



Article

Synergistic potential of *Trichoderma* and *Bacillus* isolates for suppressing *Rhizoctonia solani*, the causal agent of Damping-off in common Bean

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Abstract: Common bean (*Phaseolus vulgaris* L.) is considered a very common vegetable crop in many countries worldwide. It is the world's most widely consumed food legume, providing an essential global source of dietary protein, complex carbohydrates and micronutrients. Damping-off caused by *Rhizoctonia solani* is a major constraint to common bean production, highlighting the need for effective and environmentally compatible disease management strategies. This study evaluated the antagonistic activity and biocontrol efficacy of three *Trichoderma* isolates and three *Bacillus* isolates against *R. solani* under laboratory, greenhouse, and field conditions. Three *R. solani* isolates were obtained from diseased bean roots collected from El-Fayoum, El-Sharkia, and El-Giza governorates and evaluated for pathogenicity on three common bean cultivars. The antagonistic activity of the bioagents was assessed using a dual-culture assay, followed by greenhouse evaluation of individual and combined treatments applied as powder and suspension formulations at different rates (2.5, 5, and 10 ml or g/kg seeds). The most effective treatments were subsequently evaluated under field conditions. All bioagents (*Trichoderma harzianum* T2, *T. koningii* T1, *T. asperellum* T3, *Bacillus subtilis* B2, *B. velezensis* B1, and *B. subtilis* B3) significantly inhibited the mycelial growth of the three *R. solani* isolates *in vitro*, with *T. harzianum* (T2) showing the highest inhibition. Paulista exhibited the highest pre-emergence damping-off and was therefore selected for subsequent experiments. Increasing application rates generally improved disease suppression, while suspension formulations generally provided greater protection than powder formulations. Among the tested treatments, the combined application of *B. subtilis* (B2) and *T. harzianum* (T2) provided the greatest reduction in damping-off and the highest plant survival in greenhouse and field experiments. Under field conditions, this combination achieved 90.7±2 % plant survival when applied as a suspension formulation compared with 26.7±3 % in the untreated control, restricted pre-emergence damping-off to 9.3±1%, and entirely

suppressed post-emergence incidence ($0.0\pm0\%$). It also produced the highest shoot and root biomass, pod production, yield, and several biochemical parameters (total phenol content 15.4 mg/g, total chlorophyll 16.7 mg/g, soluble proteins levels 7.9 mg/g, resulting in a maximum yield 3.93 ± 0.02 ton/fed compared to 1.71 ± 0.09 ton/fed in the untreated control). These findings indicate that selected combinations of *Trichoderma* and *Bacillus*, particularly B2 + T2, have considerable potential and highly effective biological for integrated management strategy of *R. solani*-induced damping-off in common bean.

Key words: *Phaseolus vulgaris*. Common bean; *Rhizoctonia solani*; *Trichoderma*; *Bacillus*; Biological control. Damping-off.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most vital grain legume crops globally, serving as a primary source of dietary protein, minerals, and economic revenue for millions of smallholder farmers (Mayo-Prieto *et al.*, 2019). Despite their agricultural and nutritional significance, common bean plants are attacked by numerous diseases. These diseases can attack all plant parts, including roots, leaves, stems, and pods (Mayo *et al.*, 2015). Among these, *Rhizoctonia solani* Kühn stands out as a devastating necrotrophic pathogen capable of causing severe pre- and post-emergence damping-off, root rot, and hypocotyl lesions, ultimately leading to massive stand reductions and yield losses worldwide (Sturrock *et al.*, 2015; Mayo-Prieto *et al.*, 2019).

Conventionally, the management of *R. solani* relies heavily on synthetic chemical fungicides. However, escalating environmental concerns, the emergence of fungicide-resistant pathogen strains, and the imperative for sustainable agricultural practices have driven an urgent shift toward eco-friendly alternatives (Mayo *et al.*, 2015). Biological control using rhizosphere-competent microorganisms presents a highly promising, sustainable strategy for suppressing soil-borne diseases while enhancing overall crop performance (Mayo-Prieto *et al.*, 2019).

Among biological agents, fungal species of the genus *Trichoderma* and bacterial strains of *Bacillus* have emerged as premier biocontrol agents (BCAs). *Trichoderma* spp. (such as *T. harzianum*, *T. koningii*, and *T. asperellum*) exert robust antagonism against *R. solani* through mycoparasitism, spatial competition, the secretion of cell wall-degrading extracellular enzymes (chitinases and glucanases), and the production of bioactive secondary metabolites (Mayo *et al.*, 2015; Mayo-Prieto *et al.*, 2019). Concurrently, plant growth-promoting rhizobacteria (PGPR) like *Bacillus subtilis* contribute substantially to disease suppression via the synthesis of lipopeptides, antibiotics, and the induction of systemic resistance (ISR) in host plants. Furthermore, recent studies highlight that co-inoculating or combining compatible microbial consortia (e.g., dual applications of *Bacillus* and *Trichoderma* strains) can yield synergistic protective effects, significantly outperform single-strain applications, and provide superior, stable control against damping-off complexes in common beans (Braga *et al.*, 2025; Rigobelo *et al.*, 2024).

This work aimed to study the role of the tested bioagents, each alone or in combination as powder or suspension, in managing bean damping-off caused by *R. solani*. The study evaluated the effect of the tested bioagents on some crop parameters of bean plants grown in soil infested by the pathogenic fungus. Also, the work was expanded to assess some biochemical changes associated with the use of these bioagents in managing bean damping-off.

2. Materials and Methods

2.1. Isolation, purification, and identification of the causal organisms

Samples of common bean plants showing typical root-rot symptoms were collected from different governorates, namely El-Fayoum, El-Sharkia, and El-Giza, during the 2025 and 2026 growing seasons. The infected roots were carefully washed with tap water and then cut into small pieces. The infected root pieces were air-dried, then surface-sterilized using a 2% sodium hypochlorite solution for 3 minutes.

The surface-sterilized root pieces were washed several times with sterile water and dried between folds of sterilized filter paper. The pieces were transferred onto Petri dishes containing water agar. The inoculated plates were then incubated at 25°C and examined periodically. The obtained colonies were purified using the hyphal tip technique (Hawker, 1960; Griffin, 1994). The purified fungal isolates were identified based on their morphological and microscopic characteristics using the descriptions provided by Sneh *et al.* (1991). The fungal isolates were maintained on sterilized potato dextrose agar (PDA) slants and kept in a refrigerator at 5 °C for further studies.

2.2. Source of the biological control agents.

Six isolates of biological control agents (*Trichoderma harzianum* (T2), *Trichoderma koningii* (T1), *Trichoderma asperellum* (T3), *Bacillus subtilis* (B2), *B. velezensis* (B1), and *B. subtilis* (B3) were obtained from the Central Laboratory of Organic Agriculture, Agricultural Research Center (ARC). The accession number of each isolate was recorded.

2.3. Laboratory experiments

Antagonistic effect of different *Bacillus* spp. and *Trichoderma* spp. isolates against *R. solani*.

Screening of different bacterial isolates for their antagonistic capacities against *R. solani* was carried out using the dual culture plate method (Siddiqui *et al.*, 2001). A loopful of 48-hour-old culture of *Bacillus* spp. grown on nutrient glucose broth (NGB) was streaked on one side of Petri plates (9 cm in diameter) containing NGA medium. Petri dishes containing 15 mL of Gliotoxin Fermentation agar (GFA) medium were inoculated on one side with a disc (0.5 cm in diameter) of one of the *Trichoderma* spp. isolates, obtained from 4 days old culture.

