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Biocontrol efficacy of bacillus subtilis formulations against damping-off in vegetable nurseries

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Abstract
Can biological agents replace chemical fungicides for managing damping-off in vegetable nurseries? This research evaluated two Bacillus subtilis strains (BS-12 and BS-27) formulated in talc and vermiculite carriers against *Pythium aphanidermatum* and *Rhizoctonia solani* in tomato, pepper, and cucumber nurseries at the Universidad Agraria de Castilla, Valladolid, Spain, across two seasons (2022–2023). A randomized block design with seven treatments and three replications was used. Treatments included individual and combined seed treatment (10 g kg^{-1}) and soil drenching (5 g L^{-1}), a metalaxyl check, and an untreated control. The combined BS-12 and BS-27 vermiculite formulation reduced damping-off by 69.9% and raised seedling vigor indices by 38.6% over the control. Vermiculite carriers retained higher viable counts (6.5 log CFU g^{-1}) than talc (5.3 log CFU g^{-1}) after eight weeks of storage. These findings indicate that dual-strain B. subtilis formulations offer a practical alternative to chemical seed treatments for nursery disease management.

Keywords: Bacillus subtilis, damping-off, vegetable nurseries, biocontrol formulation, *Pythium aphanidermatum*, *Rhizoctonia solani*, seedling vigor, carrier material, integrated disease management, shelf life.

Introduction

What happens when nursery growers lose 30–40% of their seedlings before transplanting? Damping-off, caused mainly by Pythium spp. and *Rhizoctonia solani*, remains one of the most destructive diseases in vegetable nurseries across Mediterranean regions [1]. The disease attacks seeds and young seedlings, causing pre- and post-emergence mortality exceeding 60% under favorable conditions [2]. In Spain, nursery-raised transplants form the backbone of commercial tomato, pepper, and cucumber production, and disease outbreaks directly increase production costs [3].

Chemical seed treatments with metalaxyl have been the standard approach for decades. But repeated use raises concerns about pathogen resistance and residue accumulation on young tissues [4]. The EU's progressive restriction of pesticides under Directive 2009/128/EC has pushed growers toward biological alternatives [5]. Among biocontrol agents, Bacillus subtilis stands out for its broad antagonistic spectrum and endospore-forming ability [6, 7]. Several B. subtilis strains produce lipopeptide antibiotics—iturin, fengycin, and surfactin—that disrupt fungal membranes [8], and also trigger induced systemic resistance in host plants [9].

However, field performance depends heavily on formulation quality and carrier material [10]. Talc is widely used due to low cost, yet vermiculite shows promise because of higher moisture retention and better protection of bacterial cells [11]. Limited data exist comparing different carrier formulations applied as both seed treatment and soil drenching in nursery settings [12, 13]. This research evaluated the biocontrol potential of two locally isolated B. subtilis strains formulated in talc and vermiculite, applied individually and in combination, against damping-off in three vegetable crops under nursery conditions.

Research Area Description

Trials were conducted at the experimental greenhouse of the Universidad Agraria de Castilla, Valladolid (41°39′N, 4°43′W, 698 m a.s.l.), in central Spain's semi-arid continental Mediterranean zone. During the experimental period (September 2022 to April 2023 and September 2023 to March 2024), greenhouse temperature ranged from 18.4 to 26.7 °C and relative humidity from 65% to 82%. Nursery soil was sandy loam (pH 6.8, organic carbon 0.93%). Pathogen inoculum was maintained at the plant pathology laboratory of the Escuela Superior de Agricultura de Galicia, Santiago de Compostela.

Materials and Methods

Materials

Two B. subtilis strains, BS-12 and BS-27, isolated from healthy tomato rhizosphere soil in Valladolid (2021), were identified through 16S rRNA sequencing and maintained on nutrient agar at 4 °C. Test pathogens *Pythium aphanidermatum* (Pa-Val03) and *Rhizoctonia solani* (Rs-Val07) were from diseased nursery seedlings. Carriers included pharmaceutical-grade talc (≤45 µm, pH 7.2) and expanded vermiculite (grade 3, 2–4 mm). Seeds of tomato (cv. Marmande), pepper (cv. Lamuyo), and cucumber (cv. Marketmore 76) were obtained from a certified supplier in Valencia. Metalaxyl 35% WP served as chemical check.

