

AGROSYM

# BOOK OF PROCEEDINGS



*XV International Scientific Agriculture Symposium  
"Agrosym 2024"  
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## APPLICATION OF BACILLUS SPP. IN PLANT PROTECTION AND GROWTH PROMOTION OF CEREALS

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### Abstract

The research objective is the application of new bacterial isolates as an alternative to chemical pesticides for controlling plant diseases caused by *Fusarium* spp., aiming to reduce reliance on conventional interventions, minimize environmental impacts, thus fostering a shift towards environmentally friendly plant disease management strategies. In this study, the effects of four *Bacillus* spp. (BHC 2.3, BHC 2.4, BHC 4.5, BHC 4.7) isolated from two soil samples on suppressing the *Fusarium* spp. plant pathogens (*F. poae*, *F. proliferatum*, *F. oxysporum* and *F. graminearum*), as well as their effect on seed germination (wheat, oat and barley) during the fungal infection were determined. Basic chemical analyses of soil samples used for bacterial isolation and plant growth promoting experiments were performed. Antifungal activity of isolates was tested *in vitro* on PDA medium, seed germination test was performed in Petri dishes on filter paper, while the ability of isolates to promote plant growth under semi-controlled conditions was also tested. Inhibition percentage of mycelial growth of *F. poae* was up to 20% (for isolate BHC 4.5) and up to 15% (for isolate BHC 4.7). Seed germination test indicated the effectiveness of seed inoculation by selected bacterial isolates. In the case of infected seeds, germination percentage was up to 80% (wheat) and up to 90% (barley and oat), without bacterial inoculation. On the other hand, germination percentage for infected seeds inoculated by all four applied isolates was increased. Results of the experiment performed under semi-controlled conditions showed that bacterial isolate BHC 4.5 had the best overall effect on the shoot length of all used cereals. The results of this study indicate the potential of different *Bacillus* spp. soil isolates for formulating new biofertilizers to be used in plant protection and plant growth promotion of cereals.

**Keywords:** *Bacillus* spp., PGP bacteria, seed germination, oat, barley and wheat seedling shoots.

### Introduction

The effects of climate change and global warming has a strong impact on the agricultural sector. Higher air temperatures, relatively less rainfall, the rarity of continuous rainfall, the frequency of torrential rains can promote more frequent plant infections caused by pathogens (Modrzewska et al., 2022). A warmer climate and frequent extreme phenomena increase the incidence of pathogens, mostly fungi of the *Fusarium* genus, which are responsible for causing fusariosis in cereals (Siebold and von Tiedemann, 2012, West et al., 2012). The most commonly grown cereal plants include maize, wheat and rice (FAO, 2021) and diseases of these cereals result in a loss of nearly 1/6 of crops before harvest (Popp and Hantos, 2011). The major *Fusarium* species that infect many crops and represent some of the most critical soil-borne plant pathogen and are responsible for massive crop losses include *F. oxysporum*, *F. proliferatum* and *F. poae* (Baard et al., 2023).

Synthetic pesticides have a constant importance for protection of cereal crops, and chemical control is one of the most commonly used methods for preventing crops diseases. However, conventional plant protection strategies are costly and have been the subject of debate due to the possible soil pollution and the presence of residues in food (Braun et al., 2018; Ntalli & Menkissoglu-Spiroudi, 2011). Biological control has been considered as an effective alternative method to chemical control, as it poses no health risks and has an insignificant impact on the environment (Xu et al., 2020).

Rhizosphere soils from healthy plants that survived in a plot infested with phytopathogens are a good source for the isolation of microorganisms with biostimulator and biopesticides potential (Modrzewska et al., 2022, Huang at al., 2013). Using plant growth-promoting rhizobacteria (PGPR) in biological control of phytopathogens represent an eco-friendly strategy which is reported in variety of research papers (Mulk et al., 2022, Sharma et al., 2020). Microorganisms offer many advantages, as they can improve the overall fitness of plants by promoting their growth and resistance to abiotic stress and promote the suppression of pathogens (Modrzewska et al., 2022). The commercial application of bioinoculants is still limited, due to a lack of consistent field efficacy and poor product shelf life (Mulk et al., 2022, Valente et al., 2020).

