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Trichoderma and Bacillus on Faba-Bean Growth and Yield Under Soil-Borne Disease Pressure

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ABSTRACT

Faba-bean (*Vicia faba* L.) production serves human consumption and is critical for food security; however, yields can become exceptionally low due to soil-borne diseases, poor fertility and adverse climatic factors. This study evaluated the effect of seed inoculation with the fungus *Trichoderma* spp., the bacterium *Bacillus subtilis*, and their dual-consortium on disease suppression and yield improvement in faba-bean under Bolivian Andean Altiplano conditions. A randomized complete-block design with four treatments (*Trichoderma* alone, *B. subtilis* alone, consortium of both, uninoculated control) was employed. At the start of the trial, rice-trap monitoring identified three major soil pathogens (*Phytophthora* spp., *Fusarium* spp., *Rhizoctonia* spp.). At crop maturity, only the consortium treatment resulted in the consistent detection of *Trichoderma* + *B. subtilis*, along with suppression of *Phytophthora*. The consortium treatment maximised pods per plant (21 pods), seeds per pod (3), root weight (≈ 68 g) and stems per plant (8), achieving a total pod yield of 29.25 t ha^{-1}. These results demonstrate that the *Trichoderma-B. subtilis* consortium is a viable and sustainable technological alternative for increasing faba-bean productivity under the challenging soil and climatic conditions of the Bolivian highlands. Future work should quantify microbial population dynamics, elucidate mechanisms of action and evaluate performance across varieties and management systems.

KEYWORDS: biological control, microbial consortium, seed inoculation, sustainable agriculture, *Vicia faba*.

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INTRODUCTION

The faba-bean (*Vicia faba* L.) is a globally strategic legume owing to its high protein content (~ 25-30%) and adaptability to diverse climates (Maalouf et al. 2019). In 2023, South America cultivated approximately 54,668 hectares of faba-bean, averaging 3.54 t ha^{-1}, whereas Bolivia's 26,314 ha yielded only 1.37 t ha^{-1} (FAOSTAT 2025; INE 2025). In highland regions of Bolivia, the crop is crucial for food security among smallholder farmers (INE 2025; Torrez 2019).

The low yields are attributable to multiple constraints, prominently root-borne pathogens such as *Phytophthora* spp., *Fusarium* spp. and *Rhizoctonia* spp., which may cause up to 100% losses (OAP 2025; Viracocha-Mamani & Cadena-Miranda 2023). These pathogens disrupt xylem and phloem function, causing root rot, lodging and foliar chlorosis (Garcés-Fiallos & Vera-Alcívar 2014). Chemical control is often ineffective due to persistent resistance structures and the limited soil mobility plus rapid degradation of fungicides (Silva et al. 2011; De Corato 2020).

Therefore, integrated management increasingly recommends biological control via beneficial microorganisms (Altieri & Nichols 2005). Specifically, *Trichoderma* spp. and *Bacillus subtilis* have demonstrated capability to produce antimicrobial

compounds, stimulate plant growth and induce systemic resistance, thereby significantly reducing disease incidence (Zhu et al. 2022; Abdelaziz et al. 2023). Recent investigations further report that combined inoculation of these microbes enhances nutrient availability, root architecture and yields under low-input conditions (Wang et al. 2019; Maitra et al. 2021; da Silva et al. 2024).

Beyond disease pressure, the soils of the Bolivian Altiplano are often low in fertility, necessitating fertilizer application - a challenge given high costs and limited access. While legumes fix atmospheric nitrogen via rhizobia symbiosis (Castillo et al. 2014), phosphorous and other nutrients frequently limit yield. In this regard, *Trichoderma* spp. acts as both biocontrol agent and bio-fertilizer through phosphate and micronutrient solubilization and rhizosphere colonization, promoting root elongation and uptake (Zhao et al. 2020). Similarly, *Bacillus subtilis* improves availability of nitrogen and phosphorus via fixation and solubilization mechanisms (Liu et al. 2021).

