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IMPACT OF *BACILLUS MYCOIDES* INOCULATION ON GROWTH AND DEVELOPMENT OF MAIZE (*ZEA MAYS* L.)

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Abstract

The increasing demand for sustainable agricultural practices highlights the importance of biofertilizers and biocontrol agents as alternatives to chemical inputs. This study evaluates the potential of *Bacillus mycoides*, a rhizosphere-associated bacterium, to promote maize (*Zea mays L.*) growth and enhance plant resistance under controlled experimental conditions. A soil-based inoculation experiment was conducted using maize hybrid seeds grown in sterile substrate, with treatments including control and *B. mycoides* inoculation (10^8 CFU mL^{-1} applied at sowing and biweekly thereafter). Growth parameters such as germination rate, plant height, leaf number, stem diameter, and fresh and dry biomass of shoots and roots were recorded and statistically analysed (ANOVA, $p < 0.05$). The results demonstrated that *B. mycoides* significantly improved maize growth performance compared to the control. Treated plants exhibited increased stem elongation, greater leaf area, enhanced root development, and higher cob size and grain number. Inoculated plants showed improved tolerance to the fungal pathogen *Fusarium graminearum*, completing their life cycle successfully despite infection pressure. Biochemical assays confirmed the ability of *B. mycoides* to produce indole-3-acetic acid (IAA), supporting its classification as a plant growth-promoting bacterium (PGPB). These findings highlight the potential application of *B. mycoides* as a biofertilizer and biocontrol agent in maize cultivation. By stimulating growth, improving nutrient uptake, and enhancing resistance to pathogens, this bacterium represents a promising tool for sustainable crop production. Further field-scale studies are required to validate these results and assess their integration into agroecological practices.

Keywords: *Bacillus mycoides*, plant growth-promoting bacteria (PGPB), biofertilizer, maize, *Fusarium graminearum*

INTRODUCTION

Maize occupies a strategic position in Bulgaria's agricultural sector. According to data from 2022, it represents approximately 12–13% of the total value of agricultural production, ranking among the most important field crops in the country. Over the past two decades, a steady expansion of maize-growing areas has been observed—from about 214,000 ha in 2007 to over 580,000 ha in 2020. In parallel, average yields have increased, reaching 700–800 kg per dka (≈7–8 t/ha) in favourable years. One of the most significant pathogens affecting maize is *Fusarium graminearum*, which causes diseases such as ear and stalk rot. This pathogen is widely distributed geographically and leads to substantial economic losses due to reduced yields and contamination of grain with mycotoxins (Bryła et al., 2022). The economic and health importance of *F. graminearum* stems from its ability to produce toxic secondary metabolites. Biological control of *F. graminearum* relies on the use of antagonistic microorganisms such as *Trichoderma spp.* and *Bacillus spp*., which have shown promising results in suppressing the pathogen and reducing mycotoxin levels (Moonjely et al., 2023). Despite significant progress in understanding the ecology and epidemiology of *F. graminearum*, challenges remain in developing stable and resistant maize hybrids, as well as applying integrated strategies that combine genetic, agronomic, and biological approaches (Lipps et al., 2025). Inoculation with *B. mycoides* increases tolerance of wheat, maize, and rice to abiotic stress. The bacterium stimulates plant growth and enhances tolerance to heavy metals, offering potential for higher yields and improved crop quality. These characteristics underpin the growing interest in *B. mycoides* in the context of sustainable agriculture and biotechnology. It is capable of synthesizing indole-3-acetic acid (IAA) and other phytohormones that stimulate root system development and improve nutrient uptake (Miljaković et al., 2020). In addition, some strains possess the ability to solubilize phosphates and minerals, increasing their availability to plants and supporting growth under nutrient-limited conditions (Sharma et al., 2013). Neher et al. (2009) demonstrated that the *B. mycoides* strain BmJ effectively suppresses cucumber anthracnose caused by *Colletotrichum orbiculare*. Huang et al. (2018) showed that *B. mycoides* produces volatile organic compounds that inhibit *Rhizoctonia solani* and *Pythium aphanidermatum*, pathogens responsible for damping-off in cabbage seedlings. In mixed applications with yeasts such as *Pichia guillermondii*, *B. mycoides* improves strawberry resistance and reduces disease incidence (Di Francesco et al., 2015). The aim of the present study is to investigate the influence of the bacterium *Bacillus mycoides* on maize growth and development and to assess its potential for application as a biofertilizer and biocontrol agent in sustainable agriculture.