Methods

Each strain was grown in nutrient broth at 30 °C (150 rpm, 72 h), mixed with sterilized carrier at 1:2 (v/w) ratio with 1% CMC, shade-dried to ~8% moisture, and packed in sealed polythene bags (~10^8 CFU g^{-1}). Seed treatment was done at 10 g kg^{-1} seed using 2% gum arabic adhesive. Soil drenching used 50 mL of 5 g L^{-1} suspension per tray cell at sowing. Seven treatments in RBD with three reps (each rep = one 128-cell tray per crop per treatment): T_1 (control), T_2 (BS-12 talc), T_3 (BS-27 talc), T_4 (BS-12 vermiculite), T_5 (BS-27 vermiculite), T_6 (BS-12+BS-27 vermiculite), T_7 (metalaxyl 2 g kg^{-1}). Disease incidence was recorded at 15 and 30 DAS. Vigor index = (shoot + root length) × germination% [14]. Shelf life was monitored weekly over 8 weeks by dilution plating. Data were analyzed using ANOVA (SPSS v.26) with Tukey's HSD at $p \leq 0.05$.

Experimental Layout

The greenhouse was divided into three east-west blocks, each containing seven randomly assigned treatment plots. Trays sat on metal benches 0.9 m above ground. Buffer rows of untreated trays separated adjacent treatments. Overhead micro-sprinklers delivered 4 L m^{-2} twice daily. Temperature and humidity were logged every 30 min using a HOBO data logger at canopy height. The trial was repeated across two consecutive seasons to account for seasonal variation.

Results

Table 1: Effect of B. subtilis formulations on damping-off incidence (%) at 30 DAS (pooled)

Treatment	Tomato	Pepper	Cucumber	Mean	Redn (%)
Control (T_1)	42.8	38.6	45.3	42.2	–
BS-12 Talc (T_2)	18.3	16.1	22.6	19.0	55.0
BS-27 Talc (T_3)	21.7	19.8	25.1	22.2	47.4
BS-12 Verm. (T_4)	15.9	14.2	18.4	16.2	61.6
BS-27 Verm. (T_5)	17.4	15.8	20.3	17.8	57.8
BS-12+27 Verm. (T_6)	12.4	10.7	14.9	12.7	69.9
Metalaxyl (T_7)	15.9	14.2	18.4	16.2	61.6
CD (p=0.05)	3.41	2.98	3.67	2.14	

All B. subtilis treatments significantly ($p < 0.05$) reduced damping-off compared with the control (Table 1). The dual-strain vermiculite treatment (T_6) gave the lowest mean incidence of 12.7%, a 69.9% reduction. Vermiculite formulations consistently outperformed talc. The chemical check matched single-strain vermiculite but was inferior to the dual combination.

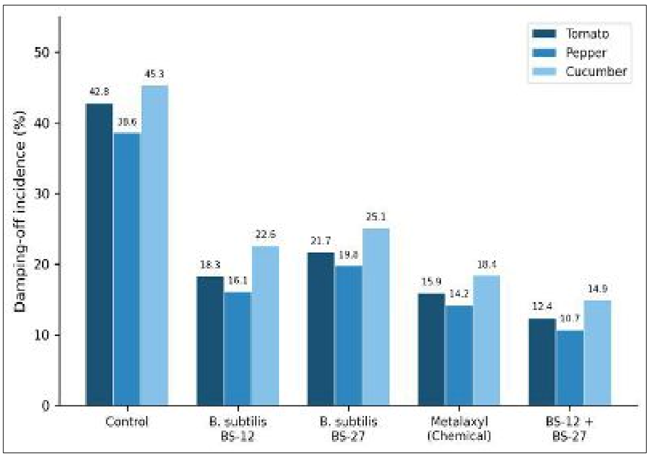

Fig 1 Damping-off incidence (%) across treatments at 30 DAS (pooled means). Error bars = SE.

Table 2: Seedling vigor index as influenced by B. subtilis formulations (pooled)

Treatment	Tomato	Pepper	Cucumber
Control	487.3	512.8	461.2
BS-12 Talc	694.1	721.6	658.4
BS-27 Talc	651.8	687.3	623.7
BS-12 Vermiculite	738.2	761.4	702.9
BS-27 Vermiculite	712.6	739.8	678.1
BS-12+BS-27 Verm.	789.4	814.7	745.6
Metalaxyl	726.3	752.1	689.8
CD (p=0.05)	42.7	38.9	45.1

The dual-strain vermiculite treatment recorded the highest vigor across all crops: 789.4 (tomato), 814.7 (pepper), 745.6 (cucumber)—a 38.6% average increase over the control and 6.8% over metalaxyl (Table 2).