The other side of each plate was inoculated with a disk (0.5 cm in diameter) of the tested pathogen taken from the periphery of a 4-day-old colony grown on PDA medium. Plates inoculated only with the pathogen were used as controls. Three replicates were used for each treatment. The plates were incubated at 25°C, and any resulting inhibition zones were measured once the mycelium in the control plates had fully overgrown the medium surface. The percentage of reduction in the mycelial growth of the pathogenic fungi was calculated as follows:

$$\% \text{ reduction of linear growth} = 100 - [G_2 / G_1 \times 100]$$

Where;

G_1 = is the growth diameter of the pathogenic fungus in the check plates (mm) and

G_2 = is the growth diameter of the pathogenic fungus in treated plates (mm).

Isolates that produced a highly clear zone of inhibition or caused lysis of the pathogenic fungal mycelium were considered bioagents and used for further studies.

2.4. Greenhouse experiments

All greenhouse experiments were carried out in a greenhouse belonging to the Central Lab of Organic Agriculture, ARC, Giza, Egypt. Plastic pots filled with clean sand, clay and peat moss were used. Three replicates for each treatment were used. Five pots were used for each replicate. Six bean seeds were sown in each pot. Each treatment included 30 bean plants. All pots received the same treatments regarding irrigation and nutrition regimen. Planted pots were kept under normal open greenhouse conditions.

a. Susceptibility of three bean cultivars against the tested pathogenic fungi under greenhouse conditions

(1) Inoculum preparation and soil infestation

Inoculum of *R. solani* was prepared in sterilized bottles by adding 250 g of corn sand meal medium supplemented with 0.2 % peptone solution (Abd-El-Moity, 1985; Paulitz and Schroeder, 2005). Bottles were separately inoculated using equal agar disks (0.5 cm diameter) taken from the edge of an active *R. solani* culture. Inoculated bottles were incubated at 25 °C for 15 days. Plastic pots (25 cm in diameter), each contained growing medium (clean sand, clay and peat moss(1:1:1w:w:w)), were

infested with the prepared inoculum at the rate of 10 g of fungal inoculum per 1kg of growing medium, according to **Spurlock et al. (2015)** and **Dörfors et al. (2022)**. The infestation process was carried out 5 days before sowing bean seeds. The three different bean cultivars (Bronco, Paulista, Auxira) were separately sown in the infested pots at the rate of six seeds/pot. Pots containing non-infested peat moss were used as controls. Three replicates for each treatment. Five pots were used for each replicate.

Disease assessment

The percentages of pre- and post-emergence damping-off were calculated 7 and 15 days after the sowing date, respectively, while the surviving plants were recorded 30 days after sowing. All percentages of disease assessment were calculated as follows (**El-Helaly et al., 1970**):

% Pre-emergence = $\frac{\text{Number of non-germinated seeds}}{\text{Total number of sown seeds}} \times 100$.

Total number of sown seeds

% Post-emergence = $\frac{\text{Number of dead seedlings}}{\text{Total number of sown seeds}} \times 100$.

Total number of sown seeds

% Survived plants = $\frac{\text{Number of survived plants}}{\text{Total number of sown seeds}} \times 100$.

Total number of sown seeds.

(2). Preparation of the bioagent inocula as powder and suspension

Three *Bacillus* spp. isolates and three *Trichoderma* spp. isolates were prepared as powder and suspension formulations. The different *Bacillus* spp. isolates were grown separately in liquid NG for 2 days at 30°C ±2. The different *Trichoderma* spp. isolates were grown separately in liquid GF medium for 11 days under complete darkness conditions at 25°C (**Abd-El-Moity and Shatla, 1981**).

Powder Formulation

The bioagents were grown on liquid fermentation medium until high propagule density was reached, typically achieving 10⁸ to 10⁹ spores/g for *Trichoderma* and 10⁸ to 10⁹ CFU/g for *Bacillus* (**Kumar et al., 2023**). The harvested fungal spores or bacterial cell biomass were thoroughly mixed with sterilized, finely ground talcum powder (1:2 ratio) under laminar airflow conditions. Carboxymethyl cellulose (CMC) was added at 0.5% to 1.0% (w/w) as an adhesive to enhance the adherence of the powder to the seed coat. The mixture was dried at room temperature and then packed in airtight bags for storage. For application, the powder was used as seed treatment (**Guzmán-Guzmán et al., 2023**).

Suspension Formulation

The bioagents were grown in an optimized liquid growth medium (such as nutrient broth or GF medium) using a rotary shaker or fermenter under controlled temperature and aeration. The resulting liquid culture/spore suspension was adjusted with sterile distilled water or buffer to a standardized concentration, typically 10⁸ to 10⁹ spores/mL for *Trichoderma* and 10⁸ to 10⁹ CFU/mL for *Bacillus* (**Kumar et al., 2023**). Protective adjuvants, such as glycerol, along with biocompatible wetting agents, were blended into the liquid matrix to minimize metabolic stress, prevent clumping, and extend shelf life (**Guzmán-Guzmán et al., 2023**). The liquid suspension was applied directly to seeds at designated volumetric rates, followed by gentle mixing and uniform shade-drying before planting.

Isolates involved in one mixture were chosen according to a compatibility experiment. Each of the tested isolates was inoculated into sterilized, melted NGA medium, and the inoculated medium was then poured into Petri dishes. After solidification, the second tested antagonist was placed at the center of the medium. Plates were incubated at 25°C for fungi and 30°C for bacteria for 4 and 2 days, respectively. The zone of inhibition (if present) was observed and measured. Each pair of cultures showing no antagonism was considered a compatible pair (**Dugassa et al., 2021**). Compatible pairs were selected to prepare the powder and suspension formulations.

b. Effect of different doses of the bioagents, applied singly or in mixtures as powder or suspension formulations

Bean seeds cv. Paulista were dressed using formulated bioagents as powder or suspension, separately, at different rates (2.5, 5 and 10 g/kg of seeds for powder or 2.5, 5 and 10 mL/kg seeds for suspension). A 0.5% Arabic gum solution was used as an adhesive agent. Six dressed seeds were sown in each pot infested with *R. solani*. Five pots were used for each replicate. Three replicates for each treatment. Non-treated seeds were sown in pots to act as a control treatment. All pots were examined periodically, and the percentages of pre- and post-emergence damping-off, as well as surviving plants, were determined at 7, 15 and 30 days, respectively.

2.5. Field experiment

In all field experiments, the experiments were carried out during the 2025/2026 season at a farm located in El-Fayoum Governorate with naturally infested soil. The soil at this farm was light sandy soil with an alkaline reaction (pH 7.5). Paulista bean cv. was used. Five rows were used for each replicate, with 30 bean seeds sown in each row (4 × 0.6 m). This means 150 seeds were used for each replicate. Three replicates were used for each treatment. All plants received the same fertilizers and irrigation regime. According to the results obtained from the greenhouse experiments, only the most effective single isolates or mixtures of the tested bioagents were used in the field studies, and the most effective application rate determined from the greenhouse experiments was used, either as powder at 10 g/kg seeds or as suspension at 10 mL/kg seeds.

a. Effect of using the tested bioagents, formulated as powder and suspension, on controlling bean damping-off under field conditions

Three different mixtures of the bioagents *T. harzianum* (T2) with *B. subtilis* (B2), *T. koningii* (T1) with *T. harzianum* (T2), and *B. velezensis* (B)1 with *B. subtilis* (B2) were tested for their efficacy on the incidence of damping-off disease. Single or mixtures of the bioagents were prepared as powder or suspension as described before. Bean seeds cv. Paulista were treated with the prepared antagonistic formula. Treated seeds were then sown in naturally infested soil. Non-treated seeds were sown in separate plots to act as a control treatment. All treatments were examined periodically, and percentages of pre- and post-emergence damping-off were calculated 7 and 15 days after sowing, respectively. Meanwhile, the surviving plants were recorded 30 days after sowing.

b. Effect of using the tested bioagents, formulated as powder and suspension, on agronomic characteristics of treated bean plants under field conditions

This experiment was carried out to understand the role of the different treatments, using different bioagents, on the fresh and dry weights of the surviving plants. Pod yield is the main target of any treatment, so the pod yield in each treatment was determined to evaluate its effect on pod production. The percentage of dry matter of pods was also recorded.

c. Effect of using the tested bioagents formulated as powder and suspension on some chemical components in treated plants under field conditions

This experiment was carried out to understand the role of the two formulas (powder and suspension) and the different bioagents in the chemical components of the treated bean plants compared with those of non-treated bean plants. Samples of the treated plants were collected from different tested treatments to assess their chemical contents (phenols, sugars, protein, and total chlorophyll) in the different treatments. Collected samples were washed with tap water and then prepared to determine reducing and non-reducing sugars using the method developed by **Thomas and Dutcher (1924)**. Free and conjugated phenols were also determined using a method developed by **Simons and Ross (1971)**. Protein and chlorophyll content were also determined using a method developed by **Bradford (1976)** for protein and a method developed by **Nornai (1982)** for chlorophyll content.