The variety of microorganisms that might potentially be used as biological agents and promoters of cereal crop growth include bacteria and fungi, and they have proven to be effective in reducing fungal infection in the greenhouse and field trials (Modrzewska et al., 2022, Huang at al., 2013). Various PGPRs have been successfully isolated from the rhizosphere soils of healthy plants and the most studied ones belong to *Bacillus*, *Pseudomonas* and *Streptomyces* genus (Huang at al., 2013).

The objective of this paper is to test and compare biocontrol and plant growth promoting potential of bacteria isolated from two rhizosphere soil samples with different acidity. Biocontrol potential experiments against four *Fusarium* species is conducted, while the plant growth promoting potential of bacterial isolates through the seed germination test and seedling parameters of tree cereal plants (oat, wheat and barley) is also examined.

## Material and Methods

The isolation of bacterial isolates was conducted from soil sampled in the territory of Lazarevac (soil sample BHC 2) and Smederevska Palanka (soil sample BHC 4). The basic soil chemical parameters are shown in Table 1:

Table 1. Basic soil chemical parameters

| Soil sample | pH   |                  | CaCO <sub>3</sub><br>% | P <sub>2</sub> O <sub>5</sub><br>mg·100 g <sup>-1</sup> | K <sub>2</sub> O | Humus content<br>% |
|-------------|------|------------------|------------------------|---|------------------|--------------------|
|             | KCl  | H <sub>2</sub> O |                        |   |                  |                    |
| BHC 2       | 5.49 | 7.33             | < LOD*                 | 2.94  | 25.9             | 2.59               |
| BHC 4       | 7.80 | 8.37             | 13.2                   | 28.4  | 39.3             | 3.79               |

\*The limit of detection (LOD) 0,04%

For the isolation of bacteria from soil samples, a series of decimal dilutions of the soil suspension from 10<sup>-2</sup> to 10<sup>-6</sup> was prepared. To ensure the isolation of bacteria from the genus *Bacillus*, tubes with dilutions from 10<sup>-4</sup> to 10<sup>-6</sup> were incubated for 15 min at 80 °C in a water bath. One ml of these dilutions was then inoculated onto Petri dishes with Nutrient agar. Petri dishes were incubated at 28 °C for 72 h, and pure cultures of bacterial isolates were obtained by several rounds streaking on Nutrient agar. The obtained pure cultures were stored on slanted Nutrient agar at 4 °C.

The antagonistic effect of bacterial isolates against different *Fusarium* species (*F. poae*, *F. proliferatum*, *F. oxysporum* and *F. graminearum*) was evaluated on potato dextrose agar (PDA). Fungi used for testing the antagonistic activity of bacterial isolates were cultured on PDA medium for 72 h at 28 °C, from which sterile plugs of fungal mycelium (approximately 2 mm in diameter) were excised for further use. A plug of fungal mycelium was placed in the centre of a Petri dish, and 20 µL of overnight bacterial cultures was inoculated approximately 1 cm from the edges of the Petri dish (on two sides of the Petri dish). Petri dishes were then incubated at 28 °C, and the results were obtained after 7 days of incubation. After incubation, the diameter of fungal colony growth was measured in millimetres and compared with the bacteria-free control. Tests were conducted in triplicate, and the antagonistic activity of bacterial isolates was determined based on the following equation (Ogbebor & Adekunle, 2005): Inhibition (%) = [(Fungal control (mm) - Treatment (mm))/Fungal control (mm)] x 100.

The ability of bacterial isolates to induce germination of wheat (*Triticum aestivum*), oat (*Avena sativa*) and barley (*Hordeum vulgare*) seeds (infected and non-infected by *F. poae*, *F. proliferatum*, *F. oxysporum* and *F. graminearum*) was accessed *in vitro* on Petri dishes by using filter paper method. Ten seeds of each plant species were soaked in over-night bacterial culture for each isolate (BHC 2.3, BHC 2.4, BHC 4.5 and 4.7), while non-inoculated seeds were used as the control sample. Further, seeds infected with each fungal pathogen were used as fungal infection control, while the seeds infected by fungi and inoculated by each bacterial isolate were also used. Petri dishes were kept for two weeks in a transparent sealed box. Relative seed germination index (RSGI%) was estimated as described by Knezevic et al. (2021). All tests were done in three independent replications.

