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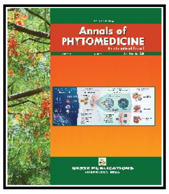

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Harnessing *Bacillus* spp. for biocontrol of *Rhizoctonia solani*: A promising solution for black scurf management in potatoes

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Abstract

Potato is a vital non-cereal food crop and is vulnerable to black scurf (c.o. *Rhizoctonia solani* Kuhn AG-3), which is particularly detrimental. In pursuit of eco-friendly disease management approaches, 26 isolates of Bacillus species were evaluated *in vitro* for their antagonistic effects on *Rhizoctonia solani*. Among them, *Bacillus subtilis* B4 demonstrated the highest suppression of fungal mycelial growth (87.90 %) and substantial production of volatile antifungal compounds (75.00 %). This strain also showed notable activity of cell wall-degrading enzymes, including chitinase and β-1,3-glucanase. Field and pot house evaluations confirmed the efficacy of talc-based bioformulations of *Bacillus subtilis* B4, with treatment such as tuber dip 15 g + soil 3.5 kg significantly reducing disease severity by 90.91% and enhancing yield (220.60 g/pot; 151.03 qtl/acre). The bioformulations maintained high rhizosphere competency and viable cell counts throughout the crop period. Talc-based bioformulation of *B. subtilis* B4 bioformulations emerged as promising biocontrol agents for managing black scurf and associated diseases in potatoes, offering sustainable and effective alternatives to chemical treatments.

1. Introduction

Potato belonging to the Solanaceae family, stands as the most significant food crop globally after cereals, ranking fourth, following rice, wheat, and maize (Rafiq *et al.,* 2024). Its ability to grow under a wide range of climatic and soil conditions enables its cultivation in temperate, subtropical, and tropical areas (Vilvert *et al*., 2022). Compared to many other crops, potato yields higher energy and protein per unit of land (Terefe, 2021) and are rich in carbohydrates (6-20%), essential minerals (*e.g*., K, Mg, P, Fe and Zn), and vitamins (C, B1, and B2) (Mahr, 2021). Often referred to as a "hidden treasure," potatoes play a critical role in global food security (Kumar *et al*., 2013).

China and India dominate global potato production, contributing approximately 33% of the total production. India saw a 6.85% production increase over five years, with potatoes occupying 25% (2.18 million hectares) of the total vegetable-cultivated area (Lal *et al*., 2022). Although, potato is a crucial staple, its production is hampered by a wide array of diseases. More than 160 distinct ailments and disorders have been documented, including about 50 fungal, 10 bacterial, and 40 viral pathogens, while the rest arise from abiotic stresses or unidentified factors (Arora and Khurana, 2004). Among the most economically damaging are bacterial wilt, late and early blight, black scurf, soft rot, and powdery scab, with soil and tuber borne pathogens causing greater losses (Singh *et al*., 2025).

Among soil-borne diseases, black scurf (*Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk)), poses a threat to the potato cultivation around the globe. Pathogen belongs to the anastomosis group AG-3 and is characterized by its ability to infect multiple hosts and survive as sclerotia in the soil for extended periods, even up to six years at ambient temperatures (Salamone *et al*., 2018). Symptoms include the formation of irregular black sclerotia on tuber surfaces, cracked and deformed tubers, and sunken brown lesions on stolon, stems, and roots. The disease also delays sprouting, reduces stem number, and causes malformed tubers, leading to both quantitative and qualitative yield losses (Tsror, 2010). In hills, yield losses can reach up to 25%, while in plains, losses are around 10% (Hussain *et al*., 2021).

Controlling black scurf has long been a priority in both agricultural research and field practices. Traditional methods have included deploying resistant potato varieties, maintaining optimal soil moisture levels, lowering soil pH, applying chemical pesticides, practicing crop rotation, and enriching soil with organic amendments (Apaliya *et al*., 2017). Despite these efforts, the outcomes have often been inconsistent and environmentally harmful. Notably, chemical control is highly inefficient, with less than 0.1 per cent of total applied pesticides reaching the target, while the rest contributes to environmental contamination of air, water, soil, and surrounding plant life (Hussain and Khan, 2020). These limitations have prompted a growing shift toward microbial biocontrol agents (BCAs) as a more sustainable solution. BCAs provide an environmentally friendly and self-sustaining method of disease management, often functioning with minimal intervention and supporting ecosystem stability, while also acting as biofertilizers (Dukare *et al*., 2019). Microorganisms such as *Bacillus* spp., non-pathogenic *Streptomyces* spp., and *Pseudomonas* spp. suppress plant pathogens through various mechanisms, including competition for various types of

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nutrients and ecological niches, production of compounds like antibiotics, siderophores, bacteriocins, secretion of lytic enzymes, and induction of SR (systemic resistance) in the host plants (Lal *et al*., 2022). Additionally, these agents enhance plant nutrition, bolster plant immunity, and can lead to yield increases of more than 20-30% (Maslennikova *et al.,* 2023).

The genus *Bacillus*, first described by Cohn in 1872, comprises over 200 species of Gram-positive, rod-shaped, endospore-forming bacteria known for their widespread presence in nature. These organisms are commonly found in diverse environments such as soil, water, air, the plant rhizosphere, and even extreme habitats (Connor *et al*., 2010). Within soil microbial communities, *Bacillus* species can make up as much as 95% of the Gram-positive bacterial population (Miljakoviæ *et al*., 2020). Several strains - *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. mycoides*, and *B. pumilus* - have shown potent antagonistic effects against various phytopathogens (Bacon *et al*., 2015). *Bacillus* spp. suppresses plant diseases using multiple mechanisms. These include the synthesis of antimicrobial substances such as fengycin, bacillomycin D, surfactin, difficidin, macrolactin, and bacilysin (Kankam *et al*., 2021), the production of CWDEs (cell wall-degrading enzymes) like chitinase that directly target pathogens such as *Rhizoctonia solani* (Miljakoviæ *et al*., 2020), activation of induced systemic resistance in host plants (Lal *et al*., 2022), which inhibits the establishment of harmful microbes (Ongena and Jacques, 2008).

Among the various species, *B. subtilis* and *B. amyloliquefaciens* stand out for their strong biocontrol capabilities specifically against *R. solani* (Khan *et al*., 2021). Their ability to form endospores contributes to the long shelf life of commercial formulations, making them suitable for use in agriculture (Abriouel *et al*., 2011). Biopesticides derived from these strains can remain viable for up to two years (Larkin, 2020). The transition from synthetic agrochemicals to biological agents reflects a major shift toward sustainable farming practices. Leveraging the biocontrol potential of *Bacillus* spp. supports both effective plant disease control and environmental stewardship (Abbas *et al*., 2019). The primary aim of this study was to isolate and characterize effective *Bacillus* spp. strains with antagonistic potential against black scurf disease in potato. Additionally, the research focused on formulating *Bacillus*-based bioproducts and optimizing their application methods for enhanced disease management.

