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Potential Biocontrol Activity of *Brevibacillus brevis* against Damping-Off and Root-Rot Diseases in Sunflower (*Helianthus annuus*)

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ABSTRACT

Root-rot and damping-off diseases pose significant challenges to sunflower cultivation, particularly in newly reclaimed and arid regions. This study aimed to isolate and evaluate native endophytic bacteria from sunflower plants for their biocontrol potential against major soilborne pathogens and their ability to enhance plant growth. A total of 25 endophytic bacterial isolates were recovered from healthy sunflower leaves, of which five strains *Bacillus cereus*, *Bacillus* sp., *B. pseudomycoides*, *B. paramycoides*, and *Brevibacillus brevis* were selected based on their consistent antagonistic activity against *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, and *Macrophomina phaseolina*. *In vitro* assays confirmed that the selected isolates effectively inhibited fungal growth through multiple mechanisms, including secretion of antifungal metabolites, production of volatile compounds, and enzymatic activity. In greenhouse and field experiments conducted over two consecutive seasons 2022 and 2023, individual and mixed applications of these bacteria significantly reduced disease incidence and severity. Notably, a mixed formulation applied as a soil drench led to a reduction in disease severity by up to 36.3% and markedly improved plant biomass parameters. Beyond disease suppression, the bacterial treatments also promoted plant growth, with measurable increases in shoot and root length and weight, particularly under combined stress conditions. These effects are likely mediated through production of phytohormones, siderophores, and enhanced nutrient uptake. The integration of these endophytic strains into a bioformulation represents a promising approach for sustainable sunflower production in challenging environments. These results represent a continuation of research efforts to evaluate biological control agents for disease management, thereby contributing to the search for safe alternatives that help reduce reliance on chemical pesticides.

Keywords: biocontrol agents, Sunflower root diseases, *Fusarium oxysporum, Fusarium. moniliforme, Rhizoctonia solani*.

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide. In Egypt, it is particularly valued for its edible oil, which is rich in unsaturated fatty acids and considered beneficial to human health. The crop is characterized by a short growth cycle of approximately three to four months, allowing for multiple cropping within a year. Furthermore, sunflower demonstrates considerable tolerance to salinity up to 3000 ppm, and has relatively low requirements for fertilizers, making it an economically and environmentally favorable crop. In addition to its oil, sunflower by-products are also utilized in animal feed and various industrial applications, enhancing its overall agricultural value (Marek *et al.,* 2020).

Despite its agronomic advantages, sunflowers are susceptible to a range of diseases that significantly affect yield and quality. Among the most destructive are soilborne fungal pathogens that cause damping-off and root-rot diseases. Key pathogens responsible for these diseases include *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, and *Sclerotium rolfsii* (Jain *et al.,* 2015; Fahimee *et al.,* 2021). Damping-off can occur both before and after seedling emergence. Pre-

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emergence damping-off leads to seed decay prior to germination, while post-emergence damping-off results in seedling collapse due to stem base necrosis or root decay. *R. solani*, for instance, typically causes sunken brown lesions at the root collar, leading to plant wilt, whereas *S. rolfsii* induces aggressive root rot and rapid seedling death.

The application of synthetic fungicides remains a primary method for managing these diseases. However, their frequent and long-term use has led to environmental pollution, soil microbial imbalance, and the development of resistant pathogen strains (Chen *et al.,* 2020). Considering these concerns, biological control has gained increasing attention as an effective and sustainable strategy. It involves the use of beneficial microorganisms to suppress phytopathogens and enhance plant resistance, without the harmful residues associated with chemicals (Zhou *et al.,* 2021).

Among these beneficial microbes, Plant Growth-Promoting Rhizobacteria (PGPR) are of particular importance. First defined by Kloepper *et al.* (1980), the concept has since expanded to include diverse rhizobacteria that support plant development and mitigate biotic stress. PGPR can act through multiple mechanisms, including production of secondary metabolites such as antibiotics and siderophores, competition for root niches, modulation of plant hormonal pathways, and induction of systemic resistance (Huang *et al.,* 2013; Backer *et al.,* 2018; Vurukonda *et al.,* 2020). These interactions not only limit pathogen infection but also contribute to improved plant vigor and productivity.

Species from the genus Bacillus notably *Brevibacillus brevis* have shown exceptional potential as biocontrol agents, due to their robust environmental adaptability and ability to produce a wide array of lipopeptides and hydrolytic enzymes (Borriss, 2015; Radhakrishnan *et al.,* 2017; Chai *et al.,* 2022). *B. brevis* is now recognized within the *Brevibacillus* genus, which comprises more than twenty validly published species (Shida *et al.,* 1996; Goto *et al.,* 2004; Ahmed *et al*. 2018).

The present study aims to isolate and identify endophytic bacteria including *Brevibacillus brevis* strains from healthy sunflower plants grown in newly reclaimed soils. The antifungal potential of these strains is evaluated against major root disease pathogens under laboratory, greenhouse, and field conditions. Furthermore, the study investigates the biochemical traits of these isolates and their mechanisms of antagonism, with the objective of assessing their applicability in integrated disease management strategies for sunflower cultivation.

