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Influence of the application of biofertilizers on the control of Fusarium root rot and Fusarium wilt and on the growth of common bean plants

Influência da aplicação de biofertilizantes no controle da podridão radicular seca e da murcha de Fusarium e no crescimento de feijoeiro

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Highlights

Biofertilizers based on *Bacillus* and *Trichoderma* control dry root rot and Fusarium wilt.
Biofertilizers and compost additives, as well as their mixtures, counteracted the growth reduction of common bean plants infected with *Fusarium solani* f. sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli.*

Abstract

Beans hold significant food and socioeconomic value for the global population. However, bean production often faces reductions due to diseases instigated by soil pathogens. The extensive use of chemicals to control these diseases presents numerous disadvantages, such as environmental pollution, harm to human health, and imbalances in disease and pest dynamics. Certain commercial products, registered as biofertilizers or compost additives, can control plant pathogens. This study aimed to evaluate the performance of biofertilizers and compost additives, along with their mixtures, in controlling dry root rot (caused by *Fusarium solani* f. sp. *phaseoli*) and Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *phaseoli*) in bean plants in a greenhouse setting. Additionally, the study examined the effect of these products and their mixtures on bean growth. A completely randomized design, with six replicates, was used. The treatments included Soil-Plex Trust®, Soil-Plex Ready®, Soil-Plex Active®, and Nem-Out®, along with their mixtures, a chemical fungicide Captan® (positive control), water (negative control), and a control group of plants without pathogen inoculation. Soil-Plex Trust® effectively reduced the severity of dry root rot. Soil-Plex Trust®, Soil-Plex Active®, and a Soil-Plex Ready + Soil-Plex Active® mixture promoted dry root weight equivalent to that of plants without *Fusarium solani* f. sp. *phaseoli* inoculation. Considering

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both inoculation methods, the application of Soil-Plex Trust® alone via in-furrow application reduced the severity of Fusarium wilt in common bean plants. Plants treated with Soil-Plex Trust® + SoilPlex Active®, and Soil-Plex Ready® + Soil-Plex Active® and inoculated with *F. oxysporum* f. sp. *phaseoli* by mycelial disk method, also exhibited lower severity of Fusarium wilt. The blends of Soil-Plex Trust® and Soil-Plex Active®, Soil-Plex Ready® and Soil-Plex Active®, and Nem-Out® and Soil-Plex Active® resulted in a root length equivalent to that of plants without *Fusarium oxysporum* f. sp. *phaseoli* inoculation. The results suggest that biofertilizers and compost additives based on *Bacillus* and *Trichoderma* can serve as a strategy to control diseases caused by *Fusarium* spp., and to mitigate the reductions in bean plants growth caused by these fungi.
Key words: Alternative control. *Fusarium oxysporum* f. sp. *phaseoli. Fusarium solani* f. sp. *phaseoli.*

Resumo

O feijão tem uma grande importância alimentar e socioeconômica para a população mundial. No entanto, a produção de feijão sofre reduções por causa de doenças causadas por patógenos de solo. O uso intensivo de produtos químicos para o controle destes tem várias desvantagens, incluindo danos à saúde humana e ao meio ambiente além de provocar desequilíbrios na dinâmica de doenças e pragas da cultura. Alguns produtos comerciais, registrados como biofertilizantes ou aditivos de compostagem têm apresentado controle de fitopatógenos. Este trabalho teve como objetivo a avaliação do desempenho de biofertilizantes e aditivo de compostagem e suas misturas no controle da podridão radicular seca (causada por *Fusarium solani* f. sp. *phaseoli*) e da murcha de Fusarium (causada por *Fusarium oxysporum* f. sp. *phaseoli*) em plantas de feijoeiro em casa-de-vegetação. Adicionalmente, este estudo investigou o efeito desses produtos e suas misturas no desenvolvimento do feijoeiro. O delineamento experimental foi inteiramente casualizado com seis repetições. Foram testados os produtos Soil-Plex Trust®, Soil-Plex Ready®, Soil-Plex Active® e Nem-Out® e suas misturas, fungicida químico Captan® (controle positivo), água (controle negativo) e um controle constituído por plantas não inoculadas com o patógeno. O produto Soil-Plex Trust® reduziu a severidade da podridão radicular seca. Os produtos Soil-Plex Trust®, Soil-Plex Active® e a mistura (Soil-Plex Ready® + Soil-Plex Active®) promoveram um peso seco da raiz igual ao de plantas sem inocular. Considerando ambos os métodos de inoculação, a aplicação de Soil-Plex Trust® sozinho no sulco de plantio reduziu a severidade da murcha de Fusarium em feijoeiro. Plantas tratadas com Soil-Plex Trust® + Soil-Plex Active® e Soil-Plex Ready® + Soil-Plex Active® e inoculadas com *F. oxysporum* f. sp. *phaseoli* pelo método do disco micelial, também apresentaram menor severidade de murcha de Fusarium. As misturas Soil-Plex Trust® + Soil-Plex Active®, Soil-Plex Ready® + Soil-Plex Active®e Nem-Out® Soil-Plex Active®, determinaram um comprimento de raiz igual ao de plantas sem inóculo. Os resultados indicam que biofertilizantes e aditivos de compostagem à base de *Bacillus* e *Trichoderma* podem ser utilizados na estratégia de controle de doenças causadas por *Fusarium* spp. e ainda contrarrestar as reduções de crescimento do feijoeiro ocasionadas por esses fungos.
Palavras-chave: Controle alternativo. *Fusarium oxysporum* f. sp. *phaseoli. Fusarium solani* f. sp. *phaseoli.*

Introduction

Soil-borne pathogens are recognized as significant limiting factors for crop yield, thereby undermining agricultural productivity and food security (Eke et al., 2020).