2. Materials and Methods

2.1 Biocontrol agents used

Six antagonistic strains of *Bacillus* spp., previously isolated and molecularly identified, were utilized in this study. These strains included: *Bacillus subtilis* B2(ON479589), *B. subtilis* B4 (ON479713), *Bacillus amyloliquefaciens* B7 (ON489306), *B. subtilis* B10 (ON479485), *B. subtilis* B12 (ON489076), and *B. subtilis* B22 (ON479313). The isolates were preserved on nutrient agar slants for further experimentation (Singh *et al*., 2025).

2.2 Isolation and identification of the black scurf pathogen

Rhizoctonia solani was isolated from sclerotial crusts found on infected potato tubers collected during November 2018-19 from five districts of Punjab, India - Ferozepur, Jalandhar, Patiala, Ludhiana, and Hoshiarpur. The pathogen was cultured on PDA (Himedia). For molecular identification, genomic DNA was extracted using the CTAB method (Choudhary *et al*., 2021). The ITS region of rDNA was amplified using ITS1 (forward) and ITS4 (reverse) primers, and the amplified product was sequenced by Barcode Biosciences Pvt. Ltd., India.

2.3 Dual culture assay

The antagonistic activity of the six *Bacillus* isolates against *R. solani* was tested using a dual culture technique following the protocol by Hussain and Khan (2020). The per-cent inhibition of mycelium growth was calculated as: Inhibition (%) = ((A - B)/A) × 100, where A represents fungal growth diameter in control plates and B represents *R. solani* R2 growth in the presence of *Bacillus* isolates.

2.4 Volatile antifungal activity

The ability of *Bacillus* strains to produce inhibitory volatile compounds was assessed according to the method described by Stoppacher *et al*. (2010). Fungal growth inhibition due to volatiles was measured using the same formula as the dual culture assay (Mrabet *et al*., 2015).

2.5 Chitinase activity assay

Colloidal chitin was prepared as described by Moka *et al*. (2021). Chitinolytic activity of the *Bacillus* isolates was assessed using a spot assay method on nutrient broth medium supplemented with 1.0% (w/v) colloidal chitin, following the protocol described by Hammami *et al*. (2013). Inoculum of each isolate was spotted at four locations on the surface of the medium in Petri dishes and incubated at 28°C. Each isolate was tested in four replications. The development of clear zones around the inoculation spots indicated chitin degradation, as by Islam *et al*. (2012).

For quantifying chitinase activity, 1 ml of culture supernatant was incubated with 1% colloidal chitin in phosphate buffer (pH 7.0) at 50°C for 30 min. After incubation, dinitrosalicylic acid (DNS) was added, and the mixture was heated at 100°C for 5 min. The amount of N-acetyl-D-glucosamine (NAGA) released was measured at 600/ nm using a spectrophotometer, and enzyme activity was expressed in units, where one unit corresponds to the release of 1 µmol of NAGA per minute (Lestari *et al*., 2017). All assays were conducted in triplicate.

2.6 β-1, 3-glucanase activity assay

Qualitative activity of β-glucanase was initially evaluated on β-glucan agar using the congo-red staining method as described by Dewi *et al*. (2016). For quantitative analysis, *Bacillus* isolates were cultured in nutrient broth supplemented with 0.2% laminarin (Rais *et al*., 2017). Reducing sugars produced were measured spectrophotometrically at 550 nm using the method of Miller (1959). One unit of β-1,3-glucanase activity was defined as the amount of enzyme that releases 1 µg of glucose per minute under the assay conditions (Abdelmoteleb *et al*., 2017).

2.7 Preparation of Bacillus - based bioformulations

Based on *in vitro* screening, two promising isolates- *B. subtilis* B4 and *B. amyloliquefaciens* B7 were selected for formulation. Each isolate was cultured in 600 ml nutrient broth at 28 ± 1°C for four days. The cultures were then mixed with 1 kg of sterile talc powder and dried in the shade under aseptic conditions. Carboxymethyl cellulose (1%) was added as a binder, and the formulations were packed in sterile polyethylene bags and stored at 4°C (Singh *et al.*, 2024).

2.8 Inoculum preparation of *Rhizoctonia solani* R2

For inoculum preparation of *R. solani* R2, the pathogen was first cultured on PDA and incubated at 25 ± 2°C temperature for 5-7 days. For mass multiplication, healthy barley grains were soaked in water for 12 to 16 h, excess water was drained, and the grains were autoclaved at 121°C for 30 min on two consecutive days. After cooling, the sterilized grains were inoculated with 5 mm diameter mycelial discs from the PDA culture and incubated at 25°C in the dark for 10-15 days, with occasional shaking to ensure even colonization. Once fully colonized, the grain inoculum was mixed into sterilized or field soil at a concentration of ~10^3 to 10t cfu/g of soil (5-10 g inoculum per kg of soil). The inoculated soil was then incubated for 3-5 days before planting to allow for pathogen establishment and facilitate effective screening of potato genotypes for resistance to black scurf (Sharma and Bhat, 2014).

2.9 Preparation of experimental plots and sowing

To assess the performance of talc-based formulations of *Bacillus subtilis* B4 and *Bacillus amyloliquefaciens* B7, field experiments were conducted over two successive potato-growing seasons (2019-20 and 2020-21). The trials were carried out under both controlled pot house conditions (using sterilized soil) and open field conditions at the Department of Plant Pathology, Punjab Agricultural University (PAU), Ludhiana, Punjab, India.

The evaluation involved two application strategies: tuber treatment and soil amendment. Tuber treatment was performed using two concentrations, 10 g and 15 g of the bioformulation per litre of water. For soil treatment, 2.5 kg and 3.5 kg of the bioformulation each were thoroughly mixed with 25 kg of well-decomposed farmyard manure (FYM) per acre. The FYM-bioformulation mixture was incubated for 72 h before application to enhance microbial activation. Combinations of tuber dip and soil treatment were also tested to assess any synergistic effects.

In addition to the biological treatments, a standard fungicidal treatment, Emesto Prime (Penflufen 240 FS, Bayer), was included as a chemical control, while a pathogen-only (untreated) control served as the baseline for comparison. The field trials were laid out following a Randomized Block Design (RBD), with 18 treatments replicated three times. The potato cultivar 'Kufri Pukhraj', known for its susceptibility to black scurf (Singh *et al.*, 2021), was used for all trials. Tubers were planted in plots measuring 1.0 m × 1.2 m, with a spacing of 20 cm between plants and 60 cm between rows. Light irrigation was applied post-planting to support establishment.