2.6. Statistical Analysis

Laboratory experiments were arranged in a completely randomized design (CRD), whereas greenhouse and field experiments were arranged in a randomized complete block design (RCBD). Data were subjected to analysis of variance (ANOVA) using CoStat Statistical Software (1990),

Microcomputer Program Analysis Version 4.2 (Cohort Software, Berkeley, CA, USA). Differences among treatment means were compared using the least significant difference (LSD) test at the 5% probability level.

3. Results

3.1. The damping-off pathogenic fungi

Samples of common bean plants showing typical root-rot symptoms were collected from different governorates, *i.e.*, El-Fayoum, El-Sharkia, and El-Giza. The infected roots were surface-sterilized. Isolation of the causal fungus was carried out from the roots as described in the Materials and Methods. Three purified fungal isolates were identified as *Rhizoctonia solani* Kühn based on their cultural, morphological, and microscopic characteristics (Table 1). Identification was confirmed by the Mycology and Plant Disease Survey Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt. The fungal isolates were maintained on PDA slants and kept in a refrigerator at 5 °C for further studies

Table (1). Names and sources of the pathogenic fungal isolates

Isolate	Source
<i>Rhizoctonia solani</i> 1	El-Fayoum
<i>Rhizoctonia solani</i> 2	El-Sharkia
<i>Rhizoctonia solani</i> 3	El-Giza

3.2. Names and sources of different biocontrol agents

Data in Table 2 show that six biological control agents were obtained from the Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC). Three isolates belonged to the genus *Trichoderma*, and three isolates belonged to the genus *Bacillus*. These bioagents were tested under laboratory conditions to select the most effective agents for use under greenhouse and field conditions.

Table (2). Names and accession numbers of the biocontrol agents

Biological agent	Accession number
<i>Trichoderma harzianum</i> T2	MT110634
<i>Trichoderma asperellum</i> T3	MT110638
<i>Trichoderma koningii</i> T1	MT110636
<i>Bacillus velezensis</i> B1	MT110637
<i>Bacillus subtilis</i> B2	MT110640
<i>Bacillus subtilis</i> B3	MT110633

3.3. Laboratory studies

Antagonistic effect of different *Bacillus* spp. and *Trichoderma* spp. isolates against *R. solani* isolates

The efficacy of different fungal and bacterial bioagents in inhibiting the linear mycelial growth of three *R. solani* isolates was evaluated *in vitro*. Data in Table 3 and Fig. 1 show that all tested bioagents significantly reduced the linear mycelial growth of the three *R. solani* isolates compared with the control.

T. harzianum T2 showed the highest reduction in linear mycelial growth among the tested bioagents, with reductions of 54.9±0.2 %, 53.8±0.2 %, and 52.1±0.2 % against *R. solani* isolates 1, 2, and 3, respectively. *T. koningii* T1 caused reductions of 51.0±0.1 %, 51.6±0.2 %, and 50.3±0.3 %, respectively.

whereas *T. asperellum* T3 caused reductions of $50.3 \pm 0.3\%$, $50.9 \pm 0.4\%$, and $51.1 \pm 0.1\%$ against *R. solani* isolates 1, 2, and 3, respectively.

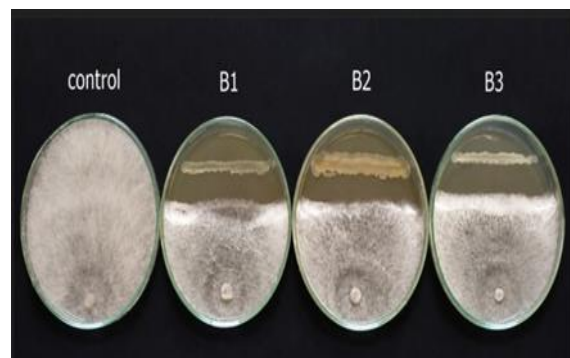
The bacterial isolates also exhibited antagonistic activity against the three *R. solani* isolates. *B. subtilis* B2 caused reductions of $51.5 \pm 0.1\%$, $48.0 \pm 0.1\%$, and $48.0 \pm 0.1\%$ against *R. solani* isolates 1, 2, and 3, respectively. *B. velezensis* B1 caused reductions of $50.1 \pm 0.1\%$, $48.4 \pm 0.1\%$, and $45.8 \pm 0.2\%$, while *B. subtilis* B3 caused reductions of $49.1 \pm 0.1\%$, $47.0 \pm 0.1\%$, and $48.4 \pm 0.2\%$ against *R. solani* isolates 1, 2, and 3, respectively. The lowest reduction was recorded for *B. velezensis* B1 against *R. solani* isolate 3 ($45.8 \pm 0.2\%$).

Table (3). Antagonistic effect of different *Bacillus* spp. and *Trichoderma* spp. isolates on the linear mycelial growth of *Rhizoctonia solani* isolates

Biological agent	% Reduction in linear mycelial growth		
	<i>R. solani</i> (1)	<i>R. solani</i> (2)	<i>R. solani</i> (3)
<i>T. koningii</i> T1	51.0 ± 0.1	51.6 ± 0.2	50.3 ± 0.3
<i>T. harzianum</i> T2	54.9 ± 0.2	53.8 ± 0.2	52.1 ± 0.2
<i>T. asperellum</i> T3	50.3 ± 0.3	50.9 ± 0.4	51.1 ± 0.1
<i>B. velezensis</i> B1	50.1 ± 0.1	48.4 ± 0.1	45.8 ± 0.2
<i>B. subtilis</i> B2	51.5 ± 0.1	48.0 ± 0.1	48.0 ± 0.1
<i>B. subtilis</i> B3	49.1 ± 0.1	47.0 ± 0.1	48.4 ± 0.2
Control	0.0 ± 0	0.0 ± 0	0.0 ± 0
LSD _{0.05}	0.27	0.34	0.31



Trichoderma spp. isolates



Bacillus spp. isolates

Fig. (1). Antagonistic effect of *Trichoderma* spp. and *Bacillus* spp. isolates on the linear mycelial growth of *Rhizoctonia solani* isolates

4. Greenhouse studies

a. Susceptibility of different bean cultivars to *R. solani* isolates under greenhouse conditions

Inoculation with *R. solani* isolates significantly increased both pre- and post-emergence damping-off incidence in all tested cultivars (Paulista, Bronco, and Auxira), resulting in a marked reduction in plant survival compared with the uninoculated control.

Data in Table 4 show that pre-emergence damping-off varied according to the *R. solani* isolate and bean cultivar. *R. solani* 1 caused the highest pre-emergence damping-off in Paulista ($54.4 \pm 2\%$), whereas the incidence in Auxira was $26.6 \pm 3\%$ under the same inoculation. Similarly, *R. solani* 2 caused $47.7 \pm 1\%$ pre-emergence damping-off in Paulista, compared with $34.4 \pm 1.5\%$ and $30.0 \pm 1.3\%$ in Bronco

and Auxira, respectively. *R. solani* 3 caused pre-emergence damping-off, ranging from 16.7±1.69 % in Auxira to 33.3±1 % in Paulista.