The common bean (*Phaseolus vulgaris*) is susceptible to numerous phytopathogenic fungi and bacteria found in the soil. Notably, species of the *Fusarium* genus are of particular concern due to their substantial impact on crop productivity. Two significant diseases, Fusarium wilt (caused by *Fusarium oxysporum* [Schlecht.] f. sp. *phaseoli* Kendrick & Snyder) and dry root rot (caused by *Fusarium solani* [Mart.] Sacc. f. sp. *phaseoli* [Burk.] W.C. Snyder & H.M. Hans), result in considerable losses in common bean cultivation areas (Naseri & Hamadani, 2017).

Fusarium oxysporum commences its infection process via the roots, proceeding to colonize the xylem vessels. This colonization subsequently results in leaf wilting, vascular discoloration, inhibited growth, and premature plant death. The infection process is also influenced by various other factors, including root lesions induced by nematodes, soil pests, soil conditions, or natural injuries (Paulino et al., 2020; Leitão et al., 2020).

Fusarium root rot is a disease of the common bean's root system caused by *Fusarium solani,* which induces depressed lesions with a reddish to dark brown color on the roots, thereby promoting the soft rot of lateral roots. As the disease advances, the lesions on the lower hypocotyl merge, culminating in the total decay of the root system. If not managed effectively, Fusarium root rot can result in a production decrease of up to 84% (Zitnick-Anderson et al., 2020).

The Fusarium genus is often responsible for diseases that pose significant management challenges due to the fungus's variability. Dry root rot management is particularly difficult due to the prolonged viability of chlamydospores in soil and plant debris (Katan, 2017). Present management strategies encompass chemical seed treatments, avoidance of planting in infested fields, crop rotation, and the use of certified seeds. Nevertheless, genetic resistance offers the most sustainable and enduring solution for disease control (Rubiales et al., 2014).

The extensive application of synthetic chemical products to control phytopathogenic fungi presents numerous drawbacks, such as handling hazards, environmental pollution, and food contamination. Furthermore, the intensive utilization of these chemicals disrupts the microbial community's balance, potentially hindering the activity of beneficial organisms and fostering the emergence of resistant pathogen strains (Kriaa et al., 2015). Consequently, it is crucial to implement integrated disease management strategies that combine alternative control methods to inhibit pathogen development.

Several commercial products, classified as biofertilizers or compost additives, have been evaluated for their effectiveness in managing phytopathogens (Miamoto et al., 2017; Soares et al., 2021; Moura et al., 2022; Rodrigues et al., 2016; Ferreira & Tebaldi, 2019). Nonetheless, research in this area remains limited, given that these products are primarily registered for nutrient provision to plants, not for pathogen control.

According to data from Alltech Crop Science® (Table 1), Soil-Plex Trust® is a commercial biofertilizer formulated with metabolites from *Bacillus licheniformis, B. subtilis,* and *Trichoderma longibrachiatum,* supplemented with *T. longibrachiatum* protease. Soil-Plex Ready® is another biofertilizer, which is based on metabolites from *Enterococcus faecium, Lactobacillus plantarum,* and *B. subtilis.* Soil-Plex Active®, on the other hand, is a biofertilizer derived from fermented extracts of fungi and bacteria, enriched with additional nutrients. Nem-Out®is a product registered as a compost additive, and it is composed of *B. licheniformis, B. subtilis, T. longibrachiatum* (total count $3.75x10^8$ CFU g^{-1}), supplemented with *T. longibrachiatum* protease, xylanase, and cellulase, although the manufacturer does not specify the quantities of these additions.

Bacteria from the *Bacillus* genus are recognized as effective agents in controlling phytopathogens, thanks to their secondary metabolism that produces a broad range of antagonistic substances. These microorganisms also serve as plant growth promoters (Fira et al., 2018). *Bacillus* species are known to produce indole acetic acid (Wagi & Ahmed, 2019), siderophores (Cerqueira et al., 2015), form biofilms (Pasvolsky et al., 2014), and they have the ability to solubilize phosphorus (Saeid et al., 2018). These characteristics significantly contribute to their plant growth-promoting capabilities. Furthermore, the *Bacillus* genus has the capacity to produce endospores, which enables these microorganisms to survive under adverse conditions (Podile & Kishore, 2006; Batista et al., 2018).

The biocontrol mechanisms employed by *Trichoderma* spp. can be either direct or indirect. Direct mechanisms involve interactions with the pathogen through micoparasitism, competition, or antibiosis. Indirect mechanisms, on the other hand, bolster the plant's defense systems against the pathogen. Furthermore, *Trichoderma* spp. can enhance plant growth by producing molecules such as phytohormones (Guzmán-Guzmán et al., 2023). The activities of rhizobacteria from the *Bacillus* genus and fungi from the *Trichoderma* genus have been primarily investigated for their role in controlling phytopathogens. However, many products marketed as soil conditioners, which are based on these organisms, have not been adequately studied for their impact on soil pathogens.