To ensure uniform disease pressure, all plots - including untreated controls - were artificially inoculated with *Rhizoctonia solani* at a concentration of 10^3-10t CFU/g of soil using mycelial culture, following the protocol of Ritchie *et al.* (2013). The untreated control plots received only the pathogen inoculation and served as a reference for treatment comparison.

Disease severity of black scurf was evaluated based on a 0-5 scale as described by Siddique *et al.* (2020). The per-cent disease severity and disease control efficiency were calculated in relation to the untreated control. In addition to disease metrics, observations on per-cent tuber sprouting and total yield were also recorded to evaluate the agronomic impact of the treatments.

$$\text{Disease severity } \% = \frac{\text{Sum of numerical rating}}{\text{Total no. of tubers examined} \times \text{Maximum grade}} \times 100$$

2.10 Statistical analysis

Statistical evaluations were conducted using R software version 4.4.2 (64-bit). Analysis of Variance (ANOVA) was carried out employing appropriate experimental designs - Completely Randomized Design (CRD) for lab analysis and Randomized Block Design (RBD) for field experiments - using relevant R packages. Post-hoc comparisons among treatment means were performed using Tukey's Honest Significant Difference (HSD) test and Fisher's Least Significant Difference (LSD) at a significance level of $\alpha \geq 0.05$ (Debez *et al.*, 2024).

3. Results

3.1 Molecular characterization of *Rhizoctonia solani* R2

Genomic DNA of *Rhizoctonia solani* R2 was subjected to universal primers (ITS1 and ITS4) for amplification of the ITS region of R2, which gave an amplified product of 680 bp (approx.). The nucleotide sequences obtained were subjected to BLAST analysis and showed nearly 98% resemblance to *Rhizoctonia solani* (KJ188645). This sequence of R2 isolate was submitted to NCBI GenBank and was identified as *Rhizoctonia solani* (Accession number ON426381) (Figure 1).

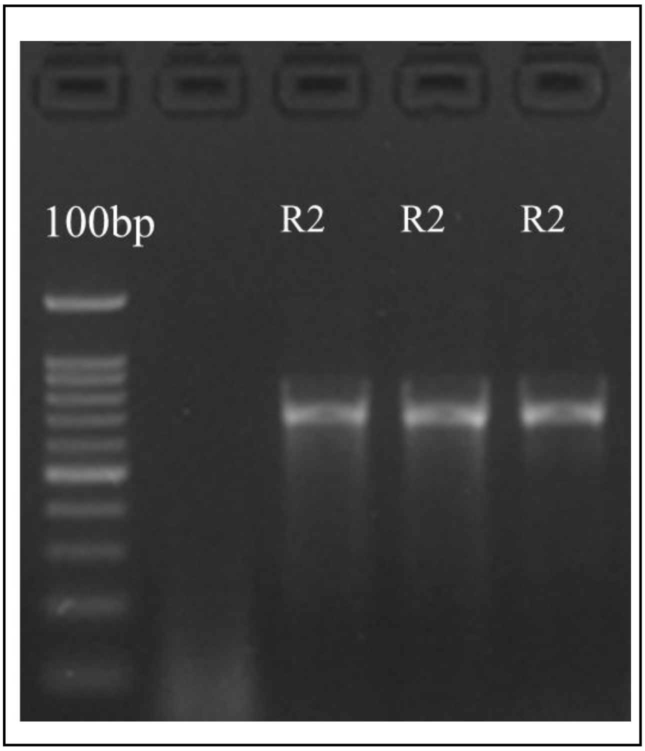

Figure 1: DNA profile generated by ITS primers with *Rhizoctonia solani* R2.

3.2 *In vitro* bioassay of isolated *Bacillus* spp. isolates against *R. solani* R2

In the dual confrontation assay, *Bacillus* spp. isolates (Figure 2) effectively inhibited *R. solani* growth, with inhibition rates between 48.40% to 56.67 %. *Bacillus subtilis* B4 showed the highest inhibition at 56.67%, followed by *B. amyloliquefaciens* B7 (56.30%) (Figure 3).

Similarly, in a volatile assay, the range of inhibition varies from maximum mycelial growth inhibition of *R. solani* R2 was observed against *B. subtilis* B4, *i.e.*, 59.98% which was followed by *B. amyloliquefaciens* B7 (56.88%) (Figure 4).

Figure 2: Dual culture assay of *Bacillus subtilis* B4 against *R. solani* R2.