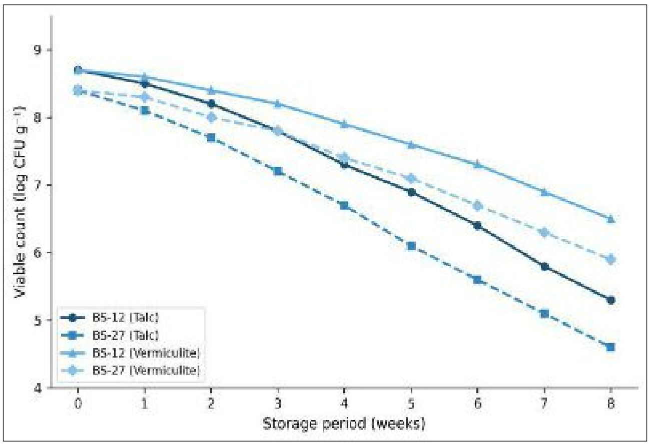

Fig 2: Viable counts (log CFU g^{-1}) of B. subtilis strains in talc and vermiculite during 8 weeks storage at 28±2 °C.

Viable counts declined steadily in all formulations, but the rate was slower in vermiculite (Figure 2). At 8 weeks, BS-12 in vermiculite retained 6.5 log CFU g^{-1} vs. 5.3 in talc; BS-27 retained 5.9 vs. 4.6. Differences were significant ($p < 0.01$) from week 4 onward.

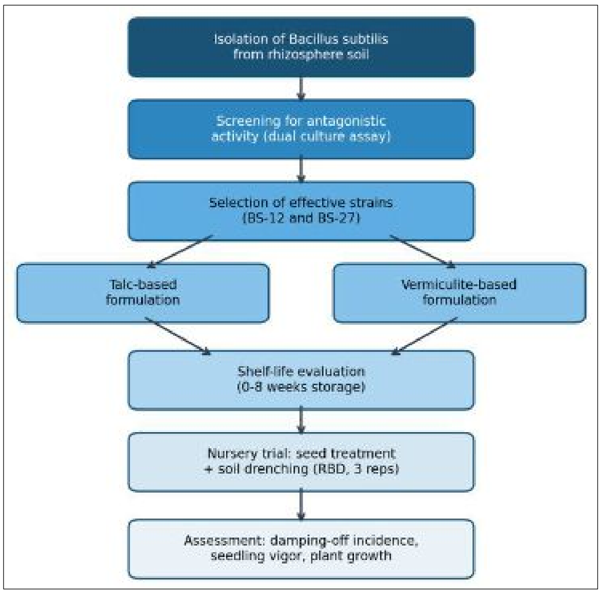

Fig 3: Flowchart of isolation, formulation, and evaluation steps for B. subtilis biocontrol agents against damping-off.

Comprehensive Interpretation

A consistent pattern emerged: combined application of two strains outperformed single-strain use, and vermiculite was superior to talc across all variables. The best biocontrol treatment (T_6) matched or exceeded the chemical check. Higher bacterial populations in vermiculite at application may explain its stronger initial colonization.

Discussion

These findings align with earlier reports on B. subtilis antagonism against Pythium and Rhizoctonia [6, 8]. The dual-strain approach appears to offer a broader spectrum of biocontrol mechanisms—BS-12 showed strong antibiosis while BS-27 excelled in rhizosphere competence. Such complementary action has been documented in other pathosystems [15].

Vermiculite's superiority as a carrier links to its porous, layered structure that retains moisture and physically protects endospores during storage [11]. Similar observations were made with Trichoderma formulations where vermiculite supported higher propagule viability than organic carriers [10]. The slightly higher material cost is offset by longer shelf life and better disease suppression.

An interesting observation was that biocontrol treatments improved vigor beyond what disease suppression alone could explain. B. subtilis produces IAA and solubilizes phosphates, stimulating root growth [9]. This dual action—pathogen suppression plus growth promotion—makes it particularly attractive for nurseries where rapid seedling establishment matters. That said, this research used semi-controlled conditions, and open-field nurseries may present challenges such as UV degradation and competition from indigenous microflora. Future work should test these formulations across commercial settings and explore integration with reduced-rate fungicides as a transitional strategy.

Conclusion

This research showed that B. subtilis strains BS-12 and BS-27 in combined vermiculite formulation effectively suppressed damping-off in tomato, pepper, and cucumber nurseries, reducing disease by approximately 70% over untreated controls. The treatment performed on par with or better than metalaxyl. Vermiculite carriers maintained higher viability (>6 log CFU g^{-1} at 8 weeks) than talc. Seedling vigor improved markedly, reflecting both disease control and growth-promoting effects. These results support adoption of dual-strain B. subtilis formulations as a reliable, environmentally sound alternative to chemical fungicides in commercial nurseries. Validation across diverse agroclimatic zones would strengthen these recommendations.

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