The objective of this study was to assess the efficacy of biofertilizers, compost additives, and their combinations in mitigating dry root rot and Fusarium wilt in common bean plants. These plants were treated with the aforementioned products and inoculated with *Fusarium solani* f. sp. *phaseoli* and *Fusarium oxysporum* f. sp. *phaseoli,* respectively. Furthermore, the impact of these products on the growth of the common bean was evaluated.

Table 1
Description of treatments

	Treatments	Composition[1]	Dose[2]	Application Method
1	Water	-	-	-
2	Non-inoculated plants	-	-	-
3	Captan® Fungicide	Captan	300 mL / 100 kg of seeds	Seeds
4	Soil-Plex Trust®	Metabolites of *Bacillus licheniformis, B. subtilis,* and *Trichoderma longibrachiatum* + *T. longibrachiatum* protease	2 L/ha	Planting furrow
5	Soil-Plex Ready®	Metabolites of *Enterococcus faecium, Lactobacillus plantarum,* and *B. subtilis*	2 L/ha	Planting furrow
6	Soil-Plex Active®	Fermented extracts (fungi and bacteria) and nutrients	1 L/ha	Planting furrow
7	Nem-Out®	*B. licheniformis, B. subtilis, T. longibrachiatum* (total count of 3.75 x 10^8 CFU g^{-1}) + T. longibrachiatum protease, xylanase, and cellulase (quantities not specified by the manufacturer)	2 Kg/ha	Planting furrow
8	Soil-Plex Trust®+ Soil-Plex Active®	(Metabolites of *Bacillus licheniformis, B. subtilis,* and *Trichoderma longibrachiatum* + *T. longibrachiatum* protease) + (Fermented extracts of fungi and bacteria and nutrients)	1 L/ha + 1 L/ha	Planting furrow
9	Soil-Plex Ready®+ Soil-Plex Active®	(Metabolites of *Enterococcus faecium, Lactobacillus plantarum,* and *B. subtilis*) + (Fermented extracts of fungi and bacteria and nutrients)	1 L/ha + 1 L/ha	Planting furrow
10	Nem-Out®+ Soil-Plex Active®	(*B. licheniformis, B. subtilis, T. longibrachiatum* + protease, xylanase, and cellulase) + (Fermented extracts of fungi and bacteria and nutrients)	1 Kg/ha + 1 L/ha	Planting furrow

[1]Product compositions were provided by the manufacturer Alltech Crop Science®.
[2]Product doses followed the manufacturer's recommendations.

Materials and Methods

Origin of pathogens and plant material

The Laboratory of Phytopathology at the State University of Londrina provided *F. solani* f. sp. *phaseoli* (*Fus* 145 and *Fs* 1) and *F. oxysporum* f. sp. *phaseoli* (IAC 9453 and Fox 1) isolates from its phytopathogen collection for use in this study. These isolates were preserved on potato dextrose agar (PDA) medium at a temperature of 25°C, under a 12/12-hour photoperiod.

IPR Uirapuru bean cultivar was chosen for their known susceptibility to these pathogens.

Inoculation method adjustment and selection of fungal isolate

Three inoculation methodologies were evaluated to determine the most effective for both pathogens. Furthermore, the virulence of two isolates from each pathogen, specifically *Fus* 145 and *Fs* 1 for *F. solani* f. sp. *phaseoli,* and IAC 9453 and Fox 1 for *F. oxysporum* f. sp. *phaseoli,* was also assessed.

Fusarium solani f. sp. phaseoli

Three inoculation techniques were evaluated: i) infesting the substrate with colonized sorghum (Tolêdo-Souza et al., 2009), ii) infesting the substrate with colonized rice (Michereff et al., 1996), and iii) incorporating spore suspension into the soil (Porteous-Álvarez et al., 2020).

In the method involving a substrate infested with colonized sorghum, sorghum grains were submerged in distilled water at a 2:1 ratio within glass containers, which were subsequently autoclaved. Sterilized sorghum grains were inoculated with discs of *F. solani* mycelium (grown on PDA medium) and maintained at 24°C under a 12/12-hour light-dark photoperiod for 12 days. Following this period, the sorghum grains, now serving as the inoculum, were pulverized, sifted, and blended with sterilized commercial seedling substrate at a ratio of 1.4 kg of substrate to 4, 8, and 10 g of inoculum. This mixture was then placed into the pots for the ensuing sowing.

In the method of substrate infestation with colonized rice (adapted from Michereff et al., 1996), rice grains were soaked in distilled water, placed in polypropylene bags and sterilized in an autoclave at 120°C for 30 minutes. This sterilization process was repeated after a 24-hour interval. Once the grains had cooled, 20 discs of *F. solani* mycelium, previously grown on PDA medium, were transferred to the bags containing the rice. These bags were then maintained at a temperature of 25°C for 14 days to allow for the complete colonization of the rice by the mycelium. Following this, ten grams of the inoculum were spread on the surface of the commercial seedling substrate used for bean sowing. This substrate was then incubated in darkness for 48 hours prior to planting.