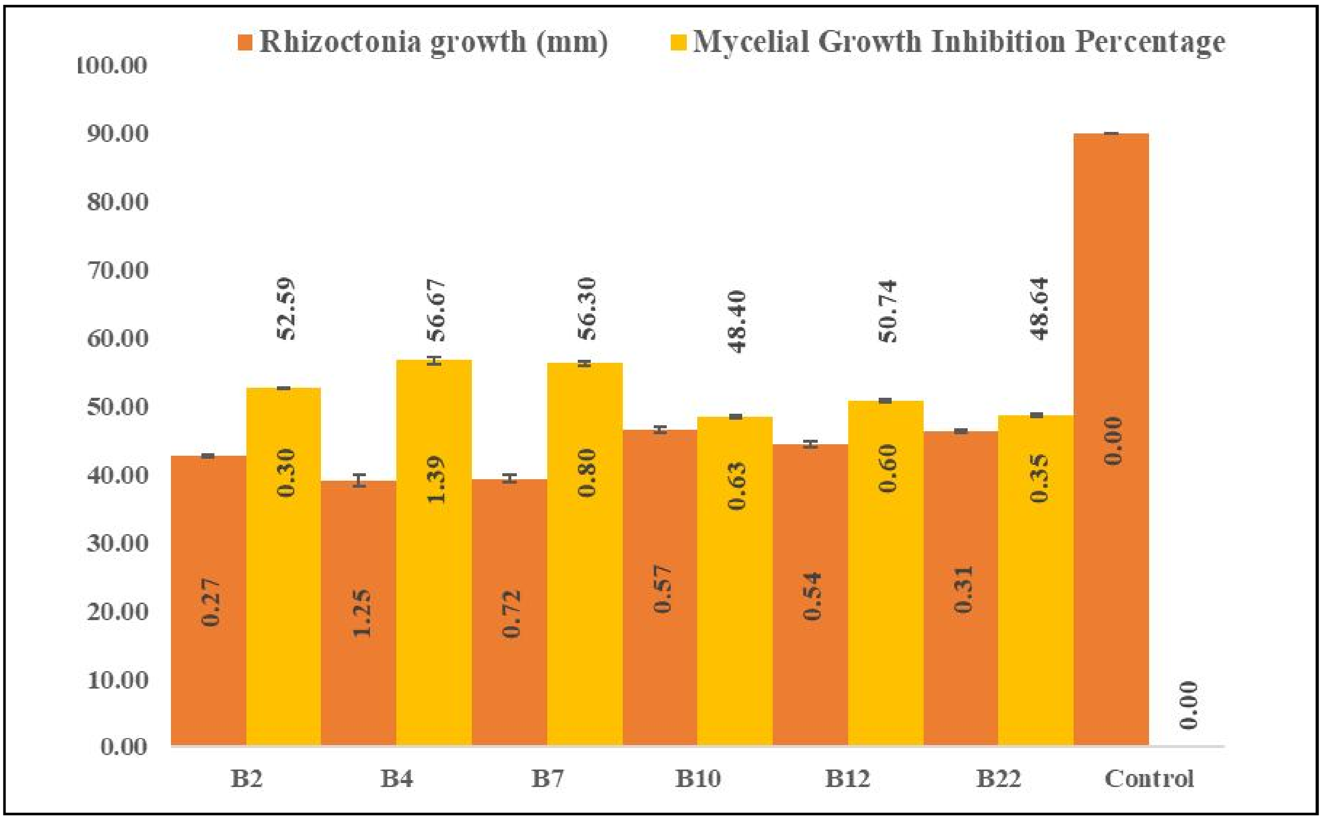

Figure 3: The bar graph here represents the *Rhizoctonia* growth (mm) and per cent mycelial growth inhibition under dual culture assay along with error bars representing the standard error of the respective mean. The values in the bars represent their respective standard deviations, and values above per cent mycelial growth inhibition bars represent their arc sine transformation values (B2, B4, B7, B10, B12, and B22 are *Bacillus* isolates).

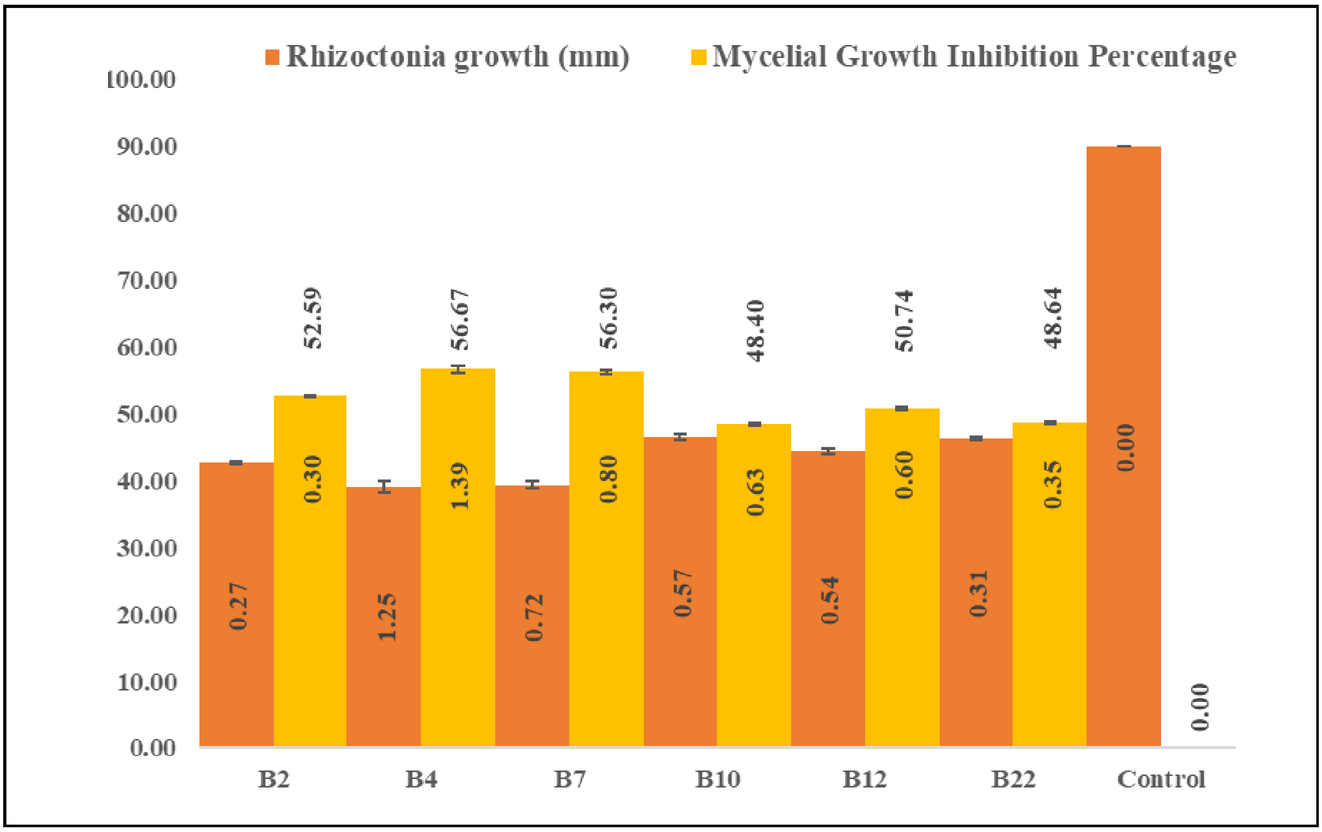

Figure 4: The bar graph here represents the *Rhizoctonia* growth (mm) and per cent mycelial growth inhibition under volatile production culture assay along with error bars representing standard error of respective mean. The values in the bars represent their respective standard deviations, and values above per cent mycelial growth inhibition bars represent their arc sine transformation values (B2, B4, B7, B10, B12, and B22 are *Bacillus* isolates).

3.3 Estimation of chitinase activity

3.3.1 Qualitative chitinase activity

The ability of *Bacillus* isolates to produce chitinase qualitatively was assessed by the formation of clear zones on 1% colloidal chitin agar plates. Among the six tested strains, isolate B4 exhibited the largest halo zone measuring 15.67 mm, followed closely by B7 with a zone diameter of 14.33 mm, indicating strong chitinolytic activity (Figure 5).