2. Materials and Methods

2.1. Fungal pathogens isolation and identification

During the 2022 growing season, naturally infected sunflower plants exhibiting symptoms of seedling damping-off and root rot were collected from three different sites in New Valley Governorate, Egypt. Samples were obtained at three growth stages: 30, 60, and 90 days post-sowing. Diseased root tissues showing pronounced symptoms were excised (5–10 mm segments), surface-sterilized using 1% sodium hypochlorite for four minutes, rinsed three times in sterile distilled water, dried on sterile filter paper, and plated on potato dextrose agar (PDA; 37 g/L, pH 7.2). Cultures were incubated at 25°C for 7–8 days. Pure cultures were obtained using single-spore and hyphal tip techniques. Ten Petri dishes per sample were used to assess fungal frequency. The purified isolates were stored at 4°C for subsequent studies.

2.2. Isolation and identification of endophytic bacteria

Healthy sunflower leaves were used to isolate endophytic bacteria. Leaves were surface-sterilized sequentially with 70% ethanol (30 seconds), 1% sodium hypochlorite (2–3 minutes), and again with 70% ethanol (30 seconds), followed by rinsing with sterile distilled water. Tissues were macerated in sterile water and spread on nutrient agar plates, incubated at 37°C for 24 hours. Colonies were purified via the streak plate method and stored at 4°C (Ullah *et al.,* 2018). Preliminary identification was conducted using Gram staining (Giuliano *et al.,* 2019) and genus-level identification was based on Bergey's Manual of Determinative Bacteriology (Juni, 1986). The dual culture method was used to screen isolates for antifungal activity. Only the most effective isolates were selected for further study.

2.3. In vitro antagonism assay

Antifungal activity of selected endophytic isolates was evaluated against six pathogenic fungi: *Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Sclerotium rolfsii,* and *Rhizoctonia solani*. A 5 mm fungal disc was placed on one side of a PDA plate, and 10 µL of bacterial

suspension (10^8 CFU/mL) was spotted 2 cm opposite the fungal disc. Plates were incubated at 25°C, and four replicates were used. Percentage inhibition of fungal growth was calculated as described by Noumavo *et al*. (2015):

Inhibition (%) = $[(d_1 - d_2) / d_1] \times 100$, where d_1 is fungal diameter in control and d_2 in dual culture.

2.4. Assessment of Extracellular Metabolites via Agar Disc Diffusion Assay

The antifungal activity of extracellular metabolites produced by *Brevibacillus brevis* was evaluated using the agar disc diffusion method, following the protocol described by Korejo *et al*. (2017) with minor modifications to enhance reproducibility. The bacterial strain was cultured in 200 mL of sterile King's B broth, contained in 500 mL Erlenmeyer flasks, and incubated at 28°C on a rotary shaker set to 150 rpm for seven days to ensure optimal metabolite production.

Following incubation, bacterial cultures were centrifuged at 4000 rpm for 20 minutes at room temperature to separate the cell-free supernatant from the bacterial biomass. The resulting supernatant was carefully decanted and subjected to partial drying under sterile conditions using a laminar airflow chamber, allowing for concentration of extracellular metabolites while avoiding thermal degradation.

Sterile Whatman No. 1 filter paper discs (6 mm diameter) were aseptically loaded with three different volumes (15, 30, and 60 µL) of the concentrated filtrate. The discs were then gently placed onto the surface of Czapek Dox agar plates, which had been pre-inoculated with a 5 mm diameter mycelial plug of the target pathogenic fungus placed centrally on the medium (Korejo, *et al*. 20217; Kumari *et al*. 2021).

The plates were incubated at 28°C for 5 to 7 days under dark conditions. After the incubation period, the antifungal activity was assessed by measuring the diameter of the inhibition zones formed around the discs in millimeters. Each treatment was performed in triplicate to ensure consistency and statistical validity of the results.

2.5. Screening for PGP and enzymatic activity

Bacterial isolates were tested for plant growth-promoting (PGP) traits and enzymatic activity. Indole acetic acid (IAA) production was detected in nutrient broth with 0.1 g/L tryptophan after 7 days (Gumiere *et al.,* 2014). Salkowski's reagent was added to culture supernatant; color development from red to pink indicated IAA production. Siderophore production was indicated by a color change from green to blue. Phosphate solubilization was performed on Pikovskaya's agar (Jang, 2006), with solubilization index (SI) calculated per Shakeela *et al*. (2017):

$$SI (\%) = [(\text{Halo diameter} + \text{Colony diameter}) / \text{Colony diameter}] \times 100$$

Bacterial strains were also tested for hydrogen cyanide (HCN), exopolysaccharides (EPS), ammonia production (NH_3), and hydrolytic enzymes including catalase, protease, and lipase following protocols by Krithika and Chellaram (2016), Etminani and Harighi (2018), and Kalyani and Rajesh (2018).

2.6. Volatile antifungal compound production

The production of volatile compounds was assessed using the sealed-plate method (Fiddaman and Rossall, 1993). A 5 mm plug of the test fungus was placed on PDA and inverted over a nutrient agar plate previously inoculated with the bacterial isolate. The two plates were sealed with parafilm and incubated at 25°C for 5 days. Fungal growth was compared with the unexposed control.

2.7. Greenhouse pathogenicity assay

Greenhouse experiments were conducted in sterile sandy clay soil artificially infested with 5 g/kg soil of barley grain inoculum of the test pathogens. Surface-sterilized seeds of Helianthus annuus cv. Sakha 53 were sown in 22 cm pots with sterile soil (1:1 sand:clay), 5 seeds per pot, with three replicates per pathogen. Seedlings were irrigated and maintained under standard conditions. Disease symptoms were monitored at 15 and 30 days post-sowing. Re-isolation of pathogens was performed to confirm Koch's postulates.