Post-emergence damping-off also varied among cultivars and *R. solani* isolates. *R. solani* 3 caused the highest post-emergence damping-off in Paulista (36.7%), followed by Auxira (33.3±3.35 %) and Bronco (20.0±3.35%). *R. solani* 1 caused post-emergence damping-off of 30.0±3.3 %, 23.3±3.35%, and 26.7±3.35 % in Paulista, Bronco, and Auxira, respectively, whereas *R. solani* 2 caused 36.7±3.35 %, 22.2±1.91 %, and 26.7±3.3 %, respectively.

Table (4). Susceptibility of different bean cultivars to *Rhizoctonia solani* isolates under greenhouse conditions

Pathogen	Percentage of damping-off incidence						% Survived plants		
	Pre-emergence			Post-emergence					
	Paulista	Bronco	Auxira	Paulista	Bronco	Auxira	Paulista	Bronco	Auxira
<i>R. solani</i> 1	54.4±2	36.7±1	26.6±3.3	30.0±3.3	23.3±3.35	26.7±3.35	15.6±1.96	40.0±1.96	46.7±3.3
<i>R. solani</i> 2	47.7±1	34.4±1.5	30±4.13	36.7±3.35	22.2±1.91	26.7±3.3	15.6±3.67	43.3±3.35	43.3±3.3
<i>R. solani</i> 3	33.3±1	20.0±3.3	16.7±1.69	36.7±3.35	20.0±3.35	33.3±3.35	30.0±3.34	60.0±3.35	50.0±3.3
Control	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	100±0	100±0	100±0
LSD _{0.05}	4.65			5.19			5.04		

The uninoculated control maintained 100% plant survival in all three cultivars. Among the inoculated treatments, the lowest survival percentage (15.6±3.67 %) was recorded in Paulista inoculated with *R. solani* (1) and *R. solani* 2, whereas the highest survival percentage (60.0±3.35%) was recorded in Bronco inoculated with *R. solani* (3). Percentages of survived plants are also presented in Fig. (2) to illustrate the variation in survival among the three bean cultivars following inoculation with the tested *R. solani* isolates.

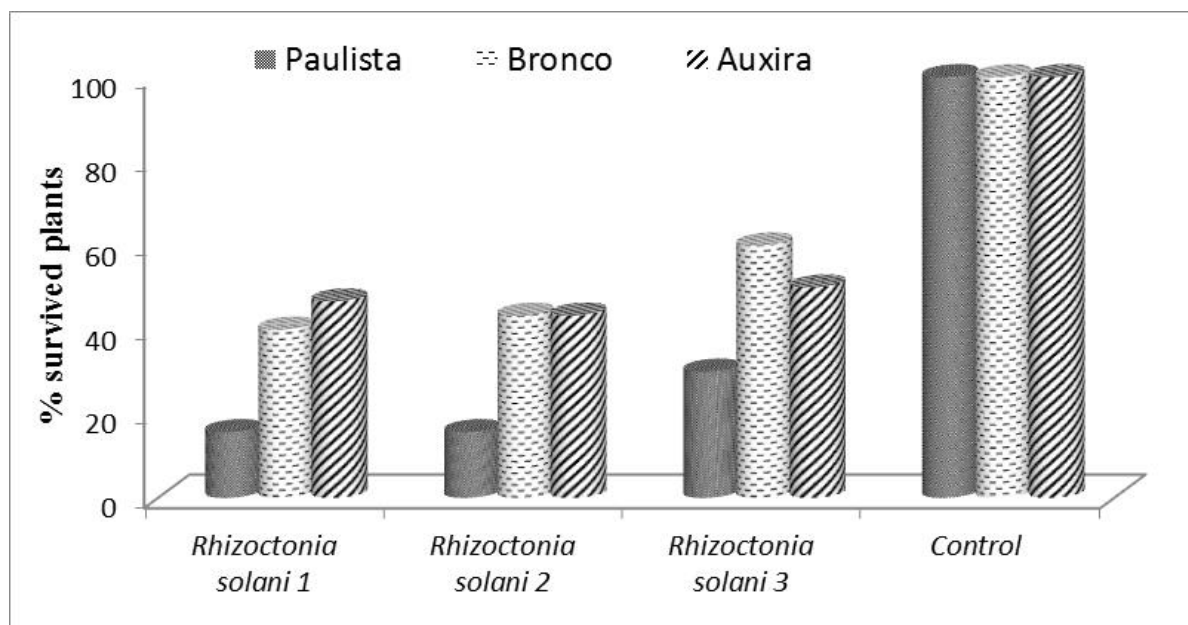


Fig. (2). Percentage of survived plants of different bean cultivars inoculated with *Rhizoctonia solani* isolates under greenhouse conditions

b. Effect of different doses of different bioagents, used as single or mixed treatments, formulated as powder on damping-off disease and survival of bean plants under greenhouse conditions.

The application of single and combined bioagents (Table 5) generally reduced pre- and post-emergence damping-off incidence while improving plant survival compared to the untreated control, which exhibited a severely limited survival rate of 6.7 ± 2 % alongside high pre-emergence (60.0 ± 3 %) and post-emergence (33.3 ± 3 %) disease incidence

The individual treatment *B. subtilis* B2 at 10.0 g/kg reduced pre-emergence damping-off incidence to 16.7 ± 2 %, while *B. velezensis* B1 gave 20.0 ± 2 %. Greater efficacy was observed with dual combinations of *B. subtilis* B2 + *T. harzianum* T2 at 10.0 g/kg, which significantly reduced pre-emergence damping-off incidence to the lowest value of 10.0 ± 1 %, closely followed by *B. velezensis* B1 + *B. subtilis* B2 at 13.3 ± 2 %. Post-emergence damping-off was heavily suppressed across nearly all treatments. At 5 and 10 g/kg, post-emergence damping-off was 0.0 ± 0 % for most individual bioagents and all combined treatments. Overall plant survival increased markedly across all bioagent-treated seeds. The highest plant survival rate (90.0 ± 1 %) was recorded under the combined treatment of *B. subtilis* B2 + *T. harzianum* T2 at 10.0 g/kg of seeds. The combined treatments *B. velezensis* B1 + *B. subtilis* B2 and *T. koningii* T1 + *T. harzianum* T2 at 10 g/kg resulted in survival rates of 86.7 ± 1 % and 83.3 ± 1 %, respectively. Among individual applications, *B. subtilis* B2 resulted in 83.3 ± 1 % survival at the highest tested dosage (10.0 g/kg).