For PGP test, seeds of wheat, oat and barley were placed in seedling trays (three seeds per one hole) in triplicates. The following treatments were applied: control (Ø, no fungal or bacterial inoculation), fungal control (FØ, fungal infection), bacterial inoculation by four isolates (BHC 2.3, BHC 2.4, BHC 4.5 and BHC 4.7), as well as mixed inoculation by *F. poae* and bacterial isolates (F-BHC 2.3, F-BHC 2.4, F-BHC 4.5 and F-BHC 4.7) for each cereal. The experiment was kept under semi-controlled conditions for 21 days, after which the shoots of all seedling were measured.

## Results and Discussion

The soils used in this research (BHC 2 and BHC 4) belong to a group between acid and moderately acidic (pH value in KCl 5.49) and alkaline (pH value in KCl 7.80) soils, respectively (Manojlović et al., 1969). A total of four *Bacillus* spp. isolates (BHC 2.3, BHC 2.4, BHC 4.5 and BHC 4.7) isolated from soil samples were used in this research. In literature, effectiveness of different *Bacillus* species has been previously demonstrated against *F. graminearum*, *F. poae* and other fusarium head blight (FHB) causative agents in cereals, such as barley and wheat (Mischler et al., 2024; Zanon et al., 2024). Among tested fungal pathogens, used bacterial isolates showed the strongest antagonistic effect against *F. poae*. The percentage of *F. poae* inhibition was 20%, 15% 12% and 7% by BHC 4.5, BHC 4.7, BHC 2.4 and BHC 2.3, respectively. In addition, BHC 4.7 and BHC 4.5 showed weak antagonistic effect against *F. graminearum* (Figure 1). Similarly, Bjelic et al. (2017) reported effectiveness of *Bacillus* spp. isolated from the soils of Province of Vojvodina against *F. proliferatum* and *F. oxysporum*.

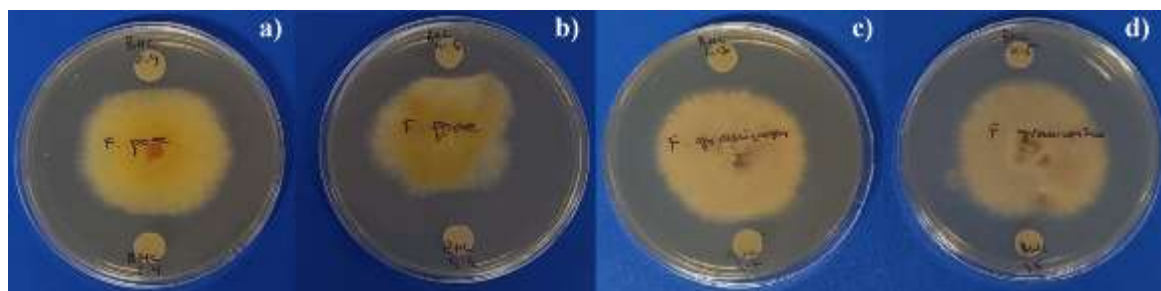


Figure 1. Antifungal potential of *Bacillus* spp. isolates against *F. poae* and *F. graminearum*. a) BHC 2.4 + *F. poae*; b) BHC 4.5 + *F. poae*; c) BHC 4.7 + *F. graminearum*; d) BHC 4.5 + *F. graminearum*

Relative seed germination index percentage (RSGI, %) of seeds infected by *F. poae* was 70% (barley), 75% (oat) and 37.5% (wheat). Application of all four bacterial treatments increased RSGI of all cereals. BHC 2.4 treatment increased seed germination for infected barley and oat seeds, with the strongest effect on wheat seeds, in comparison to the uninfected control. Furthermore, application of all bacterial treatments (except F-BHC 2.3 in oat) increased the root length of all cereals, in comparison to infected control (Figure 2).