The third inoculation method involved incorporating a spore suspension into the soil. The inoculum was a spore suspension harvested from seven-day-old fungal colonies. Each pot was first hydrated with 250 mL of water, followed by the addition of 50 mL of the spore suspension. Prior to sowing, the inoculated pots were kept in darkness for a period of eight days.

Fusarium oxysporum f. sp. phaseoli

The following methods were tested: i) root-dip (Pastor-Corrales & Abawi, 1987), ii) colonized toothpick (Coelho & Dhingra, 1996), and iii) mycelial disk (Fischer et al., 2010).

The root-dip method was employed for inoculation once the bean seedlings exhibited their first trifoliate leaf. The plants were delicately extracted from the substrate, and following a thorough root wash, a quarter

of their length was trimmed. The roots were then immersed in a spore suspension with a concentration of $1x10^6$ conidia mL^{-1} for a duration of twenty minutes. Seedlings that were submerged in distilled water served as the control treatment. Following this, the seedlings were relocated into pots filled with sterilized commercial substrate and maintained in the greenhouse.

The second method employed was the toothpick inoculation technique. In this process, a toothpick colonized by the fungus was inserted into the seedling's stem. The inoculum was prepared in advance on Petri dishes filled with PDA medium. After the medium solidified, toothpicks and a mycelial disk were added. Following a 14-day incubation period at 25°C with a 12/12-hour photoperiod, the fungus-colonized toothpicks were removed and inserted into the plant stems at the V2 development stage.

The mycelial disk method served as the third inoculation technique. The fungus was grown on PDA medium, in the previously mentioned conditions. Mycelial disks, each with a diameter of five millimeters, were positioned on the plant stems, which had been pre-wounded using a sterile scalpel, and were then secured with adhesive tape. The plants underwent inoculation upon reaching the V2 phenological stage.

Control of dry root rot and Fusarium wilt experiments

The method of rice grain colonization was selected for the inoculation with *F. solani* f. sp. *phaseoli,* using the *Fus* 145 isolate. For the inoculation with *F. oxysporum* f. sp. *phaseoli,* two methods were chosen: the toothpick method and the mycelial disk method, utilizing the IAC 9453 isolate.

In the assay for dry root rot, plants were cultivated in sterile sand. Conversely, for the Fusarium wilt assay, a blend of sterile soil and substrate was used for plant growth. Both assays utilized polypropylene pots with a one-liter volume, with two seeds sown per pot.

The plants were maintained in a greenhouse at a consistent temperature of 20°C ± 2, with daily manual watering. In the Fusarium wilt assay, a supplemental fertigation solution was administered to the pots twenty days post-planting.

Table 1 outlines the treatments. The efficacy of three commercial biofertilizers (Soil-Plex Trust®, Soil-Plex Ready®, Soil-Plex Active®), one compost additive (Nem-Out®), and their combinations was evaluated. These products were administered in the planting furrow. To achieve this, a solution was prepared and adjusted for application in the furrow using a micropipette, with bean sowing occurring immediately afterwards. For the chemical control treatment, the fungicide Captan SC® (Captan 480 g/L - 48.0% w/v) was used for seed treatment at the recommended dosage of 300 mL per 100 kg of seeds.

Evaluation of the incidence and severity of diseases and bean growth parameters

Twenty days post-sowing, the plants were removed from the pots and washed for the evaluation of the incidence and severity of dry root rot, utilizing the CIAT rating scale (Schoonhoven & Pastor-Corrales, 1987). This scale comprises nine grades:1 = absence

of visible symptoms; 3 = mild discoloration devoid of necrotic lesions or with roughly 10% of the hypocotyl and root tissues exhibiting lesions; 5 = lesions on approximately 25% of root and hypocotyl tissues, yet tissues remain firm with pronounced severe discoloration; 7 = lesions covering approximately 50% of hypocotyl and root tissues, accompanied by significant softening, decay, and root system reduction; and 9 = advanced decay in approximately 75% or more of hypocotyl and root tissues, coupled with severe root system reduction. Following the evaluations, the fresh and dry weights of the aboveground and root components were ascertained.

The Fusarium wilt experiment involved an evaluation of wilt incidence thirty days post-inoculation. Concurrently, the count of deceased plants and the severity of the disease were assessed using the CIAT scale (Schoonhoven & Pastor-Corrales, 1987). This scale is comprised of nine grades:1 = no visible symptoms; 3 = very few wilted leaves (1-3 leaves, representing less than 10% of the total) coupled with minor vascular discoloration in root and hypocotyl tissues; 5 = approximately 25% of leaves and branches exhibiting wilting and chlorosis; 7 = roughly 50% of leaves and branches showing wilting, chlorosis, and minor necrosis, with the plant demonstrating reduced development; and 9 = approximately 75% or more of leaves and branches displaying wilting, severe reduction in plant development, necrosis, and often premature defoliation, which frequently results in plant death.

The analysis of vascular necrosis percentage was conducted post-extraction of plants from their pots. Additionally, the fresh and dry weights of both the roots and aboveground components were evaluated.

Experimental design and statistical analysis

A completely randomized design, with six replicates, was used. Each experimental unit comprised a pot containing two plants. To verify the assumptions of ANOVA, the data underwent the Shapiro-Wilk test. Any data that failed to meet these assumptions were further subjected to the non-parametric Kruskal-Wallis analysis. The means were compared using the Scott-Knott test. The data were analyzed using R software.