MATERIALS AND METHODS

For routine maintenance and investigation of *Bacillus mycoides* strains, nutrient media such as tryptic soy broth (TSB) and tryptic soy agar (TSA) were used, as they provide optimal growth conditions and characteristic colony morphology.

Quantitative determination of indole-3-acetic acid using the Salkowski reagent

For the quantitative determination of the produced indole-3-acetic acid (IAA), bacterial isolates were grown in test tubes in medium with or without 0.1% (w/v) L-tryptophan (L-Trp) and incubated in the dark at 30 °C for 5 days. One millilitre of the culture was pelleted by centrifugation at 3000 g for 5 minutes, and 0.5 mL of the supernatant was mixed with 0.5 mL of Salkowski reagent (2 mL of 0.5 M iron (III) chloride and 98 mL of 35% perchloric acid) (Gordon & Weber, 1951). After 30 minutes, colour development (red) was quantified using a spectrophotometer (Unico 1200, USA) at 530 nm. A calibration curve prepared with pure indole-3-acetic acid was used to calculate the IAA concentration. IAA production by each bacterial isolate was also determined by inoculating medium containing 0.1% (w/v) L-tryptophan and incubating it in the dark at 28 °C. After incubation, to evaluate the biosynthetic potential of *Bacillus mycoides* to produce secondary metabolites with phytohormonal activity, the strain was cultivated in liquid nutrient medium containing glucose as a carbon source, yeast extract and peptone as nitrogen sources, as well as 0.1% (w/v) L-tryptophan as a precursor for IAA synthesis. The cultures were incubated at 30 °C and 150 rpm for 48 hours, and the produced IAA was quantified spectrophotometrically.

Screening for inorganic phosphate solubilization and ZnO solubilization

Screening for inorganic phosphate solubilization and ZnO solubilization was performed using Pikovskaya's medium (PVK) (Pikovskaya, 1948). From a 24-hour culture of the isolates, 100 µL were dispensed into 10 mm wells on Petri dishes containing the medium and incubated at 30 °C for 72 hours. The diameter of the colony and the halo zones were recorded. The solubilization index was calculated using the formula: diameter of the clear zone / diameter of the colony (Ramesh et al., 2014).

Testing the antimicrobial activity against mould fungi of the genus Fusarium graminearum

The investigated bacterial strains were tested to determine their antifungal activity against *Fusarium graminearum* NBIMCC 2214. *Fusarium graminearum* was cultured at 25 °C on YEPD medium for 7 days. The fungal conidia were then collected by washing with cold sterile distilled water and used to prepare an inoculum with a concentration of 1×10^6 spores/mL. The tests for antimicrobial activity were performed using the agar diffusion method (Zhao et al., 2014).

Investigation of the effect of Bacillus mycoides on the growth and development of maize

To investigate the effect of *Bacillus mycoides* on maize growth and development, pot experiments were conducted under controlled conditions. Hybrid maize seeds were used as plant material and sown in plastic pots (5 L) filled with sterile substrate (peat: perlite mixture, 3:1). The experiment was arranged in a completely randomized block design with four replications. The following variants were tested: control (no bacteria) and *B. mycoides* – applied to the soil through watering.For inoculum preparation, the *Bacillus mycoides* strain was cultured on

TSA/TSB at 30 °C, and the bacterial suspension was standardized to 10^8 CFU mL^{-1}. For soil application, the suspension was applied at sowing and every 14 days after germination. During the vegetation period, the plants were grown under optimal conditions (25 ± 2 °C, 14 h photoperiod), with regular watering and uniform background fertilization.Throughout the pot experiments conducted between 01.06.2025 and 13.09.2025, the development of the plants and the visual changes resulting from treatment with *B. mycoides* and infection with *Fusarium graminearum* were observed and recorded.

Statistical analysis

Statistical analyses were performed using R software (version 4.3.2) and independently verified using SPSS/Statistica. All analyses were conducted on individual biological replicates (n) for each treatment. Outliers were considered only when methodologically justified and prior to unblinding of treatment groups.