2.8. Inoculum preparation of biocontrol agents

Endophytic isolates were grown in tryptone soy broth (TSB) with shaking (200 rpm) for 72 h. Cultures were centrifuged at 6000 rpm for 10 min, and cells were resuspended in phosphate buffer (100 mM, pH 7.0) and adjusted to 10^9 CFU/mL using spectrophotometry.

2.9. Field trials

Field trials were conducted over two consecutive growing seasons (2022 and 2023) in naturally infected sunflower fields located in Dakhla Oasis, New Valley Governorate, Egypt. The purpose of these experiments was to evaluate the efficacy of selected endophytic bacterial isolates in promoting plant growth and reducing root rot disease under field conditions.

A Randomized Complete Block Design (RCBD) was adopted with five replicates per treatment to minimize experimental error due to field heterogeneity. Each experimental unit consisted of a 12 m² plot (4 rows × 3 meters), with 10 planting holes per row. Five seeds were sown per hole and later thinned to two uniform plants per hole.

The tested treatments included the most effective endophytic bacterial isolates (individually and as a mixture), applied as soil drench bioformulations at 15, 30, and 45 days after planting (DAP). Control plots received no bacterial treatment. All plots received uniform agronomic management, including recommended fertilization rates and foliar boron application (2% borax solution).

Disease incidence (%) and plant growth parameters (shoot and root length and weight) were recorded at two stages (30 and 60 DAP), and disease severity was assessed at harvest using a 0–5 disease index scale. The severity of root rot was evaluated visually based on root discoloration, tissue maceration, and presence of damping-off symptoms.

2.10. Disease assessment

Five root segments (1 cm) per plant were surface sterilized and cultured on PDA to identify infecting fungi. Disease incidence was calculated as:

$$\text{Infection (\%)} = (\text{Infected plants} / \text{Total plants}) \times 100$$

Root-rot severity was rated using a 0–5 scale: 0 = Healthy roots; 1 = Slight rot (≤10%); 2 = Mild rot (11–25%); 3 = Moderate rot (26–60%); 4 = Severe rot (61–80%) and 5 = Complete rot (>80%)

2.11. Statistical analysis of data

All experiments were repeated twice. *In vitro* studies followed a completely randomized design; field trials used a randomized block design. Data were analyzed using CoStat software. Treatment means were compared at a significant level of $P < 0.05$, separately for each season (Gomez and Gomez, 1984).

Results and Discussion

A field survey was conducted during the 2022 growing season in three sunflower-producing regions of the New Valley Governorate, Egypt: Mut, Balat, and Elkasr. The objective was to isolate and identify the primary fungal pathogens associated with seedling damping-off and root rot symptoms in sunflower plants at different growth stages (30, 60, and 90 days after planting). A total of 83 pure fungal isolates were obtained from symptomatic roots and soil samples.

According to the data presented in Table 1, six fungal species were consistently isolated across the surveyed sites: *Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Sclerotium rolfsii,* and *Rhizoctonia solani*. These fungi were determined to be the predominant soilborne pathogens responsible for damping-off and root rot. Additional fungal isolates were identified as saprophytic or weakly pathogenic and were therefore excluded from further analysis.

The frequency of isolation varies by plant age and region. *Rhizoctonia solani, Fusarium oxysporum, F. moniliforme,* and *F. solani* were most frequently detected during the seedling stage (30 days), with a gradual decline in older plants. In contrast, *Macrophomina phaseolina* and *Sclerotium rolfsii* showed an increasing trend in frequency with plant age, suggesting their heightened role in late-season root infections. Notably, *S. rolfsii* was consistently detected in all stages, indicating its broad adaptability and sustained pathogenicity. Pathogen prevalence also differed between regions. For example, *Macrophomina phaseolina* had the highest isolation frequency in all sites, particularly in Mut

and Elkasr at later growth stages. These findings highlight the spatial and temporal variability in pathogen dynamics and underscore the importance of site-specific disease management strategies.

Table 1: Screening sunflower fields for occurrence of damping-off root rot pathogens.

Regions	Plant age	*Fusarium oxysporum*	*Fusarium moniliforme*	*Fusarium solani*	*Macrophomina phasolinae*	*Rhizoctonia solani*	*Sclerotium rolfsii*
Mut	**1**	26	22	13	27	9	6
	2	19	17	10	37	7	8
	3	11	8	6	42	5	11
Balat	**1**	9	10	11	15	14	5
	2	7	12	7	19	12	9
	3	6	15	4	28	9	14
Elkasr	**1**	22	11	12	13	21	9
	2	17	9	9	15	17	11
	3	5	6	7	19	12	15
Means		12.56	12.22	8.77	23.8	11.78	9.77

Different times of fields inspecting to cover the different stages of plant growth, as follows:
(1) = 30 days after planting, (2) = 60 days after planting, (3) = 90 days after planting

3.1. Field evaluation of disease incidence in sunflower

In recent years, sunflower has been increasingly cultivated in the New Valley Governorate, particularly under contract farming initiatives aimed at reducing Egypt's dependency on imported vegetable oils. However, many farmers have reported widespread issues of seedling damping-off and root rot, prompting this investigation into the disease incidence in local sunflower fields.

Field inspection data presented in Table 2 demonstrate a clear correlation between plant age and disease incidence (DI%). Across all three surveyed regions (Mut, Balat, and Elkasr), the percentage of infected plants increased progressively with crop development, reaching the highest levels at the mature growth stage (85–90 days after planting). This suggests that the risk of infection intensifies as the crop ages, likely due to prolonged exposure to soilborne pathogens and a decline in plant vigor.