Accordingly, the present study aimed to evaluate the individual and combined application of *Trichoderma* spp. and *B. subtilis* on yield and yield-component parameters of faba-bean in the Bolivian Altiplano, with the goal of proposing a simple, adoptable and sustainable strategy for small-scale farmers under region-specific conditions.

MATERIALS AND METHODS

Field Study Area

The experiment was conducted during the 2023-2024 agricultural season in the community of Tacamara, Municipality of Achacachi (Omasuyos Province), Department of La Paz, Bolivia. Tacamara is situated on the Andean Altiplano with elevations reported around 3,834 m a.s.l. (Mapcarta 2025). The terrain is defined by the relief of the Andes and the Altiplano plain. Climatic conditions in the area include an average annual temperature of approximately 8-10 °C, mean maximum temperatures of 15-17 °C and minimums of 1-3 °C, along with large diurnal thermal amplitude (sunny days, cold nights) and average annual precipitation between 500-600 mm, concentrated in the December-March period (SENAMHI 2025).

Faba-Bean Material and Inoculation

The genetic material consisted of local faba-bean seed, provided by a local farmer in the study region. This seed is an ecotype adapted to the region and locally referred to as the "Local Variety".

Prior to sowing, the faba-bean seeds were inoculated using commercial products: (i) *Trichoderma* spp. (Tricotop®, Biotop), formulated at 1×10^9 spores g^{-1}; (ii) *B. subtilis* (Bio*Bacillus*®, Biotop), formulated at 1×10^9 CFU mL^{-1}; and (iii) a combined treatment (consortium) of *Trichoderma* spp. (1×10^9 spores g^{-1}) plus *B. subtilis* (1×10^9 CFU mL^{-1}). Seed inoculation was performed 30 minutes before sowing. Seeds were placed in clean plastic basins, microorganisms were applied uniformly, and water was added gradually to create adhesion without causing waterlogging. During the inoculation procedure, the seed-microbe mixture was stirred continuously to ensure homogenous microbial coverage. All operations were carried out in a shaded area to avoid exposure to direct sunlight and thus protect the microbial inoculants from potential thermal or UV damage.

Experimental Implementation

Field operations followed the conventional agronomic management practiced locally. On 21 September 2023, the plot was prepared by ploughing and harrowing. Furrows were opened at 0.8 m spacing and sowing was conducted at 0.3 m between plants within furrows. The total experimental area covered 547 m^2. Irrigation was supplied via furrow-gravity flow, applied as needed according to crop demands. For insect pest control, thiamethoxam (active ingredient) was applied at 500 mL ha^{-1}. For foliar fungal pest control, mancozeb (active ingredient) was applied at 2 kg ha^{-1}.

The trial employed a randomized complete block design with the microorganism factor at four treatment levels [*Trichoderma* spp. (10^9 spores g^{-1}), *B. subtilis* (10^9 spores g^{-1}), *Trichoderma* spp. (10^9 spores g^{-1}) + *B. subtilis* (10^9 spores g^{-1}), untreated control]. Each treatment was replicated three times. The experimental units consisted of 9 furrows, each 4 m long, with a distance between furrows of 0.8 m. The distance between plants was 0.3 m. Each experimental unit comprised an area of 28.8 m^2.

Response Variables

For faba-bean plant evaluation, only the five central rows were selected, discarding the first and last plant in each row. The evaluated variables are described in Table 1.

Table 1. Response variables evaluated on faba-bean plants.

Variables	Description
Plant height (PH)	Recorded (cm) from the collar of the main stem to the apex of the flag leaf on three randomly selected plants per experimental unit at harvest.
Leaf length (LL)	Recorded (cm) from the base to the apex of the flag leaf of three randomly selected plants per experimental unit at harvest.
Pod length (PL)	Pod length was recorded (cm) from the thalamus to the pod tip on three plants randomly selected per experimental unit at harvest.