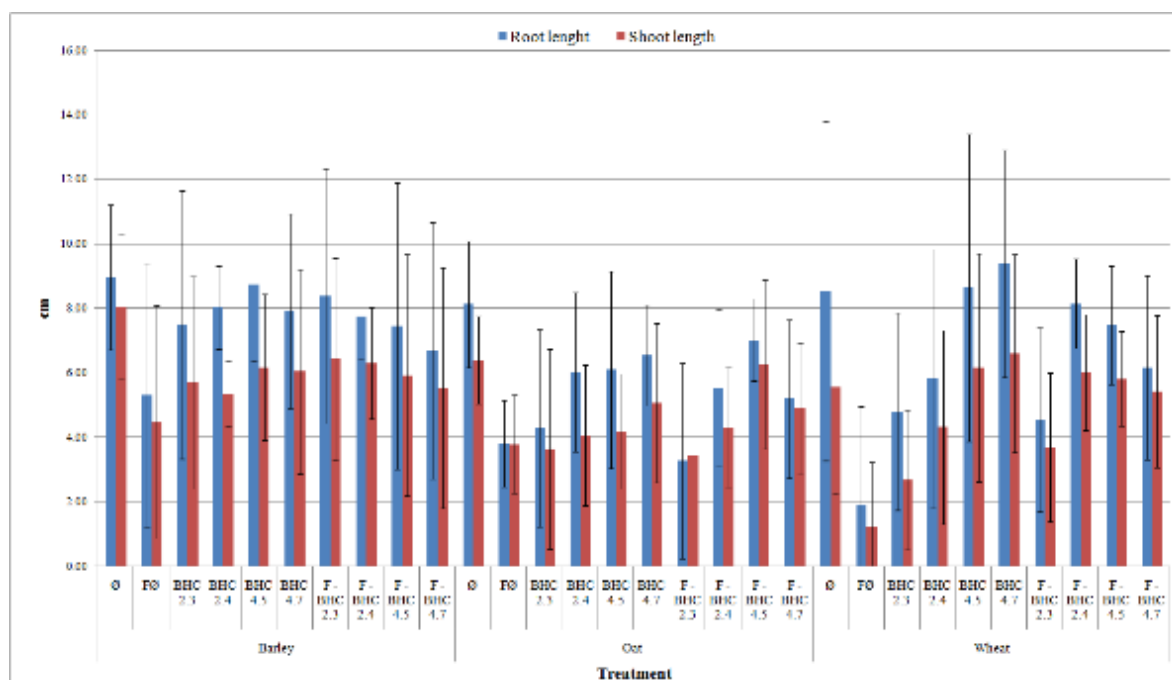


Figure 2. Impact of bacterial treatment/fungal infection on root and shoot length of barley, oat and wheat seedlings.

Similar results were obtained for the shoot length, where only treatments BHC 2.3 and F-BHC 2.3 had values of shoot length (oat) lower than infected control. Previously, it has been shown that the application of *Bacillus* spp. improved soybean seed quality compared to control, as well as the germination of garden pea seeds in the presence of *F. proliferatum* infection (Miljaković et al., 2022, Miljaković et al., 2024). In addition, *B. pseudomycoides* improved seed germination of alfalfa in the presence of *F. oxysporum* (Knezevic et al., 2021). Further, PGP test showed that applied bacterial treatments increased fresh plant biomass of infected wheat, barley and oat in comparison to the fungal control (FØ). The highest increase of fresh biomass in comparison to the infected control was recorded by BHC 4.5 treatment for oat (18.43%), followed by wheat (14.04%) and barley (2.35%), while the other bacterial

treatments increased fresh biomass in a range from 0.31 (wheat) - 3.70% (barley). Similar, it has been shown that PGP bacteria (most commonly from *Bacillus*, *Brevibacterium*, *Pseudomonas* and *Arthrobacter* genus) can increase biomass, leaf area, plant height, root length, dry weight and fresh weight of different cereals including wheat, barley, maize and rye (Wijekoon and Weerasinghe, 2024). The results of this research confirm positive effects of bacterial inoculation and the possibility of using *Bacillus* spp. for improving seed germination and seedling biomass infected by *Fusarium* species.

### Conclusions

In conclusion, this study demonstrates the promising potential of selected *Bacillus* spp. soil isolates as effective alternatives to chemical pesticides for managing plant diseases caused by *Fusarium* spp. The isolates, particularly BHC 4.5, exhibited notable antifungal activity against *Fusarium* pathogens while also promoting seed germination and enhancing plant growth in wheat, oat, and barley under semi-controlled conditions. These findings highlight the feasibility of utilizing these bacterial isolates in formulating biofertilizers, thereby offering sustainable and environmentally friendly strategies for plant disease management and crop enhancement in cereals. By reducing reliance on chemicals, these bio-based approaches hold significant promise for mitigating environmental impacts associated with conventional pesticide use allow a transition towards more eco-friendly agricultural practices.

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