RESULTS

The colonies obtained from the *Bacillus mycoides* isolate were white to creamy, matte, with characteristic mycelium-like, radial spreading, which is a distinctive morphological feature of the species. Molecular identification (16S rRNA sequencing) confirmed that the isolate belongs to the species *Bacillus mycoides*, with an accession number deposited in GenBank.Molecular identification (16S rRNA gene sequencing) confirmed the isolate as *Bacillus mycoides*; the corresponding sequence has been deposited in GenBank and will be made publicly available upon accession number assignment.

Quantitative determination of indole-3-acetic acid (IAA) using the Salkowski reagent

The results showed that *B. mycoides* is a strong producer of IAA, with concentrations in the culture filtrate significantly exceeding the values recorded for other soil isolates. This is consistent with literature reports indicating that strains of the genus *Bacillus* can be highly active producers of phytohormones that promote plant development (Spaepen, 2015; Spaepen & Vanderleyden, 2011).

Table 1. Indole-3-acetic acid (IAA) production by *Bacillus mycoides* and comparative isolates, determined using Salkowski's reagent after 48 h of cultivation at 30 °C and 150 rpm.

strain / isolate	**growth conditions**	**IAA concentration (μg mL^{-1})**	**characteristic**
Bacillus mycoides	TSB + 0,2% L-tryptofan	42.8 ± 2.1	high

Screening for Inorganic Phosphate and ZnO Solubilization

After incubation at 30 °C for 7 days, a clearly defined halo zone of transparency was observed around the bacterial colonies. The diameter of the halo ranged from 12 to 15 mm, indicating efficient solubilization of inorganic phosphates. The solubilization index (SI) reached values between 2.0 and 2.5, classifying *B. mycoides* as an active phosphate-solubilizing isolate. The results also showed the formation of halo zones around *B. mycoides* colonies with an average diameter of 8–10 mm, demonstrating the strain's ability to mobilize zinc from poorly soluble forms, albeit with lower efficiency compared to phosphate solubilization. These findings confirm that *Bacillus mycoides* possesses a well-expressed potential for mobilizing poorly available mineral forms, particularly phosphorus. Its capacity to solubilize inorganic phosphates and, to some extent, ZnO, highlights the potential application of this species as a biofertilizer agent that enhances plant nutrient status by increasing the availability of key elements such as phosphorus and zinc.

Table 2. Activity of *Bacillus mycoides* in solubilizing inorganic phosphates and ZnO on selective media (Method = (Colony diameter + Halo zone diameter) / Colony diameter)

Tested medium	**Colony diameter (mm)**	**Halo zone diameter (mm)**	**Solubilization index (SI)***	**Activity assessment**
Pikovskaya agar ($Ca_3(PO_4)_2$)	6.0 ± 0.5	12.5 ± 1.0	2.1	high
NBRIP agar(inorganic phosphate)	5.5 ± 0.4	11.0 ± 0.8	2.0	high
ZnO agar	5.8 ± 0.3	8.0 ± 0.6	1.4	Average

Antimicrobial Activity of Bacillus mycoides Against Fusarium graminearum

After incubation at 25 °C for 7 days, a clearly defined inhibition of *Fusarium graminearum* growth was observed in the interaction zone with *B. mycoides* colonies. Inhibitory zones were formed, accompanied by reduced mycelial density near the bacterial colony (Fig. 1). This confirms that *B. mycoides* secretes antimicrobial metabolites, including volatile organic compounds and lipopeptides, which restrict the development of the pathogen (Huang et al., 2018; Miljaković et al., 2020).

Effect of *Bacillus mycoides* on the growth and development of maize

Plants grown under control conditions and those subjected solely to *Fusarium graminearum* infection exhibited pronounced symptoms of growth inhibition and physiological stress. These symptoms included reduced plant height, thin stems, limited leaf expansion, premature leaf senescence, and extensive chlorosis progressing to necrosis, particularly in the lower leaves. In the *Fusarium*-infected plants, leaf drying and lodging were evident, indicating severe impairment of plant vitality.In contrast, maize plants treated with *Bacillus mycoides* displayed markedly improved growth performance. These plants showed increased stem height, thicker stems, and enhanced leaf development, with leaves retaining greener coloration and delayed senescence compared to the control.

Figure 1. Antagonistic activity of *Bacillus mycoides* against *Fusarium graminearum* in a dual-culture assay on PDA medium.