Results and Discussion

Adjustment of inoculation methods and fungal isolates

The inoculation methods for *F. solani* f. sp. *phaseoli* using colonized sorghum grains and spore suspension in the soil did not show significant levels of dry root rot. Conversely, the method utilizing colonized rice grains resulted in increased disease severity, with the *Fus* 145 isolate exhibiting greater virulence. This method and isolate were subsequently selected to evaluate the efficacy of various products in subsequent experiments.

In the *F. oxysporum* f. sp. *phaseoli* pathogen assays, the root-dip method was not selected due to the absence of visible wilting or vascular discoloration. The colonized toothpick and mycelial disk methods, however, demonstrated greater disease severity and vascular discoloration. The IAC 9453 isolate displayed higher virulence in both methods.

Nogueira et al. (2019) reported similar findings, noting that the inoculation method involving infested rice was more effective

in differentiating melon accessions. This was observed in the case of Fusarium wilt, where the colonized toothpick and mycelial disk methods resulted in high severity and stunted growth of infected plants. Paulino et al. (2020) report that in addition to the wilting symptom, the pathogen *F. oxysporum* f. sp. *phaseoli* promotes vascular discoloration in bean plants, due to the colonization of the xylem vessels by the fungus. Borba et al. (2020) assessed that the Uirapuru cultivar plants grown in soil infested with *F. oxysporum* f. sp. *phaseoli* display diminished productivity components, indicating that losses are not exclusively due to plant death.

In addition to evaluating disease severity, the selection of methods and isolates also took into account the measurements of length and weight for both aboveground and root components.

Evaluation of disease severity and plant growth promotion in bean plants

The chemical fungicide treatment demonstrated a reduced severity of dry root rot, showing statistical similar severity to treatments without inoculation and Soil-Plex Trust®. These treatments differed only from the water-treated inoculated control and the treatment with Nem-Out®+Soil-Plex Active® (Table 2).

Table 2
Severity of dry root rot in bean plants at 20 days after inoculation with *Fusarium solani* f. sp. *phaseoli* and treated with biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Severity[1]
Water	6.83[2] a
Nem-Out®+ Soil-Plex Active®	4.50 a
Nem-Out®	3.50 ab
Soil-Plex Ready®+ Soil-Plex Active®	3.50 ab
Soil-Plex Active®	3.33 ab
Soil-Plex Ready®	3.17 ab
Soil-Plex Trust®+ Soil-Plex Active®	3.17 ab
Soil-Plex Trust®	3.00 b
Captan® Fungicide	1.00 b
Non-inoculated plants	1.00 b
C.V. (%)	12.37

[1]Severity evaluated using the CIAT scale, where 1 = no visible symptoms; 3 = slight discoloration without necrotic lesions or with approximately 10% of hypocotyl and root tissues affected by lesions; 5 = approximately 25% of root and hypocotyl tissues with lesions, yet the tissues remain firm and can exhibit severe discoloration; 7 = approximately 50% of hypocotyl and root tissues covered with lesions, accompanied by significant softening, rotting, and reduction of the root system; and 9 = approximately 75% or more of hypocotyl and root tissues in an advanced state of decay, coupled with a severe reduction in the root system.
[2]Means followed by the same letter do not differ according to the Kruskal-Wallis test ($p < 0.05$).

This study demonstrated a higher severity of dry root rot when beans were inoculated with substrate previously contaminated by the *F. solani* f. sp. *phaseoli* pathogen (data not shown). The most effective treatments for disease control were those involving the application of Captan fungicide to the seeds and the standalone application of Soil-Plex Trust®, a commercial product based on metabolites from *Bacillus licheniformis, B. subtilis,* and *Trichoderma longibrachiatum* fermentation. Seed treatment with fungicide is a recommended strategy for managing dry root rot instigated by *F. solani* f. sp. *phaseoli,* and it has been shown to enhance bean production (Naseri et al., 2016).

Numerous studies have been conducted to explore biological control agents for this pathogen, with the goal of developing more environmentally sustainable control measures. Kalantari et al. (2018) reported that mixed inoculation of rhizobacteria in bean plants caused greater increases in disease suppression of dry root rot, dry and fresh matter weight of plant (aerial part) and root, plant height, and pod number per plant compared to diseased control and absolute control. Species of *Bacillus* are effective as biocontrol agents against Fusarium diseases under both *in vitro* and greenhouse conditions (Ajilogba et al., 2013). Khalil and Hassouna (2022) found that the biocontrol agents *Trichoderma* and *Bacillus* significantly inhibited the mycelial growth of *F. solani* f. sp. *phaseoli in vitro,* with inhibition rates of approximately 62% and 46%, respectively.

Numerous studies underscore the role of *Bacillus* species in mitigating *Fusarium solani* in various crops. Wang et al. (2021) documented the antifungal properties of *Bacillus velezensis* peptides against *F. solani,* which is responsible for stem basal rot in passion fruit. This results in the malformation of the germination tube and inhibits the growth of fungal mycelia *in vitro.* Similarly, other research has indicated the suppression of *F. solani* mycelial growth by *Bacillus* spp. (Kalantari et al., 2018) and *Bacillus* sp. CCeRi1-002 against *F. solani* and *Fusarium kuroshium* (causal agent of Fusarium *dieback*) in trees of the Lauraceae family (Báez-Vallejo et al., 2020).