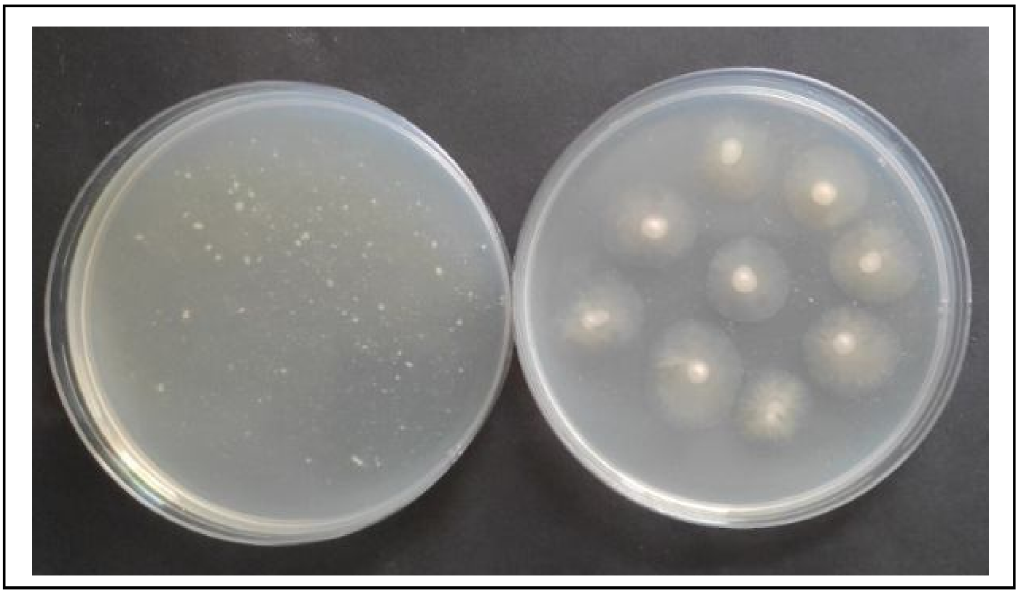

Figure 5: Qualitative analysis of chitinase production by *Bacillus subtilis* B4.

3.3.2 Quantitative chitinase activity

Quantitative assessment was based on the release of reducing sugars from colloidal chitin. *B. amyloliquefaciens* B7 recorded the highest enzymatic activity at 8.19 units/ml, while *B. subtilis* B4 followed by 8.02 units/ml. The remaining four strains - B2, B10, B12, and B22 - also demonstrated notable activity, ranging between 6.81 and 8.19 units/ml (Figures 6 and 7).

Figure 6: Quantitative analysis of chitinase production by *Bacillus subtilis* B4.

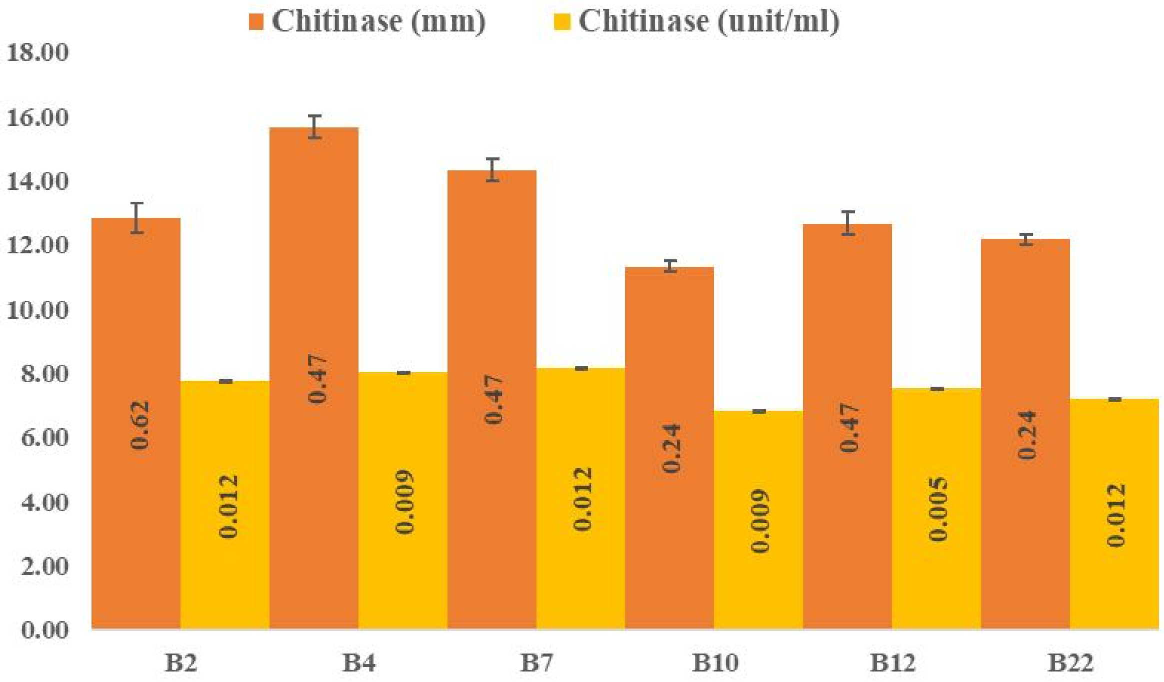

Figure 7: The bar graph here represents the qualitative chitinase activity (mm) and quantitative chitinase activity (unit/ml) assay along with error bars representing the standard error of the respective mean. The values in the bars represent their respective standard deviations (B2, B4, B7, B10, B12, and B22 are *Bacillus* isolates).

3.4 Assessment of β-1, 3-glucanase activity

3.4.1 Qualitative β 1, 3 glucanase activity

Qualitative detection of β-1, 3-glucanase production involved the observation of halo zones around colonies on β-glucan agar plates stained with Congo red and destained with NaCl. The most prominent zone was observed with *B. amyloliquefaciens* B7 (34.67 mm), statistically similar to *B. subtilis* B4 (33.42 mm). Other isolates such as B12 and B22 produced zones of 29.08 mm and 27.17 mm, respectively (Figures 8 and 9).

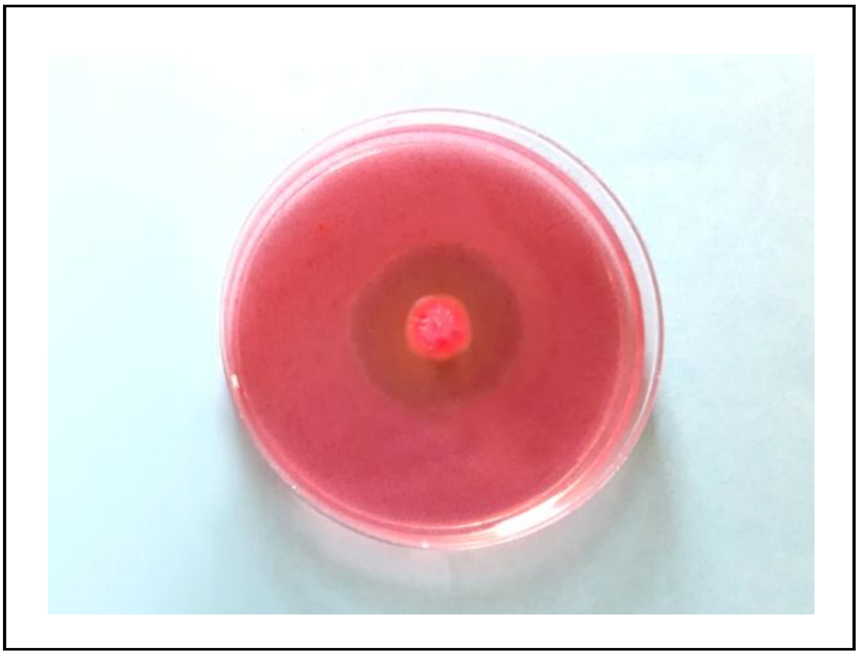

Figure 8: Qualitative β-1, 3 glucanse assay by *Bacillus subtilis* B4.