Table (5). Effect of different doses of single and mixed powder formulations of bioagents on damping-off and survival of bean plants under greenhouse conditions

Bioagent	% of Damping-off incidence						% Survived plants		
	Pre-emergence			Post-emergence					
	Dose (g/kg seeds)			Dose (g/kg seeds)			Dose (g/kg seeds)		
	2.5	5	10	2.5	5	10	2.5	5	10
<i>Bacillus velezensis</i> B1	43.3 $\pm$ 2	36.7 $\pm$ 2	20.0 $\pm$ 2	13.3 $\pm$ 1	6.6 $\pm$ 1	0.0 $\pm$ 0	43.4 $\pm$ 2	56.7 $\pm$ 2	80.0 $\pm$ 1
<i>Bacillus subtilis</i> B2	40.0 $\pm$ 3	33.3 $\pm$ 2	16.7 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	60.0 $\pm$ 3	66.7 $\pm$ 3	83.3 $\pm$ 1
<i>Bacillus subtilis</i> B3	56.7 $\pm$ 2	40.0 $\pm$ 2	26.7 $\pm$ 3	6.7 $\pm$ 1	6.7 $\pm$ 2	0.0 $\pm$ 0	36.6 $\pm$ 2	53.3 $\pm$ 2	73.3 $\pm$ 1
<i>Trichoderma koningii</i> T1	40.0 $\pm$ 2	36.7 $\pm$ 3	30.0 $\pm$ 3	3.3 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	56.7 $\pm$ 2	63.3 $\pm$ 3	70.0 $\pm$ 1
<i>Trichoderma harzianum</i> T2	43.3 $\pm$ 3	40.0 $\pm$ 2	23.3 $\pm$ 2	3.3 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	53.4 $\pm$ 3	60.0 $\pm$ 2	76.7 $\pm$ 2
<i>Trichoderma asperellum</i> T3	53.4 $\pm$ 2	50.0 $\pm$ 3	40.0 $\pm$ 2	6.6 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	40.0 $\pm$ 2	50.0 $\pm$ 2	60.0 $\pm$ 2
B1+B2	23.3 $\pm$ 2.5	16.7 $\pm$ 3	13.3 $\pm$ 2	10.0 $\pm$ 3	0.0 $\pm$ 0	0.0 $\pm$ 0	66.7 $\pm$ 2	83.3 $\pm$ 2	86.7 $\pm$ 1
T1+T2	30.0 $\pm$ 2	30.0 $\pm$ 1	16.7 $\pm$ 3	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	70.0 $\pm$ 3	70.0 $\pm$ 2	83.3 $\pm$ 1
B2+T2	20.0 $\pm$ 3	16.7 $\pm$ 3	10.0 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	80.0 $\pm$ 2	83.3 $\pm$ 3	90.0 $\pm$ 1
Control	60.0 $\pm$ 3			33.3 $\pm$ 3			6.7 $\pm$ 2		
LSD _{0.05}	3.95			1.27			3.47		

c. Effect of different doses of different bioagents, used as single or mixed treatments formulated as suspension, on damping-off disease and survival of bean plants under greenhouse conditions

All individual and combined bioagent treatments formulated as suspension formulations significantly reduced pre- and post-emergence damping-off incidence while improving overall plant survival compared to the untreated control. Table 6 shows that, in the control treatment, pre- and post-emergence damping-off reached 60.0 ± 2 % and 33.3 ± 1 %, respectively, with a plant survival rate of only 6.7 ± 2 %.

The individual bioagents demonstrated a clear dose-dependent protective effect across the tested concentrations (2.5, 5, and 10 mL/kg seeds). Increasing the application rate consistently decreased disease incidence and enhanced survival. Among the single-strain treatments, *B. subtilis* B2 and *T. harzianum* T2 at the 10.0 mL/kg dose performed best, reducing pre-emergence damping-off to 13.3 ± 2 % and 16.7 ± 1 %, respectively. Both strains successfully suppressed post-emergence damping-off to 0.0 ± 0 %, achieving a high plant survival rate of 83.3 ± 2 %. Dual inoculations (*B. velezensis* B1 + *B. subtilis* B2, *T. koningii* T1 + *T. harzianum* T2, and *B. subtilis* B2 + *T. harzianum* T2) showed greater efficacy than several single-strain treatments. The combination of *B. subtilis* B2 and *T. harzianum* T2 provided the highest level of protection across all tested doses. At the dose of 10.0 mL/kg, the *B. subtilis* B2 + *T. harzianum* T2 combination reduced pre-emergence damping-off to just 6.7 ± 1 %, completely eliminated post-emergence damping-off (0.0 ± 0 %), and resulted in the highest plant survival rate of 93.3 ± 2 %. The *B. velezensis* B1 + *B. subtilis* B2 combination closely followed, achieving a 90.0 ± 2 % survival rate at the same dose.

Table (6). Effect of different doses of single and mixed suspension formulations of bioagents on damping-off and survival of bean plants under greenhouse conditions

Bioagent	% of Damping-off incidence						% Survived plants		
	Pre-emergence			Post-emergence					
	Dose (mL/kg seeds)			Dose (mL/kg seeds)			Dose (mL/kg seeds)		
	2.5	5	10	2.5	5	10	2.5	5	10
<i>Bacillus velezensis</i> B1	43.3 $\pm$ 1.05	40.0 $\pm$ 3	20 $\pm$ 1	10.0 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	46.7 $\pm$ 2	60 $\pm$ 3	80.0 $\pm$ 1
<i>Bacillus subtilis</i> B2	36.7 $\pm$ 2	30.0 $\pm$ 1 1	13.3 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	63.3 $\pm$ 1	70.0 $\pm$ 1	83.3 $\pm$ 2.5
<i>Bacillus subtilis</i> B3	43.4 $\pm$ 1	40.0 $\pm$ 2	23.3 $\pm$ 1	6.7 $\pm$ 2	3.3 $\pm$ 0	0.0 $\pm$ 0	50.0 $\pm$ 1	56.7 $\pm$ 2	76.7 $\pm$ 1
<i>Trichoderma koningii</i> T1	53.3 $\pm$ 1	43.3 $\pm$ 2	30.0 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	46.7 $\pm$ 2	56.7 $\pm$ 1	70.0 $\pm$ 2
<i>Trichoderma harzianum</i> T2	46.7 $\pm$ 1	36.7 $\pm$ 2	16.7 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	53.4 $\pm$ 2	63.3 $\pm$ 3	83.3 $\pm$ 1
<i>Trichoderma asperellum</i> T3	46.7 $\pm$ 1	43.3 $\pm$ 2	36.7 $\pm$ 2	3.3 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	50.0 $\pm$ 1	56.7 $\pm$ 2	63.3 $\pm$ 6
B1+B2	23.3 $\pm$ 3	16.7 $\pm$ 2	10.0 $\pm$ 2	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	76.7 $\pm$ 1	83.3 $\pm$ 2	90.0 $\pm$ 2
T1+T2	26.7 $\pm$ 1	23.3 $\pm$ 1	16.7 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	73.3 $\pm$ 2	76.7 $\pm$ 2	83.3 $\pm$ 2
B2+T2	16.7 $\pm$ 1	13.3 $\pm$ 2	6.7 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0	83.3 $\pm$ 1	86.7 $\pm$ 2	93.3 $\pm$ 2
Control	60.0 $\pm$ 2			33.3 $\pm$ 1			6.7 $\pm$ 2		
LSD _{0.05}	2.76			1.07			3.47		

5. Field experiments

a. The impact of powder and suspension formulated bioagents on damping-off disease in beans under field conditions.

Data in Table 7 and Fig. (3) and Fig. (4) indicate that all used bioagents, either formulated as powder or as suspension, led to a significant reduction in disease incidence compared with the untreated control. The efficacy of combined bioagent treatments (*B. velezensis* B1 + *B. subtilis* B2, *T. koningii* T1 + *T. harzianum* T2, and *B. subtilis* B2 + *T. harzianum* T2) differed significantly between formulations for some combinations, with suspension applied at 10 mL/kg seeds or powder at 10 g/kg seeds. Overall, suspension formulations generally showed lower pre-emergence damping-off and higher plant survival than powder formulations. Post-emergence damping-off was completely suppressed (0.0 ± 0 %) across all bioagent treatments for both suspension and powder forms, compared with the untreated control. The combined application of *B. subtilis* B2 + *T. harzianum* T2 yielded the highest protective efficacy in both formulations. The suspension formulation reduced pre-emergence damping-off to 9.3 ± 1 % and achieved

a maximum plant survival rate of 90.7 ± 2 %. The powder formulation performed comparably well, with a pre-emergence damping-off rate of 10.7 ± 3.2 % and a survival rate of 89.3 ± 1 %. The combination of *B. velezensis* B1 + *B. subtilis* B2 in suspension formulation reduced pre-emergence damping-off to 14.7 ± 1 %, resulting in 85.3 ± 1 % plant survival. In comparison, the powder formulation was less effective, showing a higher pre-emergence incidence of 27.3 ± 1 % and a reduced survival rate of 72.7 ± 3 %. The mixture of *T. koningii* T1 + *T. harzianum* T2 followed a similar trend: the suspension formulation achieved 77.4 ± 2 % plant survival (22.6 ± 2 % pre-emergence damping-off), whereas the powder formulation yielded a lower survival rate of 68.7 ± 2 % (31.3 ± 1 % pre-emergence damping-off).