The analysis of aboveground variables in bean plant growth parameters suggests that uninoculated plants and those treated with Captan® chemical fungicide exhibit greater aboveground length. The uninoculated treatment resulted in a higher aboveground fresh weight, although this did not significantly differ from other treatments, with the exception of those treated with Nem-Out® + Soil-Plex Active® and water. The products Soil-Plex Trust®, Soil-Plex Active®, and the combination of Soil-Plex Ready® + Soil-Plex Active® induced a root dry weight equivalent to that of the uninoculated plants. No statistical difference was observed in root length and root fresh weight among the treatments at a 5% significance level (Table 3).

Table 3
Growth components of bean plants inoculated with *Fusarium solani* f. sp. *phaseoli* and subjected to the application of biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Aboveground			Root		
	Length (cm)	Fresh mass (g)	Dry mass (g)	Length (cm)	Fresh mass (g)	Dry mass (g)
Water	17.00[1] ab	1.64[1] b	0.22[1] cd	23.15[1] a	1.29[1] a	0.06[1] b
Non-inoculated plants	22.74 a	2.87 a	0.38 a	31.00 a	1.92 a	0.11 a
Captan® Fungicide	20.55 a	2.28 ab	0.31 ab	30.25 a	1.99 a	0.09 ab
Soil-Plex Trust®	18.40 ab	2.25 ab	0.31 ab	29.50 a	1.81 a	0.10 a
Soil-Plex Ready®	18.95 ab	1.97 ab	0.26 bcd	29.49 a	1.89 a	0.16 ab
Soil-Plex Active®	18.53 ab	2.03 ab	0.28 abcd	29.29 a	1.97 a	0.12 a
Nem-Out®	18.63 ab	2.18 ab	0.29 abc	29.00 a	2.04 a	0.09 ab
Soil-Plex Trust®+Soil-Plex Active®	18.23 ab	1.98 ab	0.25 bcd	29.89 a	1.96 a	0.10 ab
Soil-Plex Ready®+Soil-Plex Active®	17.16 ab	1.99 ab	0.29 ab	27.89 a	1.71 a	0.11 a
Nem-Out®+Soil-Plex Active®	14.74 b	1.49 b	0.19 d	25.06 a	1.40 a	0.08ab
C.V. (%)	12.88	25.90	63.25	15.84	33.82	25.13

[1]Means followed by the same letter do not differ according to the Kruskal-Wallis test ($p < 0.05$).

The effect observed with Soil-Plex Trust® may be attributed to the reduced disease severity in bean plants treated with this product (Table 2). Conversely, for Soil-Plex Active® and the combination of Soil-Plex Ready®+Soil-Plex Active®, the observed effect implies potential growth promotion, as these products did not significantly diminish the severity of dry root rot.

Bacillus employs various mechanisms for disease control and growth promotion, including the synthesis of auxins and other hormones, the production of siderophores and antibiotics (like lipopeptides), competition, and induction of resistance, among other processes (Khalil & Hassouna, 2022; Das et al., 2017; Khan et al., 2021). The lipopeptides fengicins, iturins, and surfactins, distinguished by their unique chemical structures, act on *Fusarium* species, including *F. oxysporum* and *F. solani.*

The assays inoculated with *F. oxysporum* f. sp. *phaseoli* were assessed 30 days post-inoculation. Differences in disease severity was observed among the treatments in the assay where plants were inoculated using the toothpick method. The treatment that did not involve inoculation exhibited no signs of Fusarium wilt. Treatments involving the application of a chemical fungicide to the seeds, as well as those involving products such as Soil-Plex Trust®, Soil-Plex Ready®, Nem-Out®, Soil-Plex Ready® + Soil-Plex Active®, and Nem-Out® + Soil-Plex Active®,

showed statistically equal levels of severity with each other and with uninoculated plants. The treatments involving water and the isolated application of Soil-Plex Active® failed to effectively control the pathogen (Table 4).

Table 4
Fusarium wilt severity in bean plants inoculated with *Fusarium oxysporum* f. sp. *phaseoli* using the toothpick method and treated with biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Severity[1]
Water	6.33[2] a
Soil-Plex Active®	5.50 a
Soil-Plex Trust®+ Soil-Plex Active®	4.67 ab
Nem-Out®	4.33 abc
Soil-Plex Ready®+ Soil-Plex Active®	4.00 abc
Nem-Out®+ Soil-Plex Active®	4.00 abc
Soil-Plex Ready®	3.33 abc
Captan® Fungicide	1.67 bc
Soil-Plex Trust®	1.67 bc
Non-inoculated plants	1.00 c
C.V. (%)	11.94

[1]Severity assessed using the CIAT scale, where 1 = no visible symptoms; 3 = very few wilted leaves (1-3 leaves, representing less than 10% of the total), coupled with minor vascular discoloration in root and hypocotyl tissues; 5 = approximately 25% of leaves and branches exhibiting wilting and chlorosis; 7 = approximately 50% of leaves and branches show wilting, chlorosis, and minor necrosis, with the plant demonstrating stunted growth; and 9 = approximately 75% or more of leaves and branches display severe wilting, significant reduction in plant development, necrosis, and premature defoliation, often leading to plant death.
[2]Means followed by the same letter do not differ according to the Kruskal-Wallis test ($p<0.05$).

