. Data on the use of biologics are shown in Table 1.

Regarding the productivity of agrophytocenoses formed under the influence of such biologics, it can be argued that the improved technological elements of grain sorghum cultivation are sufficiently effective. Thus, over the years of observation, no facts have been established when the introduction of biologics led to a decrease in sorghum yields compared to the technological schemes used on farms. On the positive side, the use of biologics can eliminate the use of synthetic fungicides, improve the immune status of plants and eliminate the impact of abiotic stressors. The profitability of sorghum cultivation in traditional farms ranged from 61-76%, while the introduction of biologically based technology elements varied from 66-90%.

According to the research results, pre-sowing tillage, pre-sowing seed treatment, and treatment of grain sorghum plants during the growing season with biologics proved to be a highly effective measure aimed at increasing grain productivity of the crop (Table 2).

On average, over the years of research, in the areas where biologics were used, the grain yield of the crop was 5.85 t/ha, in the variants where plants were treated with pure water – 5.21 t/ha, and in the control areas – 5.14 t/ha, which allows us to conclude that the use of biologics has a significant positive impact on the yield of grain sorghum and a noticeable effect of the refreshing effect of water in the formation of the generative part of the plant yield.

In all hybrids, we noted a pattern according to which the use of biologics increased the seed productivity of the crop compared to the untreated control: Sontsedar hybrid – by 0.73 t/ha or 14.5%; Prime – by 0.68 t/ha or 14.3%; Burggo – by 0.78 t/ha or 15.2%; Sprint W – by 0.43 t/ha or 13.2%; Dash E – by 0.86 t/ha or 12.5% and in areas where Targa hybrid was sown, the increase was 0.76 t/ha or 13.2%.

Conclusions. Agricultural enterprises of the Southern Steppe zone of Ukraine, specialising in the production of grain crops, are recommended to sow grain sorghum at a soil temperature of 8-10 °C at a seeding depth of 2-4 cm, forming a plant density in the agrophytocenosis at the level of 160 thousand units/ha with hybrids Sontsedar and Dash E. The use of biologics is an effective technological element that allows to significantly (by 0.7-0.8 t/ha) increase the grain productivity of the crop.

Table 1

Scheme of application of biologics in grain sorghum cultivation

| Terms of use of the biologics | Application rate |
|---|---|
| Pre-sowing seed treatment (2-3 days before sowing, but not more than 10 days) | 1 l of the biological product + complex of microelements per 1 t of seeds |
| Pre-sowing soil cultivation with mandatory incorporation of the product (for pre-sowing cultivation, harrowing) | 1 l/ha biological product + nutrient medium (for the vital activity of the bacterial complex) |
| Beginning of tillering | 2 l/ha biological product + nutrient medium |
| Stage of intensive vegetative growth – appearance of the apical leaf | 1 l/ha biological product + nutrient medium + complex of microelements |
| Formation of panicle | 1 l/ha biological product + nutrient medium |
| Milk ripeness | 2 l/ha biological product + nutrient medium + complex of microelements |

Source: compiled by the author

Table 2

Influence of biological treatment on grain yield of grain sorghum hybrids (soil t 8-10 °C), t/ha

| Hybrid (factor A) | Method of plant treatment (factor B) | | | | |
|------------------------|--------------------------------------|---------------------------|----------------|------------------------------------|----------------|
| | no-till – control | clean water as a backdrop | + - to control | treatment with biological products | + - to control |
| Sontsedar | 5.04 | 5.12 | 0.08 | 5.77 | 0.73 |
| Prime | 4.76 | 4.85 | 0.09 | 5.44 | 0.68 |
| Burggo | 5.14 | 5.22 | 0.08 | 5.92 | 0.78 |
| Sprint W | 3.25 | 3.30 | 0.05 | 3.68 | 0.43 |
| Dash E | 6.88 | 6.95 | 0.07 | 7.74 | 0.86 |
| Targa | 5.77 | 5.82 | 0.05 | 6.53 | 0.76 |
| Average in culture | 5.14 | 5.21 | | 5.85 | |
| SED ₀₅ t/ha | A | 0.63-0.94 | | | |
| | B | 0.39-0.66 | | | |
| | AB | 0.9-1.7 | | | |

Source: compiled by the author

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Boiko M.O. Biofungicides based on *Bacillus subtilis* as a safe alternative to chemical plant protection in agriculture