Table (7). Field evaluation of powder and suspension formulations of different bioagents on bean damping-off and plant survival

Bioagents	% of damping-off incidence				Survived plants (%)	
	Pre-emergence damping-off (%)		Post-emergence damping-off (%)			
	Suspension	Powder	Suspension	Powder	Suspension	Powder
	10mL/kg seeds	10 g/kg seeds	10mL/kg seeds	10 g/kg seeds	10mL/kg seeds	10 g/kg seeds
<i>Bacillus velezensis</i> B1 + <i>B. subtilis</i> B2	14.7 $\pm$ 1	27.3 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	85.3 $\pm$ 1	72.7 $\pm$ 3
<i>T. koningii</i> T1 + <i>T. harzianum</i> T2	22.6 $\pm$ 2	31.3 $\pm$ 1	0.0 $\pm$ 0	0.0 $\pm$ 0	77.4 $\pm$ 2	68.7 $\pm$ 2
<i>B. subtilis</i> B2 + <i>T. harzianum</i> T2	9.3 $\pm$ 1	10.7 $\pm$ 3.2	0.0 $\pm$ 0	0.0 $\pm$ 0	90.7 $\pm$ 2	89.3 $\pm$ 1
untreated control	63.3 $\pm$ 1.53		10.0 $\pm$ 2		26.7 $\pm$ 3	
LSD _{0.05}	5.69		1.23		3.74	

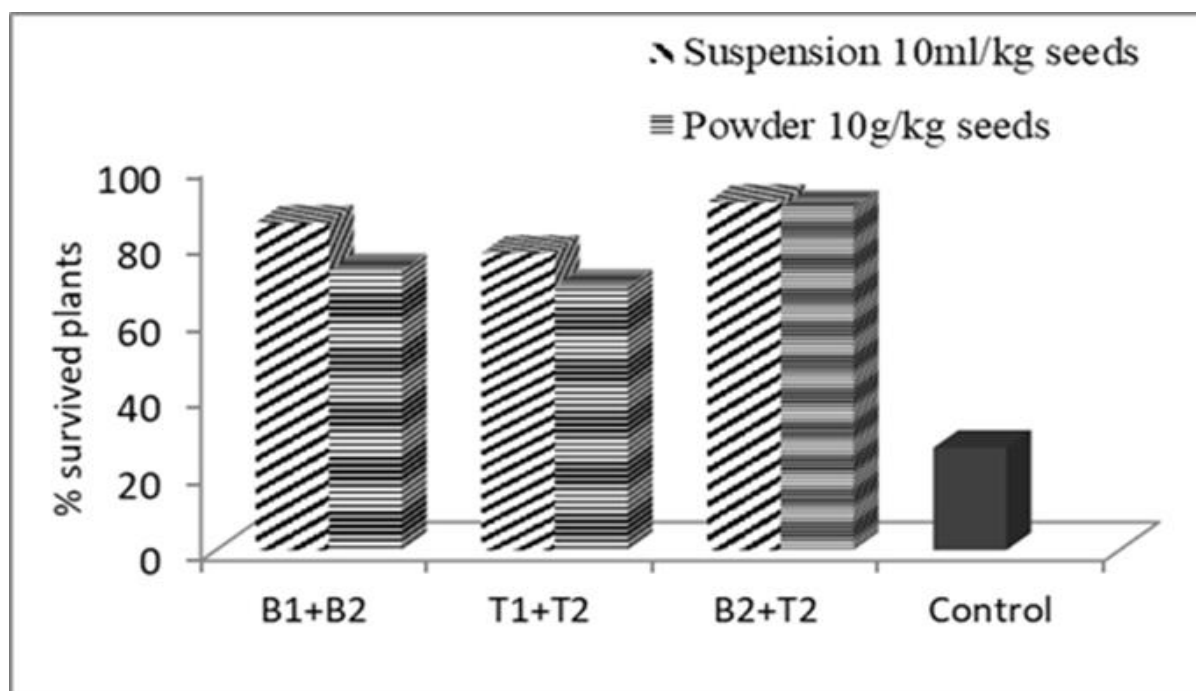


Fig. (3). Field evaluation of powder and suspension formulations of different bioagents on bean damping-off and plant survival. B1 = *B. velezensis* B1; B2 = *B. subtilis* B2; T1 = *T. koningii* T1; T2 = *T. harzianum* T2

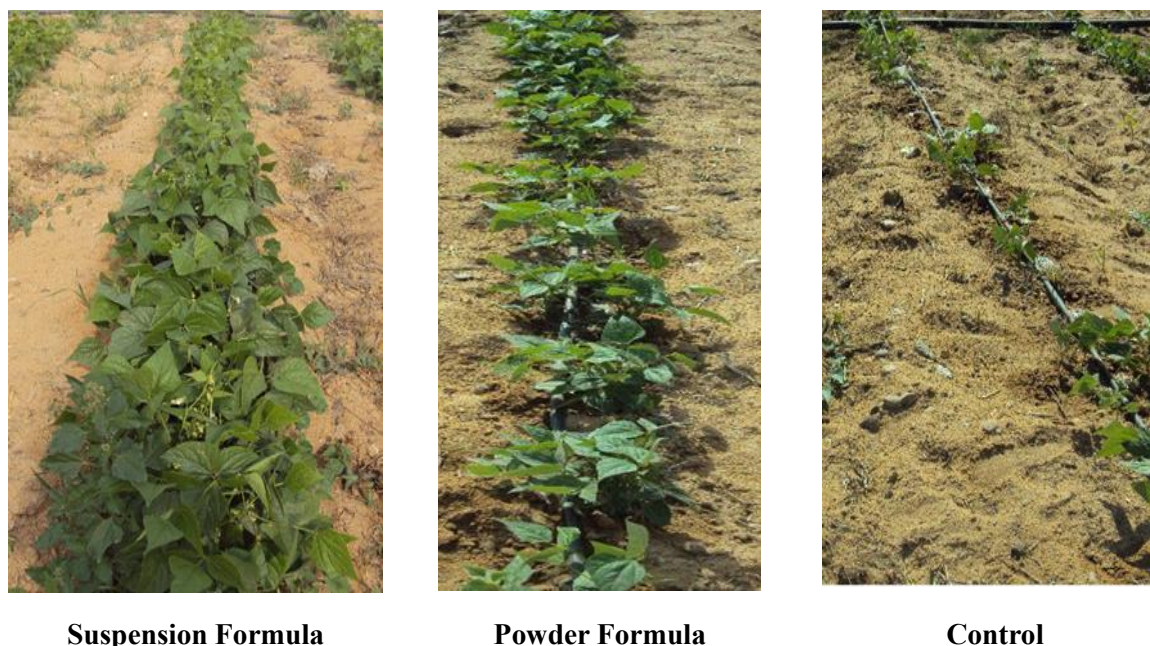


Fig. (4). Effect of using different bioagents, formulated as powder and suspension, on damping-off incidence in beans under field conditions

b. Influence of different bioagents, formulated as powder and suspension, on agronomic characteristics of bean plants under field conditions