Variables	Description
Number of pods per plant (NPP)	Number of pods per plant counted on three plants randomly selected from the central furrows of each experimental unit at harvest.
Number of seeds per pod (NSpP)	Number of seeds per pod counted on the pods from three plants randomly selected from the central furrows of each experimental unit at harvest.
Number of stems per plant (NSP)	All stems of three plants randomly selected per experimental unit were counted at harvest.
Root weight (RW)	Root weight (g) measured for three plants randomly selected from the central furrows of each experimental unit at harvest.
Yield	Pod weight measured per m^2 and extrapolated to tons per hectare (t ha^{-1}).

Statistical Analysis

A single factor data was analyzed by one-way analysis of variance (ANOVA) and - when the parametric assumptions were satisfied - mean separation was performed using Tukey's honestly significant difference test at $p \leq 0.05$. For non-parametric data - where normality or homogeneity of variance were not met - the Kruskal-Wallis test was applied, followed by the Dunn's test for multiple comparisons. In addition, the coefficient of determination (R^2) was calculated to evaluate the proportion of total variance in the response explained by the experimental factor (treatments) and model fit. Experimental precision was assessed by calculating the coefficient of variation (CV%).

RESULTS

Presence of Phytopathogens

Soil-borne phytopathogens were identified using rice-trap culture techniques following established protocols (Cuervo et al., 2019). These protocols employ color-coded identification keys: red (*Fusarium* spp.), grey (*Rhizoctonia* spp.), brown (*Phytophthora* spp.), white (*Bacillus* spp.), and green (*Trichoderma* spp.). Microorganisms present in the traps were initially identified based on color cues and subsequently confirmed through morphological observation (Figure 1).

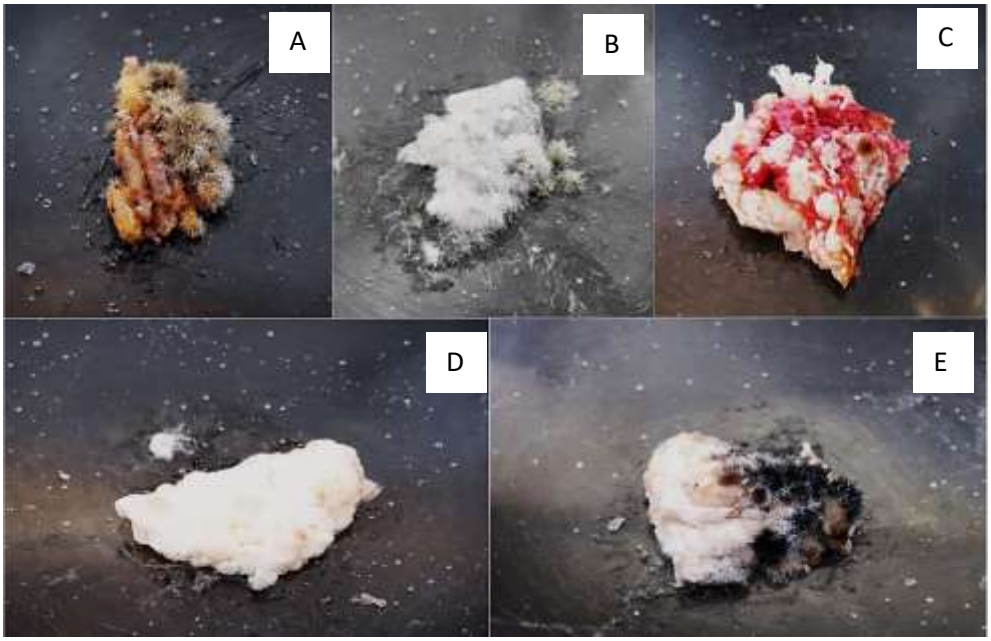

Figure 1. Visual identification of pathogenic and beneficial microorganisms captured in rice traps. Pathogens = A: *Phytophthora* spp.; B: *Fusarium* spp.; C: *Rhizoctonia* spp. Beneficial = D: *Bacillus subtilis*; E: *Trichoderma* spp.

At the end of the faba-bean production cycle, repeat inspection of the traps revealed the presence of the inoculated beneficial microorganisms. Both *Trichoderma* spp. and *B. subtilis* were detected in traps across all treated plots, confirming successful establishment and rhizosphere colonization. No detectable *Phytophthora* spp. were observed in end-cycle traps. Although *Fusarium* spp. and *Rhizoctonia* spp. were still present, their colonization of rice traps was limited.