Among the locations, the Mut region exhibited the highest mean disease incidence (15.73%), followed by Elkasr (12.57%) and Balat (9.73%). These differences may reflect site-specific variation in soil conditions, pathogen pressure, or crop management practices.

Table 2: Disease incidence (D.I.%) as recorded by field inspection

Regions	Plant age	Disease Incidence %			
		Site 1	**Site 2**	**Site 3**	**Mean**
Mut	**1**	3.5	3.3	4.1	3.63
	2	6.2	5.4	6.8	6.13
	3	18.1	16.6	12.5	15.73
Balat	**1**	2.8	3.1	4	3.30
	2	4.4	5.5	5.9	5.27
	3	8.6	9.3	11.3	9.73
Elkasr	**1**	2.2	5.5	4.7	4.13
	2	4.3	8.5	7.1	6.63
	3	12.7	14.2	10.8	12.57
LSD		0.58	0.85	0.72	0.40

Different times of fields inspecting to covering the different stages of plant growth, as follows:
(1) = 30 days after planting (2) = 60 days after planting (3) = 90 days after planting

Interestingly, the observed disease incidence did not always align with the frequency of fungal isolation reported in Table 1. For example, although certain fungi were more abundant at earlier growth stages, infection rates continued to rise in later stages. This suggests that early colonization by pathogens

may predispose plants to delayed symptoms or weaken plant defenses, making them more susceptible to damage even when pathogen loads decrease later in the season. In other words, disease severity may reflect early pathogen interference with plant physiology rather than the absolute number of pathogens present at the time of symptom observation.

These findings highlight the importance of early intervention and underscore the complexity of host-pathogen-environment interactions in determining disease outcomes in sunflowers.

3.2. Greenhouse pathogenicity assessment of fungal isolates

To validate the pathogenic potential of fungal isolates recovered from diseased sunflower plants, artificial infection experiments were conducted under controlled greenhouse conditions. All tested fungi successfully induced typical symptoms of damping-off and root rot on sunflower seedlings, confirming their virulence.

As shown in Table 3, *Rhizoctonia solani* and *Macrophomina phaseolina* were identified as the most aggressive pathogens during the pre-emergence stage, with damping-off rates of 37.5% and 32.2%, respectively. These fungi also caused substantial damage post-emergence, with *R. solani* inducing 40.2% and *M. phaseolina* 28.5% damping-off in emerged seedlings. Notably, *Sclerotium rolfsii* exhibited a severe effect during the post-emergence phase (36.3%) and was responsible for the highest root rot severity (41.1%).

In contrast, *Fusarium oxysporum* and *F. solani* demonstrated comparatively lower pathogenicity, with pre-emergence damping-off of 15.8% and 12.2%, and post-emergence damping-off of 20.2% and 14.5%, respectively. Despite their presence in early plant stages, their disease-inducing capacity under greenhouse conditions appeared limited relative to the other isolates.

By 25–30 days after germination, both *M. phaseolina* and *S. rolfsii* remained highly virulent, causing significant root rot symptoms, confirming their role as major contributors to late-stage infections in sunflower. These results are consistent with previous reports by Abd El-Hai *et al.* (2009); Durairaj et al. (2017); Eirini and Tjamos (2023), which highlighted the aggressive nature of these fungi under similar conditions.

Based on these findings, the most virulent fungal isolates particularly *R. solani, M. phaseolina,* and *S. rolfsii* were selected for subsequent experiments aimed at evaluating the biocontrol efficacy of endophytic bacterial strains.

Table 3: Pathogenicity of isolated fungi in a pot experiment

	Damping-off (%)*		Root- rot %
	Pre-emergence	Post- emergence	
Fusarium moniliforme	17.3	20.8	20.4
Fusarium oxysporum	15.8	20.2	29.1
Fusarium solani	12.2	14.5	22.6
Macrophomina phaseolina	32.2	28.5	38.3
Sclerotium rolfsii	29.2	36.3	40.5
Rhizoctonia solani	37.5	40.2	41.1
Control	0	0	0
LSD 5%	2.40	1.71	2.16

*Mean values in column showing differences greater than LSD values are significant different at $p < 0.05$.

3.3. Isolation and identification of endophytic bacteria

Endophytic bacteria were isolated from healthy sunflower (*Helianthus annuus*) leaf tissues. A total of 25 bacterial isolates were initially recovered and screened for antifungal activity. Among them, only five isolates demonstrated consistent and significant antagonistic effects against the pathogenic fungi identified in this study.

These five effective isolates were selected for further taxonomic identification. Morphological, biochemical, and physiological characteristics were examined according to the standard procedures outlined by Skinner and Lovelock (1979), Sneath *et al*. (1986), and the guidelines of Bergey's Manual

of Systematic Bacteriology (2001). Based on this polyphasic approach, the isolates were tentatively identified at the genus level as follows:

- Two isolates belonging to the genus Bacillus (designated as Bac_1 and Bac_2),
- Two isolates of Pseudomonas (Ps_1 and Ps_2),
- One isolate identified as *Brevibacillus* (*Brevi*$_1$).

These five isolates were further subjected to in vitro and in vivo evaluation to assess their antagonistic efficacy and plant growth-promoting traits. Notably, *Brevibacillus brevis* (*Brevi*$_1$) consistently exhibited the strongest antifungal activity across all assays and was thus prioritized for detailed analysis.