The application of dual-bioagent seed treatments significantly enhanced all measured growth and yield parameters compared to the untreated control. Across all evaluated microbial combinations, liquid suspension formulations generally showed higher numerical values than their corresponding powder formulations in promoting plant biomass accumulation and final yield. The dual-inoculation of *B. subtilis* B2 and *T. harzianum* T2 in suspension form in Table 8 achieved the highest overall growth performance, registering a shoot fresh weight of 96.9 ± 2 g/plant and root fresh weight of 5.7 ± 0.2 g/plant. This was closely followed by the *B. subtilis* B2 + *T. harzianum* T2 powder formulation at 93.4 ± 3 g/plant and 5.1 ± 0.1 g/plant, respectively. Mixture treatment *B. subtilis* B2 + *T. harzianum* T2 as suspension also recorded maximum dry weights of 26.6 ± 1 g/plant (shoots) and 1.8 ± 0.1 g/plant (roots), with the highest shoot dry weight and the highest numerical root dry weight. Other combinations, such as *B. velezensis* B1 + *B. subtilis* B2 suspension (18.7 ± 0.5 g/plant shoot dry weight) and *T. koningii* T1 + *T. harzianum* T2 suspension (17.8 ± 2 g/plant shoot dry weight), also provided substantial increments over the control (6.9 ± 1 g/plant). Yield components including the number of pods per plant, total yield, and pod dry matter percentage showed marked improvements following bioagent application. The *B. subtilis* B2 + *T. harzianum* T2 suspension treatment yielded a maximum of 3.93 ± 0.02 ton/fed, representing approximately 2.8 times the yield of the untreated control (1.71 ± 0.09 ton/fed). The powder form of *B. subtilis* B2 + *T. harzianum* T2 yielded 3.6 ± 0.01 ton/fed. Among the bacterial and fungal pairings, the bacterial combination *B. velezensis* B1 + *B. subtilis* B2 (suspension) produced a higher yield (3.27 ± 0.06 ton/fed) than the fungal combination *T. koningii* T1 + *T. harzianum* T2 (suspension at 2.97 ± 0.05 ton/fed). Furthermore, pod dry matter content was significantly elevated in the *B. subtilis* B2 + *T. harzianum* T2 treatments (16.9 ± 2 % for suspension and 16.3 ± 0.3 % for powder) relative to the control (8.7 ± 0.2 %).

c. Biochemical effects of powder and suspension formulated bioagents on treated bean plants under field conditions.

Data present in Table 9 reveal that all biological treatments led to a clear increase in the percentage of reducing sugars, chlorophyll, and protein compared with the control treatment. The application of dual-bioagent seed treatments formulated as either suspensions (S) or powders (P) increased some biochemical constituents, including reducing sugars, total chlorophyll, and protein content, compared to the untreated control. The highest phenolic compound accumulation was observed in the *B. subtilis* B2

+ *T. harzianum* T2 suspension treatment, which achieved a total phenol content of 15.4 mg/g (comprising 10.1 mg/g free and 5.3 mg/g conjugated phenols), followed closely by its powder formulation at 12.6 mg/g. In contrast, the untreated control recorded a total phenol level of 11.8 mg/g. Reduced sugars peaked in the *B. subtilis* B2 + *T. harzianum* T2 suspension (4.9 mg/g), compared with the control (0.8 mg/g). Total chlorophyll levels reached a maximum of 16.7 mg/g in both the *B. subtilis* B2 + *T. harzianum* T2 suspension and powder treatments, compared to just 9.0 mg/g in the control. Similarly, protein concentrations increased to 7.9 mg/g under the *B. subtilis* B2 + *T. harzianum* T2 suspension treatment relative to 2.1 mg/g in the untreated control.

Table (8). Influence of different bioagents, formulated as powder or suspension, on agronomic characteristics of bean plants under field conditions

Bioagents	Formula	Fresh Weight (g)/plant		Dry weight(g)/plant		No. of Pods/ Plant	Yield(g)/ plant	%Dry matter/100g pods	Yield Ton/fedan
		Shoots	roots	shoots	roots				
<i>B. velezensis</i> B1 + <i>B. subtilis</i> B2	suspension	85.6±1	4.5±0.1	18.7±0.5	1.5±0.5	43±3	216.1±6	14.0±1	3.27±0.06
	powder	81.2±2	4.3±0.3	17.3±1	1.0±0.1	40±1	210.0±2	13. ±42	3.07±0.03
<i>T. koningii</i> T1 + <i>T. harzianum</i> T2	suspension	85.8±1	4.4±0.3	17.8±2	1.2±0.2	40±1.15	203.4±3	13.9±1	2.97±0.05
	powder	81.5±1	4.3±0.1	16.8±1	0.9±0.1	39±4	195.5±1.15	13.5±1	2.60±0.1
<i>B. subtilis</i> B2+ <i>T. harzianum</i> T2	suspension	96.9±2	5.7±.02	26.6±1	1.8±0.1	58±2	275.3±3	16.9±2	3.93±0.02
	powder	93.4±0.30	5.1±0.1	22.8±2	1.5±0.1	53±3	251.6±2	16.3±0.3	3.59±0.01
Control	-	36.3±2.05	2.6±0.3	6.9±1	0.3±0.1	20±2	98.4±2	8.7±0.2	1.71±0.09
LSD _{0.05}	-	2.58	0.38	2.31	0.38	4.40	5.43	2.20	0.10

Table (9). Biochemical effects of powder and suspension formulated bioagents on treated bean plants under field conditions

Treatments	Formula	Phenols (mg/g)			Sugars(mg/g)		Chlorophyll (mg/g)	Protein (mg/g)
		Free	Conj	Total	Reduced	Non-red.		
<i>B. velezensis</i> B1 + <i>B. subtilis</i> B2	suspension	6.5	1.8	8.3	4.2	0.7	15.9	7.3
	Powder	6.1	1.6	7.7	3.9	0.7	15.5	6.7
<i>T. koningii</i> T1 + <i>T. harzianum</i> T2	suspension	6.8	2.3	9.1	4.5	0.5	14.9	7.1
	Powder	6.3	1.4	7.7	3.5	1.2	13.3	6.5
<i>B. subtilis</i> B2 + <i>T. harzianum</i> T2	suspension	10.1	5.3	15.4	4.9	1.4	16.7	7.9
	Powder	8.3	4.3	12.6	4.5	1.6	16.7	7.5
Control	-	4.3	7.5	11.8	0.8	1.2	9.0	2.1

4. Discussion

Biological control, as an effective means to protect cultivated crops, especially those grown under organic agriculture conditions, is considered the most important method to produce fresh, healthy, and high-quantity crops free of chemical residues (Meshram *et al.*, 2026). By replacing or minimizing hazardous synthetic inputs, biological management suppresses phytopathogens while safeguarding soil health, preserving beneficial microbial biodiversity, and yielding safe, residue-free agricultural products for consumers (Padel *et al.*, 2025).

The *in-vitro* dual-culture assay results demonstrate that all tested *Trichoderma* and *Bacillus* bioagents possess strong antagonistic activity against *R. solani*, achieving significant reductions in the pathogen's linear mycelial growth compared to the untreated control. The evaluation confirmed that fungal bioagents, particularly *T. harzianum* T2, exhibited high inhibition rates. This high antagonistic capacity aligns with established mechanisms such as mycoparasitism, nutrient competition, and the secretion of extracellular lytic enzymes (e.g., chitinases, β -glucanases, and proteases that directly hydrolyze and degrade the structural cell walls of phytopathogenic fungi such as *R. solani* (Vinale *et al.*, 2008; Harman *et al.*, 2004; Lü *et al.*, 2022; Wang *et al.*, 2024). Bacterial strains, including *B. subtilis* (B2, B3) and *B. velezensis* (B1), also demonstrated marked suppression of the mycelial growth of *R. solani*. This may be attributed to the biosynthesis of broad-spectrum antimicrobial secondary metabolites, notably cyclic lipopeptides (surfactin, iturin, and fengycin) and volatile organic compounds (VOCs) that disrupt pathogen hyphal extension (Al-Mutar *et al.*, 2023; Markelova and Chumak, 2025).