Analysis of Variance

Table 2 presents the mean squares for all evaluated variables. The block effect showed mean squares greater than zero for most variables, confirming that the randomized complete block design effectively controlled inter-block variability. Only the variable NPP required model adjustment due to residual block heterogeneity.

Table 2. Mean squares of the analysis of variance for treatment effects on faba-bean variables.

Source of Variation	*df*	PH	LL	PL	NPP	NSpP	NSP	RW	Yield
Block	3	221.75	0.85	0.88	51.06**	0.17	232.16	0.92	172.85
Treatment	3	314.08 ns	0.42 ns	1.00 ns	83.56**	0.83**	976.13*	12.75**	228.67**
Error	9	257.81	0.60	1.22	8.62	0.11	207.66	1.19	26.04
CV (%)		11.75	11.06	8.69	19.17	13.33	27.70	20.33	26.18
R^2		0.41	0.41	0.33	0.84	0.75	0.66	0.79	0.84

df = degrees of freedom, * = significant ($p \leq 0.05$), **= highly significant ($p \leq 0.01$), ns= not significant.
Plant height (PH), leaf length (LL), pod length (PL), number of pods per plant (NPP), number of seeds per pod (NSpP), number of stems per plant (NSP), root weight (RW), yield (faba-bean pod weight).

Plant Height (PH)

No significant effects ($p > 0.05$) of *Trichoderma* spp., *B. subtilis*, or their combination were observed for plant height (Table 2). Mean values ranged from 127.00 ± 11.80 cm (T1) to 145.00 ± 7.35 cm (T2) (Figure 2).

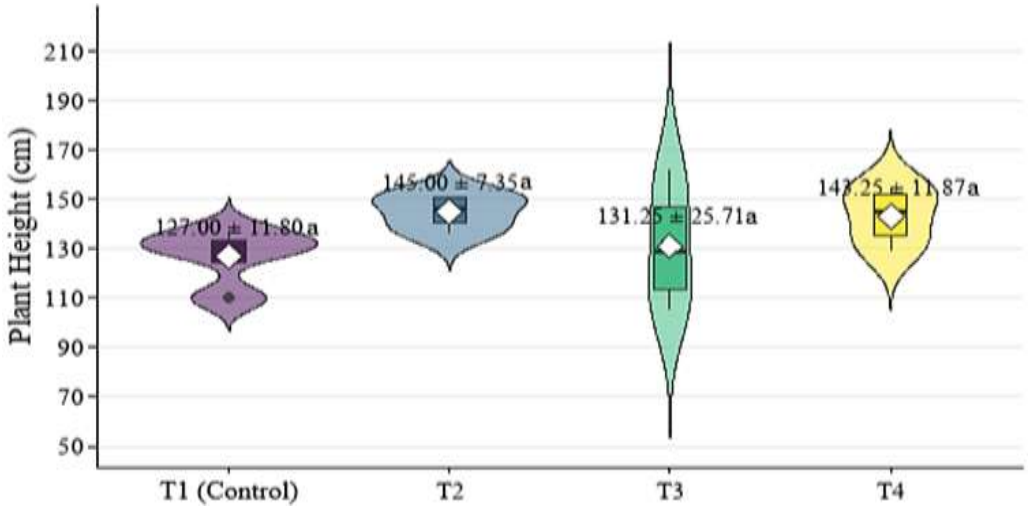

Figure 2. Plant height distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

The coefficient of variation (CV = 11.75%) indicates acceptable experimental precision.

Leaf Length (LL) and Pod Length (PL)

Leaf length and pod length were not significantly affected by treatments ($p > 0.05$), with CV values of 11.06% and 8.69%, respectively (Table 2). Leaf length ranged from 6.53 ± 1.17 cm (T2) to 7.22 ± 0.68 cm (T1) (Figure 3), while pod length ranged from 12.10 ± 1.87 cm (T2) to 13.30 ± 0.41 cm (T3) (Figure 4).