The overall plant architecture was more robust, indicating improved vegetative growth and physiological status.in the combined treatment (*B. mycoides* + *F. graminearum*), plants maintained growth characteristics comparable to or exceeding those of plants treated with *B. mycoides* alone. Despite pathogen pressure, these plants exhibited reduced disease symptoms, improved leaf turgor, and lower levels of chlorosis and necrosis. The visual reduction in disease severity suggests that *B. mycoides* effectively mitigated the negative impact of *F. graminearum* infection.

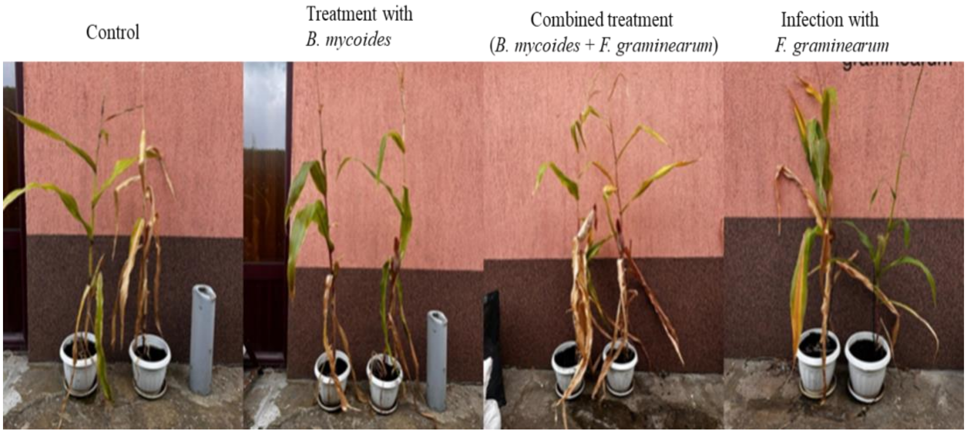

Figure 2. Effect of *Bacillus mycoides* and *Fusarium graminearum* on the growth and development of maize in pot experiments: Control, treatment with *B. mycoides*, combined treatment (*B. mycoides* + *Fusarium*), and infection with *Fusarium graminearum*

Effect on stem height

In control plants, the average stem height was 125.5 cm, whereas treatment with *B. mycoides* resulted in a significant increase to 143 cm. In the combined treatment with *B. mycoides* and *Fusarium graminearum*, stem height reached 145 cm, indicating that the bacterium partially mitigates the negative effect of the pathogen. Plants infected solely with *F. graminearum* showed the lowest height (111.5 cm), confirming the inhibitory effect of the pathogen on growth.

Effect on leaf length

Average leaf length values showed clear differences among treatments. Control plants had an average leaf length of 52.5 mm. Treatment with *Bacillus mycoides* significantly increased leaf length to 65.5 mm. A similar effect was observed in the combined treatment (*B. mycoides* + *F. graminearum*), where leaf length reached 64.5 mm. The lowest value (48 mm) was recorded in plants infected only with *F. graminearum.*

Effect on ear weight

Ear weight data revealed distinct differences among treatments. Control plants produced relatively small ears with an average weight of 14 g. Treatment with *Bacillus mycoides* increased ear weight to 28.5 g, indicating a positive effect on productivity. The highest values were observed in the combined treatment (*B. mycoides* + *F. graminearum*), reaching 58 g, demonstrating that the bacterium stimulates ear development even in the presence of the pathogen. Plants infected only with *F. graminearum* produced lighter ears (19.5 g), reflecting the negative impact of the pathogen on productivity.

Effect on the number of seeds per ear

Data showed clear differences in seed number per ear depending on the treatment. Control plants produced an average of 17 seeds per ear, indicating low productivity. Treatment with *Bacillus mycoides* significantly increased seed number to an average of 52, more than three times higher than the control. The highest seed number was observed in the combined treatment (*B. mycoides* + *F. graminearum*), with an average of 98.5 seeds per ear. This indicates that the bacterial strain stimulates generative development of maize even under pathogen stress. Plants treated solely with *F. graminearum* showed a greatly reduced seed number (average 15.5), confirming the negative effect of the pathogen on seed formation.