3.4. In vitro antagonistic activity of endophytic bacteria against root-infecting fungi

The antagonistic potential of five selected endophytic bacterial isolates *Bacillus* sp. (Bac_1 and Bac_2), *Pseudomonas* spp (Ps_1 and Ps_2), and *Brevibacillus* sp. (*Brevi*$_1$) was evaluated *in vitro* against six pathogenic fungi associated with damping-off and root rot in sunflower. The tested fungi included *Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Sclerotium rolfsii,* and *Rhizoctonia solani.*

As shown in Table 4, all five bacterial isolates exhibited varying degrees of inhibition against the tested fungi, although their efficacy differed significantly by both bacterial and fungal species. Notably, *Brevi*$_1$ displayed the highest antagonistic activity overall, recording the maximum inhibition percentages against *F. oxysporum* (69.2%), *F. moniliforme* (66.4%), *F. solani* (56.3%), and *R. solani* (50.2%). Bacillus isolates (Bac_1 and Bac_2) also showed substantial activity, particularly Bac_2, which inhibited *F. oxysporum* by 59.9% and *F. moniliforme* by 53.5%, while Bac_1 recorded 61.3% inhibition against *F. moniliforme*.

In contrast, the Pseudomonas isolates (Ps_1 and Ps_2) exhibited comparatively lower antifungal effects. Ps_1 resulted in minimal inhibition of *M. phaseolina* (16.5%) and *S. rolfsii* (18.6%), while Ps_2 showed similarly low inhibition values against these two fungi (12.5% and 16.8%, respectively).

These results suggest that the antagonistic performance of endophytic bacteria is isolate-specific and depends on the fungal target. The varying susceptibility of the fungal pathogens may reflect differences in fungal cell wall structure, growth rate, or sensitivity to the bacterial metabolites produced.

Importantly, *Brevi*$_1$ consistently outperformed all other isolates, confirming its potential as a strong biocontrol agent. The variable responses also highlight the potential advantage of combining bacterial isolates in a mixed inoculum to broaden the spectrum and consistency of antifungal activity. This principle was applied in subsequent greenhouse and field trials to assess the efficacy of endophytic bacteria under more complex conditions.

Table 4: Growth inhibition of root rotting fungi by the endophytic bacteria in dual culture plate assay.

S.No	Endophytic bacteria	Inhibition of mycelial growth (%)					
		Fusarium oxysporum	***Fusarium moniliforme***	***Fusarium solani***	***Macrophomina phaseolina***	***Sclerotium rolfsii***	***Rhizoctonia solani***
1	***Bac*$_1$**	52.9	61.3	50.5	39.5	34.6	47.3
2	***Bac*$_2$**	59.9	53.5	48.8	36.2	32.7	44.2
3	***Ps*$_1$**	27.2	37.1	36.2	16.5	18.6	32.5
4	***Ps*$_2$**	25.9	29.5	34.5	12.5	16.8	34.7
5	***Brevi*$_1$**	69.2	66.4	56.3	48.8	42.5	50.2
LSD 5%		3.36	3.10	2.14	1.75	2.37	2.83

3.5. Inhibition of Pathogenic Fungi by Cell-Free Culture Filtrates of Endophytic Bacteria

To evaluate the extracellular antifungal activity of the selected endophytic bacterial isolates, their cell-free culture filtrates were tested against six pathogenic fungi using the agar disc diffusion method. As presented in Table 5, the degree of fungal growth inhibition varied according to the bacterial isolate, fungal species, and filtrate concentration.

Across all treatments, *Brevi*$_1$ demonstrated the most potent antifungal activity, especially at the highest concentration (60 µL/disc). It produced the largest inhibition zone against *Fusarium oxysporum* (26.8 mm), followed by substantial inhibition of *F. moniliforme* (22.5 mm) and *Rhizoctonia solani* (20.4

mm). Bacillus isolates also showed notable activity; Bac_2 inhibited *F. oxysporum* and *R. solani* by 24.2 mm and 19.5 mm, respectively, while Bac_1 was particularly effective against *F. solani* (20.2 mm).

In contrast, the Pseudomonas isolates (Ps_1 and Ps_2) exhibited the lowest inhibitory effects, particularly against *Macrophomina phaseolina* and *Sclerotium rolfsii.* These two fungi were largely unresponsive to the lower concentrations (15 µL and 30 µL/disc) of filtrates from Ps_1 and Ps_2, with only minor inhibition observed at the 60 µL level (Table 5).

A consistent trend was observed across all isolates and fungal targets: the inhibition zone diameter increased proportionally with the volume of cell-free culture filtrate applied. This dose dependent response indicates that the antifungal compounds secreted into the culture medium by the bacterial isolates are effective in suppressing pathogen growth, particularly at higher concentrations.

The superior performance of *Brevi*$_1$ further supports its candidacy as a promising biocontrol agent and highlights its ability to produce potent extracellular metabolites with broad spectrum antifungal activity.

Table 5: Growth inhibition of pathogenic fungi by the cell free culture filtrates of endophytic bacteria by agar disc diffusion method.