In this assay, only the aboveground fresh weight and root fresh weight of the bean plant exhibited treatment effects. Soil-Plex Ready® was the sole treatment with the aboveground fresh weight statically equal to the uninoculated control and different from the water control. The treatments that resulted in the highest root fresh weight were Nem-Out®, Soil-Plex Ready®, and Soil-Plex Active®. These were statistically equal to the uninoculated treatment and exceeded the weight of plants treated with water (Table 5).

Table 5
Growth components of bean plants inoculated with *Fusarium oxysporum* f. sp. *phaseoli* using the toothpick method and subjected to application of biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Aboveground			Root		
	Length (cm)	Fresh mass (g)	Dry mass (g)	Length (cm)	Fresh mass (g)	Dry mass (g)
Water	25.12[1] a	8.20[1] c	1.37[1] a	31.45[1] a	1.00[1] b	0.15[1] a
Non-inoculated plants	32.35 a	18.10 a	2.32 a	41.90 a	3.17 a	0.36 a
Captan® Fungicide	34.57 a	14.95 abc	1.59 a	42.25 a	1.98 ab	0.22 a
Soil-Plex Trust®	29.02 a	11.92 abc	1.39 a	34.58 a	2.03 ab	0.24 a
Soil-Plex Ready®	35.93 a	17.08 ab	2.39 a	42.75 a	3.36 a	0.44 a
Soil-Plex Active®	27.45 a	11.27 abc	1.61 a	39.43 a	3.12 a	0.29 a
Nem-Out®	26.27 a	13.11 abc	1.54 a	46.05 a	3.70 a	0.28 a
Soil-Plex Trust®+Soil-Plex Active®	31.80 a	10.77 abc	1.20 a	32.68 a	2.39 ab	0.20 a
Soil-Plex Ready®+Soil-Plex Active®	24.97 a	9.63 bc	1.45 a	37.92 a	2.43 ab	0.22 a
Nem-Out®+Soil-Plex Active®	25.50 a	9.69 bc	1.27 a	35.06 a	2.04 ab	0.17 a
C.V. (%)	24.18	20.07	40.64	24.62	39.58	42.67

[1]Means followed by the same letter do not differ according to the Kruskal-Wallis test ($p < 0.05$).

The mycelial disk method, using *F. oxysporum* f. sp. *phaseoli,* revealed a heightened severity of Fusarium wilt in plants that were neither chemically nor biologically treated. In contrast, plants that were either not inoculated or treated with Soil-Plex Trust®, Soil-Plex Trust® + Soil-Plex Active®, and Soil-Plex Ready® + Soil-Plex Active® exhibited a lower severity of the disease compared to those subjected to other treatments (Table 6).

The effectiveness of Captan® fungicide in disease control was assessed in our tests, serving as a comparative parameter between chemical and biological control methods. In the test where inoculation was performed using the toothpick method, the product demonstrated effective control, comparable to the Soil-Plex Trust® treatment with the lowest mean severity and both did not differ significantly from the non-inoculated treatment. However, when evaluating the fungicide's impact in the test inoculated with mycelial disks, its efficiency in disease control was found to be low. Ishizuka et al. (2020) noted that biological control using *Trichoderma* species reduced *F. oxysporum* incidence in beans by up to 68%, a stark contrast to the chemical control with the fungicide, which only managed to reduce incidence by up to 30%.

In both Fusarium wilt assays, disease control was observed with products product based on metabolites from *Bacillus* and *Trichoderma* fermentation. In the assay where the toothpick method was used for

inoculation, all tested products, except for Soil-Plex Active® applied in isolation, demonstrated a positive control effect. However, when Soil-Plex Active® was used in combination with other tested products, it proved to be efficient. In the assay where mycelial disks were used for inoculation, the biological products exhibited a trend similar to that of the toothpick method, with the weakest control observed when Soil-Plex Active® was applied alone. It was also noted that the efficiency of chemical control with the fungicide Captan® significantly decreased when the mycelial disk inoculation method was used.

Table 6
Fusarium wilt severity in bean plants inoculated with *Fusarium oxysporum* f. sp. *phaseoli* using the mycelial disc method and treated with biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Severity[1]
Water	7.83[2] a
Soil-Plex Active®	5.17 b
Captan® Fungicide	4.33 c
Nem-out®	4.33 c
Nem-out®+ Soil-Plex Active®	3.50 d
Soil-Plex Ready®	3.17 d
Soil-Plex Trust®	2.33 e
Soil-Plex Trust®+ Soil-Plex Active®	2.17 e
Soil-Plex Ready®+ Soil-Plex Active®	2.17 e
Non-inoculated plants	1.00 f
C.V. (%)	13.42

[1]Severity assessed using the CIAT scale, where 1 = no visible symptoms; 3 = very few wilted leaves (1-3 leaves, representing less than 10% of the total), coupled with minor vascular discoloration in root and hypocotyl tissues; 5 = approximately 25% of leaves and branches exhibiting wilting and chlorosis; 7 = approximately 50% of leaves and branches show wilting, chlorosis, and minor necrosis, with the plant demonstrating stunted growth; and 9 = approximately 75% or more of leaves and branches display severe wilting, significant reduction in plant development, necrosis, and premature defoliation, often leading to plant death.
[2]Means followed by the same letter do not differ according to the Kruskal-Wallis test ($p<0.05$).