3.4.2 Quantitative β 1, 3 glucanase activity

Enzymatic activity levels among the six isolates varied between 3.08 and 4.09 units/ml (Figure 9). The highest level was again observed in *B. amyloliquefaciens* B7 (4.09 units/ml), which was comparable to *B. subtilis* B4 (4.06 units/ml). The other isolates - B2, B10, B12, and B22 - showed activity values of 3.38, 3.08, 3.38, and 3.63 units/ml, respectively.

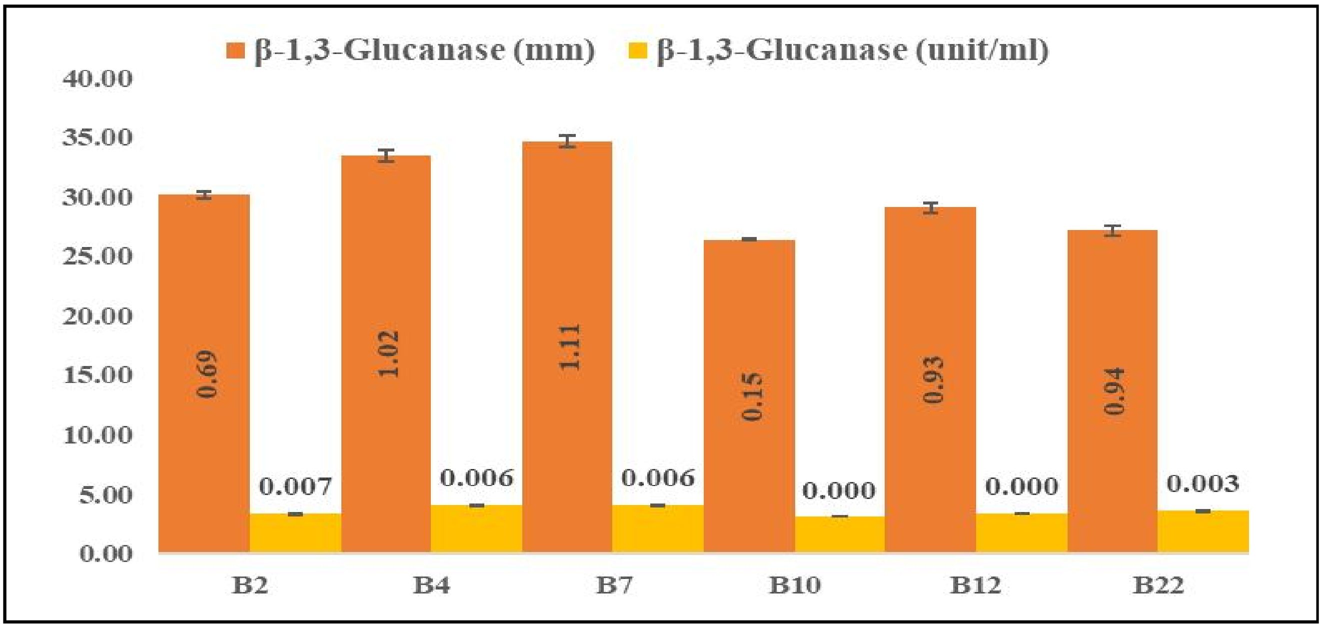

Figure 9: The bar graph here represents the qualitative β-1,3-glucanase activity (β-1,3-glucanase mm) and quantitative β-1,3-glucanase activity (unit/ml) assay along with error bars representing standard error of respective mean. The values in the bars represent their respective standard deviations (B2, B4, B7, B10, B12, and B22 are *Bacillus* isolates).

3.5 Evaluation of bioformulations against black scurf of potato

All the different treatments with bioformulations of *B. subtilis* B4 and *B. amyloliquefaciens* B7 significantly reduced the disease severity under pot house and field conditions.

3.5.1 Bioefficacy against black scurf disease severity under pot house and field conditions.

B. subtilis B4 tuber dip treatment (15 g + soil 3.5 kg) recorded the lowest disease severity, with values of 6.67, followed by *B. subtilis* B4 tuber dip (15 g + soil 2.5 kg), *i.e.*, 7.50, as compared to the untreated control, 85.00 % (Table 1).

* → Mean of three replications, B4: *B. subtilis*; B7: *B. amyloliquefaciens*. Values in the parenthesis are their respective arc sine transformed values, Values with ± are their respective standard deviations among replications. Different letters mean significantly different according to Duncan's multiple range test ($p \leq 0.05$) and values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

Table 1: **Effect of *Bacillus subtilis* B4 and *Bacillus amyloliquefaciens* B7 treatments on black scurf severity percent under pot house conditions**

Treatments	Disease severity^{DMRT} ± Stdev (arc sine, SE)	Per cent severity control
Tuber Dip 10 g (B 4)	34.17^{bc} ± 3.44 (35.73, 0.92)	59.80
Tuber Dip 15 g (B 4)	34.17^{bc} ± 3.44 (35.73, 0.92)	59.80
Soil 2.5 kg (B 4)	30.00^{de} ± 5.00 (33.13, 1.37)	64.71
Soil 3.5 kg (B 4)	28.33^{e} ± 3.73 (32.10, 1.05)	66.67
Tuber Dip 10 g + Soil 2.5 kg (B 4)	13.33^{fg} ± 3.73 (21.23, 1.38)	84.31
Tuber Dip 10 g + Soil 3.5 kg (B 4)	10.00^{ghi} ± 2.89 (18.23, 1.27)	88.24
Tuber Dip 15 g + Soil 2.5 kg (B 4)	07.50^{hij} ± 3.82 (15.47, 1.71)	91.18
Tuber Dip 15 g + Soil 3.5 kg (B 4)	06.67^{ij} ± 2.36 (14.75, 1.16)	92.16
Tuber Dip 10 g (B 7)	37.50^{b} ± 3.82 (37.72, 1.00)	55.88
Tuber Dip 15 g (B 7)	34.17^{bc} ± 3.44 (35.73, 0.92)	59.80
Soil 2.5 kg (B 7)	32.50^{cd} ± 3.82 (34.71, 1.03)	61.76
Soil 3.5 kg (B 7)	29.17^{de} ± 5.34 (32.59, 1.47)	65.69
Tuber Dip 10 g + Soil 2.5 kg (B 7)	15.00^{f} ± 4.08 (22.58, 1.49)	82.35
Tuber Dip 10 g + Soil 3.5 kg (B 7)	13.33^{fg} ± 3.73 (21.23, 1.38)	84.31
Tuber Dip 15 g + Soil 2.5 kg (B 7)	10.83^{gh} ± 1.86 (19.15, 0.73)	87.25
Tuber Dip 15 g + Soil 3.5 kg (B 7)	09.17^{hij} ± 3.44 (17.31, 1.55)	89.22
Chemical (Emesto Prime - Penflufen 240 FS)	05.83^{j} ± 1.86 (13.83, 0.92)	93.14
Control	85.00^{a} ± 6.45 (67.72, 2.45)	-
CD ($p \leq 0.05$)	3.21	-
SE (m)	1.14	-
SE (d)	1.61	-
C.V.	9.89	-