No	Endophytic bacteria	Concentration	Zone of Inhibition (mm)*					
			Fusarium oxysporum	*Fusarium moniliforme*	*Fusarium solani*	*Macrophomina phasolinae*	*Rhizoctonia solani*	*Sclerotium rolfsii*
1	*Bac*$_1$	**15µl/disc**	9.2	9.8	10.1	8.3	8.9	10.2
		30µl/disc	11.8	12.9	15.5	9.9	14.2	12.2
		60µl/disc	21.1	17.7	20.2	13.3	16.3	15.1
2	*Bac*$_2$	**15µl/disc**	12.2	11.8	13.3	8.2	12.8	10.6
		30µl/disc	14.5	14.2	17.5	11.5	16.5	13.5
		60µl/disc	24.2	18.6	12.5	14.9	19.5	15.5
3	*Ps*$_1$	**15µl/disc**	5.9	5.1	5.4	0	6.2	0
		30µl/disc	8.1	7.2	6.6	0	8.8	0
		60µl/disc	9.9	11.4	11.2	5.5	10.6	5.2
4	*Ps*$_2$	**15µl/disc**	5.7	5.1	7.6	0	5.3	0
		30µl/disc	8.4	7.6	8.1	0	5.5	6.3
		60µl/disc	10.2	9.7	8.4	5.5	6.9	8.1
5	*Brevi*$_1$	**15µl/disc**	10.5	12.6	10.3	10.8	11.1	8.8
		30µl/disc	13.4	15.5	14.7	12.2	16.5	10.5
		60µl/disc	26.8	22.5	18.5	16.2	20.4	16.5
	LSD 5%		1.13	0.80	1.21	0.63	0.90	1.14

3.6. *In vitro* evaluation of plant growth-promoting traits and hydrolytic enzyme production

The five selected endophytic bacterial isolates were assessed for their ability to promote plant growth through the production of key bioactive compounds and hydrolytic enzymes (Table 6). These traits are essential not only for improving nutrient availability but also for enhancing disease resistance and plant vigor.

3.7. Phosphate solubilization

Phosphorus is a vital macronutrient often present in insoluble forms in the soil, thus limiting its availability to plants (Satyaprakash *et al.,* 2017). Among the tested isolates, *Brevi*$_1$ and Bac_2 demonstrated the highest phosphate solubilization index (SI), recording values of 4.203% and 3.706%, respectively. These values were significantly higher ($P < 0.001$) than those recorded for the Pseudomonas isolates (2.705% and 2.482%), indicating the superior mineralization potential of *Brevibacillus brevis* and *Bacillus* spp., These findings were largely consistent with the results reported by Kirui *et al.* (2022), who observed comparable trends in phosphate solubilization efficiency among phosphate-solubilizing bacteria isolated from semi-arid agroecosystems.

3.8. Indole acetic acid (IAA), HCN, and ammonia production

All five isolates exhibited the ability to produce multiple plant growth-promoting metabolites, including indole acetic acid (IAA), hydrogen cyanide (HCN), and ammonia (NH_3). These compounds play central roles in enhancing root development, nutrient acquisition, and plant defense. *Brevi*$_1$, Bac$_1$, and Bac$_2$ showed the highest qualitative intensity of IAA production (+++), supporting their classification as strong PGPR strains. These findings are consistent with previous studies demonstrating the role of *Bacillus* and *Brevibacillus* spp. in IAA biosynthesis (Patten and Glick, 2002; Wagi & Ahmed, 2019; Nithyapriya *et al.,* 2021).

3.9. Production of Hydrolytic Enzymes

All isolates were evaluated for production of key hydrolytic enzymes, including catalase (Cat), lipase (Lip), and protease (Pro). These enzymes contribute to biological control by degrading pathogen cell walls and neutralizing oxidative stress. *Brevi*$_1$ again exhibited the highest activity across all enzyme categories (+++), followed by Bac$_1$ and Bac$_2$ with moderate to strong activity. The Pseudomonas isolates showed limited or no production of these enzymes.

Hydrolytic enzyme production, particularly by *Brevibacillus brevis*, suggests a potential mechanism of antagonism against soilborne pathogens. Previous reports have confirmed the antifungal efficacy of enzyme-producing bacteria in suppressing root-infecting fungi (Siegień and Bogatek, 2006; Mishra *et al.,* 2020; Anand *et al.,* 2021; Sehrawat *et al.,* 2022).

Overall Assessment; Taken together, these results confirm the multifunctional nature of *Brevi*$_1$, which combines strong plant growth-promotion traits with effective enzymatic activity. Such capabilities make it an excellent candidate for both biocontrol and biofertilization in integrated disease management programs.

Table 6: Average solubilization index and estimation of the production of metabolites by the endophytic bacterial isolates

S.no	Endophytic bacteria	solubilization index (%)	Qualitative production of metabolites						
			IAA	*HCN*	*NH3*	*EPS*	*Cat*	*Lip*	*Pro*
1	***Bac*$_1$**	3.442	++	++	+	+	+	++	+
2	***Bac*$_2$**	3.706	+++	++	++	+	+	++	+
3	***Ps*$_1$**	2.705	+	+	+	-	+	-	-
4	***Ps*$_2$**	2.482	+	+	+	-	-	-	-
5	***Brevi*$_1$**	4.203	+++	+++	+++	++	+	+++	++

3.10. Growth inhibition of pathogenic fungi by volatile compounds produced by endophytic bacteria

The ability of endophytic bacterial isolates to produce volatile antifungal compounds was evaluated *in vitro*, and the inhibition of mycelial growth was quantified relative to untreated controls (Table 7). The results showed a significant reduction in fungal growth, particularly in plates exposed to volatiles from *Brevi*$_1$ and Bac$_2$, confirming their strong bioactive potential.

Among all combinations, *Brevi*$_1$ exhibited the highest inhibitory effect, with a mycelial growth reduction of (-37.73%) against *Fusarium solani* and (-34.15%) against *F. moniliforme*. Similarly, Bac$_2$ showed considerable inhibition of *F. solani* and *F. moniliforme* by (-31.44% and -29.72%), respectively. *Brevi*$_1$ also caused notable suppression of *Macrophomina phaseolina* (-25.96%) and *Rhizoctonia solani* (-25.4%).