Effect on seed weight

Results clearly demonstrate the positive effect of *Bacillus mycoides* on grain biomass accumulation. In control plants, the average seed weight was 4.55 g, whereas treatment with *B. mycoides* increased it more than fourfold to 19.15 g. The highest values were recorded in the combined treatment (*B. mycoides* + *F. graminearum*), reaching 34.5 g, and showing that the strain not only enhances productivity but also mitigates the negative effect of the pathogen. Plants infected only with *F. graminearum* had strongly reduced seed weight (1.5 g), confirming the adverse impact of the pathogen on yield formation.

Effect on root system length

Control plants developed roots averaging 39.5 cm, whereas treatment with *Bacillus mycoides* increased root length to 48 cm, indicating the stimulatory effect of the strain on underground plant development. In the combined treatment (*B. mycoides* + *F. graminearum*), average root length was 43.5 cm – higher than the control but lower than in plants treated with the bacterium alone, suggesting partial mitigation of the pathogen's negative effect. The shortest roots (34.5 cm) were observed in plants infected only with *F. graminearum*, highlighting the strong inhibitory effect of the pathogen on root development.

DISCUSSION

The present study provides compelling evidence that *Bacillus mycoides* exerts a pronounced growth-promoting and protective effect on maize, significantly enhancing both vegetative development and yield-related traits. The strong stimulation of plant growth observed in *B. mycoides*–treated plants can be directly linked to the strain's high capacity for indole-3-acetic acid (IAA) production, a key phytohormone known to regulate root architecture, cell elongation, and biomass accumulation. Enhanced root system development, as evidenced by increased root length and improved overall plant vigor, likely facilitated more efficient water and nutrient uptake, thereby supporting sustained aboveground growth and productivity.

In addition to phytohormone synthesis, *B. mycoides* demonstrated a well-defined ability to solubilize inorganic phosphates and zinc compounds, thereby increasing the bioavailability of essential mineral nutrients. Phosphorus and zinc play critical roles in energy metabolism, enzyme activation, and photosynthetic efficiency; consequently, their improved availability provides a plausible explanation for the observed increases in leaf length, ear weight, seed number, and grain mass. These findings align with previous reports highlighting the multifunctional role of *Bacillus* spp. as plant growth-promoting bacteria capable of simultaneously improving nutrient acquisition and physiological performance.

The beneficial effects of *B. mycoides* extended beyond growth stimulation to include a clear biocontrol function against *Fusarium graminearum*. Under combined treatment conditions, maize plants maintained high biometric and yield parameters despite pathogen pressure, indicating that *B. mycoides* effectively mitigated the deleterious effects of Fusarium infection. This protective effect is consistent with the known ability of *Bacillus* species to produce a diverse array of antimicrobial compounds, including lipopeptides such as iturin, fengycin, and surfactin, which suppress fungal growth and trigger induced systemic resistance in host plants. The visibly reduced disease symptoms and preservation of plant vigor in the combined treatment strongly support the involvement of such mechanisms.

In contrast, maize plants exposed solely to *F. graminearum* exhibited severe growth suppression, premature senescence, and substantial reductions in yield-related traits, underscoring the aggressive phytopathogenic nature of this fungus. The stark contrast between infected plants with and without *B. mycoides* application highlights the critical role of biological control agents in sustainable crop protection. Collectively, these results demonstrate that *Bacillus mycoides* functions as a multifunctional bioinoculant, combining plant growth promotion with effective disease suppression. Such dual functionality positions this strain as a promising alternative to chemical fertilizers and fungicides and supports its potential integration into environmentally friendly maize production systems.

CONCLUSIONS

In conclusion, *Bacillus mycoides* demonstrated a dual role as both a biofertilizer and a biocontrol agent, making it a promising candidate for sustainable maize production. Such an integrated approach can contribute to the reduction of chemical fertilizers and pesticides, aligning with the principles of environmentally friendly and sustainable agriculture. The strain promotes maize growth and productivity through the synthesis of phytohormones (IAA), solubilization of poorly available phosphates and zinc compounds, and improvement of plant morphological traits. Additionally, *B. mycoides* mitigates the negative effects of the phytopathogen *Fusarium graminearum*, helping to maintain high yield parameters even under infection conditions. Based on the data obtained from the pot experiments with maize, it can be concluded that *Bacillus mycoides* can be classified as a member of the PGPB (Plant Growth-Promoting Bacteria) group, microorganisms that enhance plant development and growth.

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