The differences in susceptibility among common bean (*Phaseolus vulgaris* L.) cultivars (Paulista, Bronco, and Auxira) to different *R. solani* isolates indicated differences in host-pathogen interactions, as reflected by variations in pre- and post-emergence damping-off incidence and plant survival (Ghoneem *et al.*, 2023).

The Paulista cultivar showed the highest pre-emergence damping-off across the tested isolates and was therefore selected to be used as the host plant. The selection of the Paulista cultivar was done according to results obtained from the greenhouse experiment. This susceptibility may be due to the genetic structure of this cultivar (Parajuli *et al.*, 2025). Susceptibility or resistance of the plants may be due to root exudates that are produced by roots of the different cvs. These exudates play a role in promoting growth of certain antagonists in the rhizosphere, which lead to root protection against certain pathogens. Susceptibility also may be due to histological structure of root, which mechanically prevents the pathogens to attack plant tissues (El-Sharkawy and El-Bakatoushi, 2023).

An increase in the application dose generally increased the efficacy of the treatment; this could be explained in light of work carried out by Guzmán-Guzmán *et al.* (2023) that increase dose of antagonist caused fast spread in the course of infection and increased the rate of growth consequently increased fungal or bacterial metabolism. This increase in cellular density results in a faster secretion of active secondary metabolites (e.g. lytic enzymes and lipopeptides) and increased general metabolic rates, therefore preventing colonization of host tissue by *R. solani*. Higher initial application rates accelerate the establishment of the microbial population in the infection court, facilitating rapid spatial and nutritional competition against the pathogen.

The better performance of liquid suspension formulation versus powder carriers in damping-off suppression and plant survival maximization could be explained by several physiological and physicochemical advantages. Liquid suspensions provide immediate microbial hydration and eliminate the lag phase required for spore rehydration or bacterial cell reactivation, allowing the bioagents to colonize the spermosphere and infection court much faster than powder-bound propagules (Guzmán-Guzmán *et al.*, 2023). This rapid establishment intensifies early competition against *R. solani* and accelerates the secretion of active secondary metabolites. Furthermore, liquid formulations ensure a more uniform distribution and intimate physical contact across the irregular contours of the seed coat, minimizing gaps in protection compared to dry powder coatings that frequently suffer from uneven clumping or dusting off during handling and planting (Kumaresh *et al.*, 2024; Parajuli *et al.*, 2025).

The exceptional performance of combined *Trichoderma* and *Bacillus* (*B. subtilis* B2 + *T. harzianum* T2) mixture can be attributed to the complementary ecological, biochemical, and physiological mechanisms of the two bioagents, that create a multi-layered defense barrier against *R. solani* (Raza *et al.*, 2022).

Dual inoculation merges distinct mechanisms of pathogen suppression. *Trichoderma* species exert control primarily through rapid mycoparasitism, spatial competition, and the secretion of cell wall-degrading lytic enzymes (β -glucanases and chitinases). Simultaneously, *Bacillus* strains produce broad-spectrum antimicrobial cyclic lipopeptides (surfactin, iturin, and fengycin) and volatile organic compounds (VOCs). This multi-targeted mode of action may enhance pathogen suppression (Dimkić *et*

al., 2022). *Trichoderma* spp. and *Bacillus* spp. occupy different ecological niches in the spermosphere and root zone, potentially allowing complementary colonization of the plant-associated environment (Chowdhury *et al.*, 2015). The combined treatment may also contribute to increased accumulation of biochemical compounds associated with plant defense and growth. Co-inoculated plants exhibit significantly elevated accumulations of total phenols, soluble sugars, and defense-related proteins, activating both Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) far beyond the levels achieved by single-strain applications (Abdelrhim *et al.*, 2023). Positive physiological cross-talk between *Bacillus* and *Trichoderma* promotes overall plant vigor. The consortia protect the host's photosynthetic machinery—maintaining higher chlorophyll levels under pathogen stress—which provides the necessary energy and carbon skeletons required for rapid tissue repair and sustained seedling survival (Shoresh & Harman, 2008).

The productivity of the treated plants with the tested bioagents was determined to figure out the most effective treatment on plant production. Dry matter of pods was also considered to be sure that the increase in yield is due to the increase in nutrient substances and not due to holding more water. The obtained results indicate that the mixture of *B. subtilis* B2 + *T. harzianum* T2 resulted in the highest yield compared with any of the other treatments. This may be due to the high capacity of this mixture in protecting the root system; a healthy root system leads to an increase in the amount of absorbed nutrient substances, consequently resulting in a high yield. Similar results were obtained by (Yobo *et al.*, 2011; Ali, 2013 and Abd-El-Moneim, 2012). The percentage of dry matter of pods was also determined. The obtained data show an association between high yield and the increase in dry matter. These results also are in harmony with those obtained by (Akladios & Abbas, 2012; Ali, 2013 and El-Fiki *et al.*, 2014). The combination (*B. subtilis* B2 + *T. harzianum* T2) may enhance plant growth through complementary mechanisms, including phytohormone production and improved nutrient availability, which may contribute to improved agricultural productivity (Raza *et al.*, 2022).

The biochemical changes in common bean (*Phaseolus vulgaris* L.) plants inoculated with bioagents in the form of liquid suspensions (S) and powders (P) indicated an association between improvement of physiological parameters, accumulation of secondary metabolites, and enhancement of plant survival against *R. solani*. The highest total phenol content was recorded in the *B. subtilis* B2 + *T. harzianum* T2 treatment, which also yielded the highest survival rate. Phenols act as crucial defense-related secondary metabolites that directly inhibit pathogen growth and contribute to the structural reinforcement of host cell walls. The improvement in phenolic accumulation in liquid treatments may reflect enhanced microbial establishment and stimulation of plant defense responses (Abdelrhim *et al.*, 2023). Total soluble sugars, including reducing and non-reducing sugars, were higher under the *B. subtilis* B2 + *T. harzianum* T2 treatment. This may be due to improved carbon partitioning and physiological vigor, supporting higher energy demands for tissue repair, defense enzyme synthesis, and sustained cellular growth during pathogen stress (Shoresh & Harman, 2008). Chlorophyll and soluble protein contents were substantially higher in all microbially treated plants relative to the control. The *B. subtilis* B2 + *T. harzianum* T2 treatment showed the highest chlorophyll and protein levels, which may indicate improved photosynthetic activity and plant metabolic status (Akladios & Abbas, 2012).

5. Conclusion

The present study demonstrated the effectiveness of selected *Trichoderma* and *Bacillus* isolates in suppressing *Rhizoctonia solani*-induced damping-off and improving the performance of common bean plants. The antagonistic activity observed in vitro was reflected in greenhouse and field conditions, confirming the potential of these beneficial microorganisms as practical biological control agents. Among the tested treatments, the combination of *Bacillus subtilis* B2 and *Trichoderma harzianum* T2 consistently provided the greatest reduction in damping-off and the highest plant survival, with the liquid formulation generally performing better than the powder formulation. Under field conditions, this combination also markedly improved plant growth, biomass production, pod formation, yield, and several biochemical parameters compared with the untreated control. The superior performance of the combined treatment suggests that integrating compatible bacterial and fungal bioagents can provide a broader and more effective approach to disease suppression than relying on individual microorganisms. Overall, the findings highlight the potential of the B2 + T2 combination, particularly as a liquid seed

treatment, as a promising component of sustainable management strategies for *R. solani*-induced damping-off and for improving common bean productivity under field conditions. Further studies under diverse environmental conditions and on larger production scales are warranted to validate its consistency and practical applicability.

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