The purpose of the study is to substantiate the feasibility of using biological preparations based on saprophytic bacteria, in particular *Bacillus subtilis*, in plant protection systems as an effective alternative to chemical fungicides, taking into account their biological activity, environmental safety, impact on increasing the yield of grain sorghum, plant resistance to diseases and improving the state of agrophytocenoses. The study employed a comprehensive set of **methods**, including literature review, field trials, agrocenosis assessment, and statistical data analysis. **Results.** Biologics based on *Bacillus subtilis* are today considered as a promising tool for ecologically safe and effective plant protection, meeting the requirements of sustainable agriculture, contributing to increased crop yield and being an urgent response to modern challenges related to climate change, soil degradation and the need to reduce the chemical burden on agroecosystems. The article examines the potential of using biofungicides based on *Bacillus subtilis* bacteria as a safe and effective alternative to chemical plant protection products in modern agriculture. The relevance of the topic is due to the need to reduce the pesticide load on agroecosystems, increase the environmental safety of agricultural production and preserve soil fertility. The article proposes an optimized scheme for the use of biologics based on *Bacillus subtilis* in the cultivation of grain sorghum. The conducted studies confirm that the treatment of grain sorghum seeds with biofungicides based on *Bacillus subtilis* contributes to the activation of plant defense mechanisms, the formation of healthy agrophytocenoses, as well as the growth of crop yield. Particular attention is paid to the analysis of the ecological feasibility of biological protection of

plants, in particular its safety for the environment and people, in contrast to chemical fungicides, which have a negative residual effect. **Conclusions.** The obtained results indicate the prospects of integrating biofungicides based on *Bacillus subtilis* into the system of sustainable agricultural production and increasing the competitiveness of agriculture in the conditions of modern environmental challenges.

Key words: biofungicides, *Bacillus subtilis*, ecological feasibility, biological protection, yield increase

Бойко М.О. Біофунгіциди на основі *Bacillus subtilis* як безпечна альтернатива хімічному захисту рослин у сільському господарстві

Метою дослідження є обґрунтування доцільності застосування біопрепаратів на основі сапрофітних бактерій, зокрема *Bacillus subtilis*, у системах захисту рослин як ефективної альтернативи хімічним фунгіцидам, з урахуванням їх біологічної активності, екологічної безпеки, впливу на підвищення врожайності сорго зернового, стійкості рослин до хвороб та покращення стану агрофітоценозів. У дослідженні використано комплекс **методів**, зокрема аналіз літературних джерел, польові випробування, оцінка агрофітоценозів і статистична обробка даних. **Результати.** Біопрепарати на основі *Bacillus subtilis* сьогодні розглядаються як перспективний інструмент екологічно безпечного та ефективного захисту рослин, що відповідає вимогам сталого сільського господарства, сприяє підвищенню врожайності культур та є актуальною відповіддю на сучасні виклики, пов'язані зі зміною клімату, деградацією ґрунтів і необхідністю скорочення хімічного навантаження на агроєкосистеми. У статті досліджено потенціал використання біофунгіцидів на основі бактерій *Bacillus subtilis* як безпечної та ефективної альтернативи хімічним засобам захисту рослин у сучасному сільському господарстві. Актуальність теми зумовлена необхідністю зниження пестицидного навантаження на агроєкосистеми, підвищення екологічної безпеки виробництва сільськогосподарської продукції та збереження родючості ґрунтів. У статті запропоновано оптимізовану схему застосування біологічних препаратів на основі *Bacillus subtilis* при вирощуванні сорго зернового. Проведені дослідження підтверджують, що обробка насіння сорго зернового біофунгіцидами на основі *Bacillus subtilis* сприяє активізації захисних механізмів рослин, формуванню здорових агрофітоценозів, а також зростанню урожайності культури. Особливу увагу приділено аналізу екологічної доцільності біологічного захисту рослин, зокрема його безпечності для довкілля та людей, на відміну від хімічних фунгіцидів, які мають негативний залишковий вплив. **Висновки.** Отримані результати свідчать про перспективність інтеграції біофунгіцидів на основі *Bacillus subtilis* у систему сталого агровиробництва та підвищення конкурентоспроможності сільського господарства в умовах сучасних екологічних викликів.

Ключові слова: біофунгіциди, *Bacillus subtilis*, екологічна доцільність, біологічний захист, зростання урожайності

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