Under the field evaluation, the tuber dip 15 g + soil 3.5 kg (B 4) found to be the least value of per cent disease severity and maximum per cent of disease severity control, *i.e.,* 8.00 and 90.91, respectively followed by, tuber dip 15 g + soil 2.5 kg (B 4) *i.e.,* 9.33 and 89.39 (Table 2).

Table 2: **Effect of *Bacillus subtilis* B4 and *Bacillus amyloliquefaciens* B7 treatments on black scurf severity per cent under field conditions**

Treatments	Disease severity^{DMRT} ± Stdev (arc sine, SE)	Per cent severity control
Tuber Dip 10 g (B 4)	60.00^{b} ± 2.31 (50.75, 0.60)	31.82
Tuber Dip 15 g (B 4)	58.67^{b} ± 1.89 (49.97, 0.48)	33.33
Soil 2.5 kg (B 4)	48.67^{d} ± 3.59 (44.21, 0.92)	44.70
Soil 3.5 kg (B 4)	50.67^{d} ± 3.77 (45.36, 0.96)	42.42
Tuber Dip 10 g + Soil 2.5 kg (B 4)	23.33^{f} ± 2.75 (28.83, 0.83)	73.48

Tuber Dip 10 g + Soil 3.5 kg (B 4)	13.33^{h} ± 1.89 (21.36, 0.69)	84.85
Tuber Dip 15 g + Soil 2.5 kg (B 4)	09.33^{ij} ± 1.89 (17.70, 0.80)	89.39
Tuber Dip 15 g + Soil 3.5 kg (B 4)	08.00^{j} ± 2.31 (16.24, 1.13)	90.91
Tuber Dip 10 g (B 7)	60.00^{b} ± 4.00 (50.76, 1.04)	31.82
Tuber Dip 15 g (B 7)	60.67^{b} ± 1.49 (51.14, 0.39)	31.06
Soil 2.5 kg (B 7)	54.00^{c}± 3.06 (47.27, 0.78)	38.64
Soil 3.5 kg (B 7)	54.00^{c}± 3.83 (47.28, 0.98)	38.64
Tuber Dip 10 g + Soil 2.5 kg (B 7)	26.67^{e}± 2.98 (31.04, 0.85)	69.70
Tuber Dip 10 g + Soil 3.5 kg (B 7)	25.33^{ef}± 1.89 (30.19, 0.55)	71.21
Tuber Dip 15 g + Soil 2.5 kg (B 7)	18.67^{g} ± 3.77 (25.46, 1.28)	78.79
Tuber Dip 15 g + Soil 3.5 kg (B 7)	11.33^{hi} ± 1.49 (19.62, 0.63)	87.12
Chemical (Emesto Prime - Penflufen 240 FS)	06.67^{j} ± 1.89 (14.79, 1.03)	92.42
Control	88.00^{a} ± 4.00 (70.05, 1.80)	-
CD ($p \leq 0.05$)	2.41	-
SE (m)	0.85	-
SE (d)	1.21	-
C.V.	5.70	-

* →Mean of three replications, B4: *B. subtilis*; B7: *B. amyloliquefaciens*. Values in the parenthesis are their respective arc sine transformed values, Values with ± are their respective standard deviations among replications. Different letters mean significantly different according to Duncan's multiple range test ($p \leq 0.05$), and values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

Table 3: Effect of *Bacillus subtilis* B4 and *Bacillus amyloliquefaciens* B7 treatments on yield under pot house and field conditions

Treatments	Yield^{DMRT} ± Stdev (SE)	
	Pot house (g/pot)*	Field (qtl/ha)*
Tuber Dip 10 g (B 4)	173.55^{gh} ± 1.80 (0.80)	121.57^{i} ± 4.38 (1.96)
Tuber Dip 15 g (B 4)	176.15^{g} ± 2.87 (1.28)	124.83^{h} ± 4.57 (2.04)
Soil 2.5 kg (B 4)	182.73^{f} ± 3.14 (1.40)	128.64^{f} ± 4.69 (2.09)
Soil 3.5 kg (B 4)	187.61^{e} ± 1.92 (0.86)	129.02^{f} ± 4.70 (2.10)
Tuber Dip 10 g + Soil 2.5 kg (B 4)	201.30^{d} ± 2.84 (1.27)	142.23^{d} ± 5.52 (2.47)
Tuber Dip 10 g + Soil 3.5 kg (B 4)	209.53^{c} ± 5.26 (2.35)	146.45^{c} ± 4.70 (2.10)
Tuber Dip 15 g + Soil 2.5 kg (B 4)	214.98^{b} ± 5.62 (2.51)	148.88^{b} ± 4.32 (1.93)
Tuber Dip 15 g + Soil 3.5 kg (B 4)	220.60^{a} ± 8.86 (3.96)	151.03^{a} ± 3.52 (1.57)
Tuber Dip 10 g (B 7)	171.93^{h} ± 1.44 (0.64)	119.53^{i} ± 4.42 (1.97)
Tuber Dip 15 g (B 7)	175.78^{g} ± 2.58 (1.16)	124.02^{h} ± 4.42 (1.97)
Soil 2.5 kg (B 7)	180.26^{f} ± 2.57 (1.15)	126.69^{g} ± 4.54 (2.03)
Soil 3.5 kg (B 7)	188.44^{e} ± 1.93 (0.86)	128.80^{f} ± 4.72 (2.10)
Tuber Dip 10 g + Soil 2.5 kg (B 7)	201.13^{d} ± 3.35 (1.50)	141.92^{d} ± 5.56 (2.48)
Tuber Dip 10 g + Soil 3.5 kg (B 7)	200.82^{d} ± 2.87 (1.28)	141.35^{d} ± 5.04 (2.25)
Tuber Dip 15 g + Soil 2.5 kg (B 7)	208.28^{c} ± 4.72 (2.11)	145.95^{c} ± 4.59 (2.05)
Tuber Dip 15 g + Soil 3.5 kg (B 7)	213.75^{b} ± 5.54 (2.48)	147.21^{c} ± 4.15 (1.85)
Chemical (Emesto Prime - Penflufen 240 FS)	203.21^{d} ± 3.86 (1.73)	136.58^{e} ± 3.88 (1.73)
Control	143.00^{i} ± 5.62 (2.51)	93.23^{k} ± 3.01 (1.34)
CD ($p \leq 0.05$)	3.33	1.39
SE (m)	1.18	0.49
SE (d)	1.67	0.70
C.V.	1.51	0.91