On the other hand, the lowest inhibition rates were observed with Pseudomonas isolates, particularly Ps$_2$, which showed minimal or negligible inhibition across most fungi (e.g., −5.4% against *F. solani,* (-5.84% against *M. phaseolina*, -6.81% against *R. solani*, and only -5.50% against *S. rolfsii*).

Despite variability in sensitivity among fungal species, *F. moniliforme* was consistently affected by all isolates, including Bac$_1$ (-15.84%), Ps$_1$ (-11.3%), and even Ps$_2$ (-8.96%). This indicates its general susceptibility to volatile metabolites compared to other pathogens.

The observed antifungal effects are attributable to the production of volatile organic compounds (VOCs) by endophytes. These bioactive metabolites, which diffuse through the air space, are known to suppress pathogen growth through diverse mechanisms (Garbeva *et al.,* 2014; Effmert *et al.,* 2012;

Huang *et al.,* 2022; Nagrale *et al*. 2022). The role of VOCs in microbial antagonism has been confirmed in recent studies involving Bacillus and related genera (Xie *et al.,* 2020; Roca-Couso *et al.,* 2021; Zhao *et al.,* 2021; Hao *et al.,* 2022; Ling *et al.,* 2023).

Taken together, these findings underscore the promising biocontrol potential of *Brevibacillus brevis* through VOC production, especially against *F. solani, F. moniliforme,* and *M. phaseolina*, which are major root-rot pathogens in sunflower.

Table 7: Growth inhibition of fungi exposed to volatile antifungal compounds produced by endophytic Pseudomonas (mm)

No	Endophytic bacteria	Hyphal growth of pathogenic fungi (mm)*					
		Fusarium oxysporum	***Fusarium moniliforme***	***Fusarium solani***	***Macrophomina phasolinae***	***Rhizoctonia solani***	***Sclerotium rolfsii***
	Control	72.2	81.4	79.5	85.5	92.4	83.5
1	***Bac*$_1$**	60.3 (-16.48%)	68.5 (-15.84%)	59.3 (-25.40%)	69.2 (-19.04%)	81.1 (-12.22%)	74.4 (-10.89%)
2	***Bac*$_2$**	55.2 (-23.54%)	57.2 (-29.72%)	54.5 (-31.44%)	67.3 (-21.28%)	74.5 (-19.37%)	70.2 (-15.92%)
3	***Ps*$_1$**	63.7 (-11.77%)	72.2 (-11.30%)	65.5 (-17.61%)	79.1 (-7.48%)	85.2 (-7.79%)	76.2 (-8.74%)
4	***Ps*$_2$**	65.5 (-9.27%)	74.1 (-8.96%)	75.2 (-5.40%)	80.5 (-5.84%)	86.1 (-6.81%)	78.9 (-5.50%)
5	***Brevi*$_1$**	51.3 (-28.95%)	53.6 (-34.15%)	49.5 (-37.73%)	63.3 (-25.96%)	70.5 (-23.70%)	70.1 (-16.04%)
LSD 5%		2.34	2.84	2.46	11.65	2.77	2.48

3.11. Evaluation of Endophytic Bacteria for Growth Promotion and Disease Suppression under Field Conditions

Field trials conducted during the 2022 and 2023 growing seasons assessed the dual functionality of five selected endophytic bacterial strains in enhancing sunflower growth and suppressing root rot pathogens under natural conditions. The results demonstrated that treatment with the bacterial isolates particularly when applied as a mixture led to significant improvements in plant growth metrics, as well as a marked reduction in disease incidence and severity.

As presented in Table 8, sunflower plants treated with *Brevibacillus brevis* (*Brevi*$_1$) and *Bacillus* sp. (Bac$_2$) exhibited the most pronounced increases in shoot and root length, as well as shoot and root fresh weight, at both 30 and 60 days after sowing. While Pseudomonas isolates (Ps$_1$ and Ps$_2$) also showed some growth-promoting effects, Brevi$_1$ consistently produced the highest growth parameters, reflecting its superior plant growth-promoting rhizobacterial (PGPR) traits.

These enhancements can be attributed to several mechanisms, including the production of phytohormones, phosphate solubilization, and secondary metabolites, as supported by previous studies (Noreen *et al.,* 2018; Dutta and Thakur, 2017; Ek-Ramos *et al.,* 2019). Additionally, these endophytic strains likely contributed to systemic resistance induction, nutrient acquisition, and biocontrol through siderophore and lytic enzyme production (Rahman *et al.,* 2016; Ahmad and Kibret, 2014; Ferreira *et al.,* 2019; Ghazy and El-Nahrawy, 2021).

Following preliminary in vitro assays that confirmed the strong antagonistic potential of the most effective bacterial isolates and verified their compatibility for combined use, a consortium of these isolates was prepared and applied as a soil drench under field conditions (Table 9). The integrated treatment significantly ($P < 0.05$) reduced the incidence of *Fusarium oxysporum* (21–25%), *F. solani* (23–27%), *Macrophomina phaseolina* (33–35%), and *Rhizoctonia solani* (22–27%) across both growing seasons. Moreover, disease severity, expressed as the root rot index on a 0–5 scale, was consistently lower in treated plants (mean 1.9–2.2) compared with the untreated control (mean 3.9–4.1), indicating the potential of this bacterial consortium as an effective and sustainable strategy for managing multiple soil-borne pathogens in sunflower.

Notably, untreated plants showed severe disease symptoms such as dry brown lesions, wilting, reduced root systems, and stunted shoot growth. In contrast, plants treated with the endophytic mixture

exhibited improved vigor and minimal root damage. Across both seasons, the disease index declined by 33.3% to 36.3% compared to the control.