The causal agent of the disease, *F. oxysporum* f. sp. *phaseoli,* initiates its colonization process in the bean's root cortex, advancing to the xylem vessels before progressing to the aerial part of the plant. Enhancing cell walls can help mitigate the fungal spread within the plant's vascular system (Quadros et al., 2020). Research indicates that *Bacillus* species may facilitate the lignification and fortification of the host's cell walls and epidermal cells (Cantoro et al., 2021; Shafi et al., 2017; Liu et al., 2019).

Various studies have identified *Bacillus* spp. as effective biological control agents against phytopathogens. This biocontrol agent can inhibit the establishment and proliferation of phytopathogenic organisms through several mechanisms, including the production of antibiotics and the induction of systemic resistance (Villarreal-Delgado et al., 2018). Villa-Rodríguez et al. (2019) underscore the utility of *Bacillus* as a biocontrol agent, particularly noting its potential as a source of broad-spectrum antifungal metabolites.

In our examination of bean plant growth components, we observed that the Soil-Plex Ready® + Soil-Plex Active® mixture fostered the most optimal development in above-ground growth parameters, including length and both fresh and dry mass. With regard to root development, the three mixtures tested (Soil-Plex Trust®+Soil-Plex Active®, Soil-Plex Ready®+Soil-Plex Active®, and Nem-Out®Soil-Plex Active®) yielded root lengths comparable to those of non-inoculated plants. Additionally, the Soil-Plex Trust®+ Soil-Plex Active® mixture enhanced root dry mass. However, the variable of root fresh weight did not exhibit any statistical differences among the treatments (Table 7).

Table 7
Growth components of bean plants inoculated with *Fusarium oxysporum* f. sp. *phaseoli* using the mycelial disc method and subjected to application of biofertilizers, composting additive, and their mixtures in the planting furrow, seed chemical treatment, and water

Treatments	Aboveground			Root		
	Length (cm)	Fresh mass (g)	Dry mass (g)	Length (cm)	Fresh mass (g)	Dry mass (g)
Water	20.36[1] b	6.03[1] b	0.89[1] b	18.45[1] b	1.11[1] a	0.12[1] b
Non-inoculated plants	33.63 ab	10.30 ab	1.64 ab	42.08 a	3.01 a	0.23 ab
Captan®Fungicide	26.33 ab	11.90 ab	1.76 ab	35.24 ab	2.67 a	0.25 ab
Soil-Plex Trust®	27.53 ab	9.56 ab	1.52 ab	34.38 ab	1.58 a	0.23 ab
Soil-Plex Ready®	24.32 ab	9.85 ab	1.35 ab	32.47 ab	2.36 a	0.22 ab
Soil-Plex Active®	28.18 ab	11.69 ab	1.71 ab	35.42 ab	1.61 a	0.20 ab
Nem-Out®	24.68 ab	10.79 ab	1.77 ab	33.99 ab	1.88 a	0.23 ab
Soil-Plex Trust®+Soil-Plex Active®	25.70 ab	11.74 ab	1.79 ab	41.08 a	3.48 a	0.37 a
Soil-Plex Ready®+Soil-Plex Active®	34.71 a	16.60 a	2.79 a	42.12 a	2.05 a	0.29 ab
Nem-Out®+Soil-Plex Active®	26.98 ab	11.84 ab	1.89 ab	44.22 a	2.60 a	0.26 ab
C.V. (%)	21.45	34.27	37.41	16.19	49.10	39.09

[1]Means followed by the same letter do not differ significantly according the Kruskal-Wallis test ($p < 0.05$).

In the mycelial disk inoculation assay, products based on metabolites from *Bacillus* and *Trichoderma* positively influenced the growth of inoculated bean plants. *Bacillus*

species play an important role in enhancing plant growth, a trait directly linked to their capacity to biosynthesize plant hormones such as gibberellic acid and auxin (Shafi et al., 2017). Sabaté et al. (2018) found that *Bacillus* strains, when inoculated in seeds, fostered bean plant growth by increasing both above-ground and root length. These strains proved even more effective than seed fungicide treatments. Additionally, *Trichoderma* has been shown to enhance the growth parameters of bean plants inoculated with *F. oxysporum* (Elhelaly & Ammar, 2022).

Conclusions

The application of Soil-Plex Trust®, a biofertilizer based on metabolites from *Bacillus* and *Trichoderma,* via in-furrow application significantly mitigated the severity of dry root rot in bean plants. The level of control achieved was comparable to that of a chemical fungicide.

Upon evaluating both inoculation methods, it was observed that the use of Soil-Plex Trust® alone effectively reduced the severity of Fusarium wilt in bean plants. Plants treated with Soil-Plex Trust® + Soil-Plex Active®, and Soil-Plex Ready® + Soil-Plex Active® and inoculated with *F. oxysporum* f. sp. *phaseoli* by mycelial disk method, also exhibited lower severity of Fusarium wilt.

The application of biofertilizers and composting additives, either independently or in combination, mitigated the growth decline in bean plants infected with *Fusarium solani* f. sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli.*

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