* →Mean of three replications, B4: *B. subtilis*; B7: *B. amyloliquefaciens*. Values in the parenthesis are their respective arc sine transformed values, Values with ± are their respective standard deviations among replications. Different letters mean significantly different according to Duncan's multiple range test ($p \leq 0.05$), and values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

3.5.2 Effect of application of *Bacillus* isolates on yield under pot house and field conditions

B. subtilis B4 and *B. amyloliquefaciens* B7 significantly enhanced yield across all treatments during both years compared to the untreated control. Under both pot house and field conditions, the highest yield was recorded in tubers treated with Tuber Dip 15 g + Soil 3.5 kg (B4), resulting in 220.60 g/pot and 151.03 qtl/acre, respectively. This was followed by Tuber Dip 15 g + Soil 3.5 kg (B7) with 213.75 g/pot and Tuber Dip 15 g + Soil 2.5 kg (B7) with 147.21 qtl/acre. In contrast, the untreated control recorded the lowest yield of 143.00 g/pot and 93.23 qtl/acre, respectively (Table 3).

4. Discussion

The present study elucidates the promising potential of *Bacillus* isolates as BCAs against *R. solani*, a major phytopathogen responsible for significant crop losses, particularly in potato cultivation. Our findings reinforce the pivotal role of molecular and morphological characterization for accurate pathogen identification and provide a comprehensive analysis of various biocontrol mechanisms employed by *Bacillus* spp., including antagonistic activity, enzyme production, and growth-promoting capabilities.

The successful isolation and identification of *R. solani* are corroborated with earlier methodologies adopted by Pandey and Pundhir (2013); Mahr (2021); Al-Abedy *et al.* (2018), where hyphal tip purification and 90° hyphal branching served as key diagnostic features. Amplification of the ITS region using ITS1 and ITS4 primers, as demonstrated in our work, further validated molecular approaches reported by Ganeshamoorthi and Dubey (2013) and others, underlining the reliability of ITS-based genotyping in pathogen identification and differentiation, which is pivotal for subsequent biocontrol strategies.

Our *in vitro* assays demonstrated strong antagonistic activity by selected *Bacillus* isolates against *R. solani*, echoing the findings of Kumar *et al.* (2013) (32.43-50%), Solanki *et al.* (2015) (62.5-63%) and Lan *et al.* (2024) (82.86%). Notably, the observed inhibition percentages in this study are consistent with, or in some cases exceed, those reported in previous literature, highlighting the strain-specific potency of *B. subtilis* and *B. amyloliquefaciens*. These strains not only disrupted fungal growth but also exhibited significant morphological deformities in *R. solani*, similar to the cellular damage described by Ali *et al.* (2017) and Ekrim *et al.* (2021). These findings emphasize the importance of exploring strain-specific inhibitory effects for optimized pathogen control.

The efficacy of volatile organic compounds, chitinase, and β-1,3-glucanase adds an additional layer to the antagonistic arsenal of *Bacillus* spp. This aligns with studies by Solanki *et al.* (2015), Gao *et al.* (2023), and others who highlighted the suppressive action of antifungal volatiles. Moreover, the production of chitinase and â-1,3-glucanase observed in our isolates supports the enzymatic data of Zarei *et al.* (2012); Raut *et al.* (2021) and Solanki *et al.* (2012), reaffirming the relevance of hydrolytic enzyme activity in fungal cell wall degradation.

The clustering and molecular identification of six potent isolates (B10, B2, B22, B12, B4, B7) facilitated a targeted approach for subsequent *in vivo* trials. Field evaluations confirmed the biocontrol efficacy of these isolates, reflected in reduced disease incidence and improved agronomic parameters. The results resonate with earlier reports by Suryadi *et al.* (2021); Debez *et al.* (2024), demonstrating significant disease suppression and improved yield outcomes in *Bacillus*-treated tubers. Importantly, our study highlights the dual function of *Bacillus* spp. as both BCA's and PGPR's. Enhanced root and shoot growth, as well as increased yield in treated plots, are consistent with findings by Patel and Singh (2020); Uysal and Kantar, (2020). The ability of these isolates to solubilize phosphate, produce phytohormones, and induce systemic resistance underscores their multifaceted contribution to plant health and productivity. Various formulations, including talc-based products, have demonstrated strong disease-suppressive activity and yield benefits (Suryadi *et al.*, 2021; Kumar *et al.*, 2013; Ben Khedher *et al.*, 2015). In both pot and field studies, *Bacillus* strains significantly decreased the severity of black scurf and other diseases, while promoting healthier plant growth. The beneficial effects are attributed to multiple mechanisms, including phosphate solubilization, nitrogen fixation, hormone production, and secretion of antimicrobial compounds (Sivasakthi *et al.*, 2014). These mechanisms also enhance nutrient uptake and root development, contributing to increased plant height, root length, and ultimately, tuber yield (Ali *et al.*, 2017). In field studies, *Bacillus subtilis* B4 and *B. amyloliquefaciens* B7 exhibited strong rhizosphere persistence throughout the crop period and significantly reduced black scurf incidence, with notable improvements in plant vigour and yield (Brar *et al.*, 2025). Microbial fertilizers using *B. subtilis* and *B. amyloliquefaciens* provided yields comparable to chemical fertilizers, highlighting their potential as sustainable alternatives (Uysal and Kantar, 2020). Field trials also showed significant yield gains, with some studies reporting up to 12-19% increases over control (Shuang *et al.*, 2022).

5. Conclusion

B. subtilis B4 based talc formulation with treatment tuber dip 15 g + soil 3.5 kg significantly reduced disease severity by 90.91 % and enhanced the yield, thus offering a promising and eco-friendly approach to disease management and crop enhancement. Future research should focus on optimizing formulations, exploring microbial consortia, and conducting extensive field trials to validate their efficacy across different agro-climatic conditions.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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