Table 8: Effect of endophytic bacteria on the growth of sunflower in field plot experiment conducted in two seasons (2022 and 2023).

Treatment	Shoot length (cm)				Root length (cm)			
	2022		2023		2022		2023	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
Control	29.5	108.3	35.2	112.7	12.1	17.4	12.8	19.2
***Bac*$_1$**	43.5	97	52.9	107.2	17.5	27.2	13.3	19.1
***Bac*$_2$**	35.3	103	48.2	117.5	13.4	27.4	11.8	18.2
***Ps*$_1$**	39.2	120	42.2	127.3	15.2	20.3	12.4	20.5
***Ps*$_2$**	49.1	130	54.8	138	12.4	19	10.5	13.7
***Brevi*$_1$**	52.2	130	58.3	134.5	11.6	29.2	9.3	21.5
LSD 5%	3.33	ns	3.19	3.89	2.02	3.01	1.20	1.82
	Shoot weight (g)				**Root weight (g)**			
Control	13.5	141	27.4	155	1.7	10.1	1.9	22.4
***Bac*$_1$**	20.5	158.3	42.5	162.5	3	23.5	3.5	25.2
***Bac*$_2$**	35.3	155.2	41.7	170.3	5.1	33.1	5.3	33.3
***Ps*$_1$**	28.5	190.4	44.5	182	5.3	30.5	6.2	35.5
***Ps*$_2$**	35.2	158	23.5	152.5	4.7	33.9	5.9	39.3
***Brevi*$_1$**	38.1	163	32	158.2	5	27.3	6	40.7
LSD 5%	2.74	4.04	4.20	3.67	0.65	2.84	0.57	3.17

Table 9: The effect of endophytic bacteria mixture on the incidence of root-infecting fungi and root-rot index in sunflower plants under field conditions

Treatment	Incidence of root-infecting fungi%								Root-rot index (0–5 scale)	
	Fusarium oxysporum		*Fusarium solani*		*Macrophomina phasolinae*		*Rhizoctonia solani*			
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Control	35	38	42	48	40	46	32	35	3.9	4.1
Endophytic bacteria	21	25	23	27	33	35	27	22	2.2	1.9
LSD 5%	4.01	4.77	3.93	4.48	5.07	3.58	4.52	4.53	0.63	0.36

*Each value represents the average of 100 plants per treatment

The results of this study clearly demonstrate that the tested bacterial isolates not only suppressed root rot pathogens effectively but also significantly promoted sunflower growth. This dual function highlights the role of these isolates as plant growth-promoting rhizobacteria (PGPR). Several earlier studies have emphasized that PGPR, particularly species from *Bacillus* and *Brevibacillus*, exert their effects by producing phytohormones (e.g., indole-3-acetic acid), siderophores, and various enzymes that facilitate nutrient uptake and root development (Benedetto *et al.,* 2009; Lugtenberg and Kamilova, 2009; Kausar *et al.,* 2024; Radhakrishnan *et al.,* 2017; Wagi and Ahmed, 2019). The improved shoot and root biomass observed in treated plants may also be linked to induced systemic resistance (ISR), a mechanism through which PGPR enhance the plant's defense capacity without directly targeting the pathogen (Noreen *et al.,* 2015; Rahman *et al.,* 2016). The combination of disease suppression and growth stimulation makes these bacterial endophytes promising candidates for integrated disease management (IDM) strategies, particularly in arid or newly reclaimed soils where synthetic inputs are limited.

These findings validate the hypothesis that a synergistic application of endophytic bacteria, particularly *Brevi*$_1$ and Bac$_2$, not only enhances sunflower growth but also offers effective biological control of root rot pathogens under field conditions**.**

Conclusion

The findings of this study highlight the potential of endophytic bacteria particularly *Brevibacillus brevis*, *Bacillus cereus*, *Bacillus pseudomycoides*, *Bacillus paramycoides*, and an unidentified *Bacillus* sp. as effective biological control agents against the major soil-borne pathogens causing damping-off and root-rot diseases in sunflower, namely *Rhizoctonia solani, Fusarium solani, F. oxysporum, F. moniliforme, Macrophomina phaseolina, and Sclerotium rolfsii*.

In vitro assays demonstrated strong antagonistic activity of these isolates via multiple mechanisms, including direct growth inhibition, production of hydrolytic enzymes, phosphate solubilization, IAA production, and the release of volatile antifungal compounds. Among all tested isolates, *Brevibacillus brevis* consistently exhibited the highest levels of antifungal activity across most pathogenic fungi.

Field trials over two consecutive growing seasons (2022–2023) further validated these findings. The application of a bio-formulated bacterial mixture significantly improved plant growth parameters (shoot and root length and weight) and led to a marked reduction in disease incidence and root-rot severity, particularly in fields naturally infested with pathogenic fungi. Importantly, the disease suppression effect was more pronounced when the bacterial isolates were applied as a consortium rather than individually, suggesting synergistic interactions among strains.

Given the inconsistent performance of commercial bioagents in arid or newly reclaimed environments, the use of indigenous endophytic bacterial mixtures isolated from sunflower plants offers a promising alternative. These locally adapted strains not only suppressed disease effectively but also enhanced plant vigor under field conditions.

Therefore, the application of native endophytic bacterial consortia, especially those including *Brevibacillus brevis* can be recommended as a sustainable, eco-friendly approach for managing root diseases and promoting sunflower productivity in newly reclaimed desert regions.

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