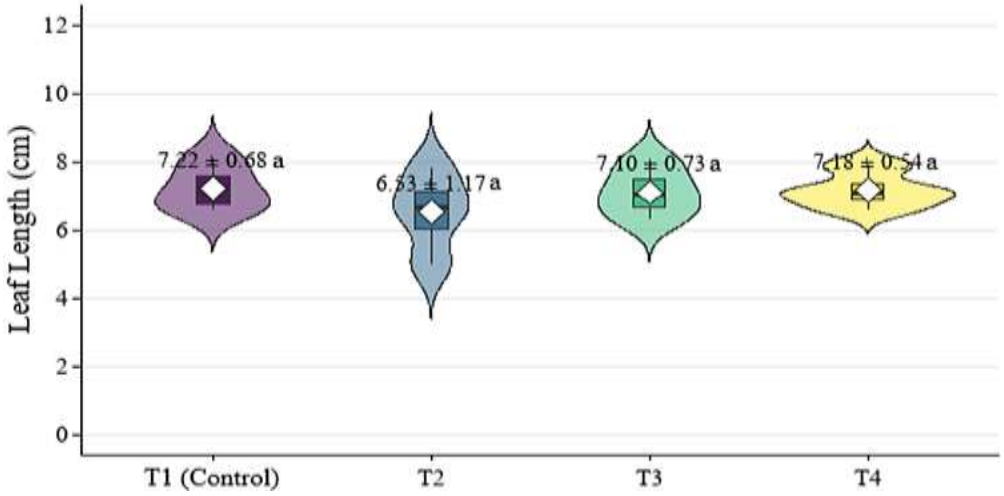

Figure 3. Leaf Length distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

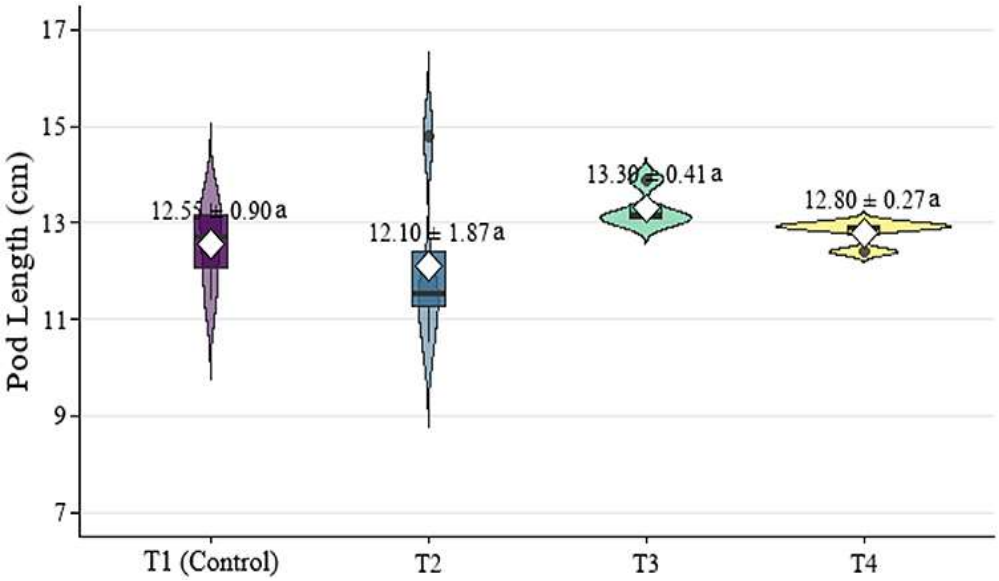

Figure 4. Pod length distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

Number of Pods per Plant (NPPP)

A significant treatment effect was observed for NPP ($p \leq 0.05$) (Table 2). Treatment T4 (*Trichoderma* spp. + *B. subtilis*) presented the highest mean (21.5 ± 5.26 pods $plant^{-1}$), approximately double that of the control (10.50 ± 1.91 pods $plant^{-1}$) (Figure 5).

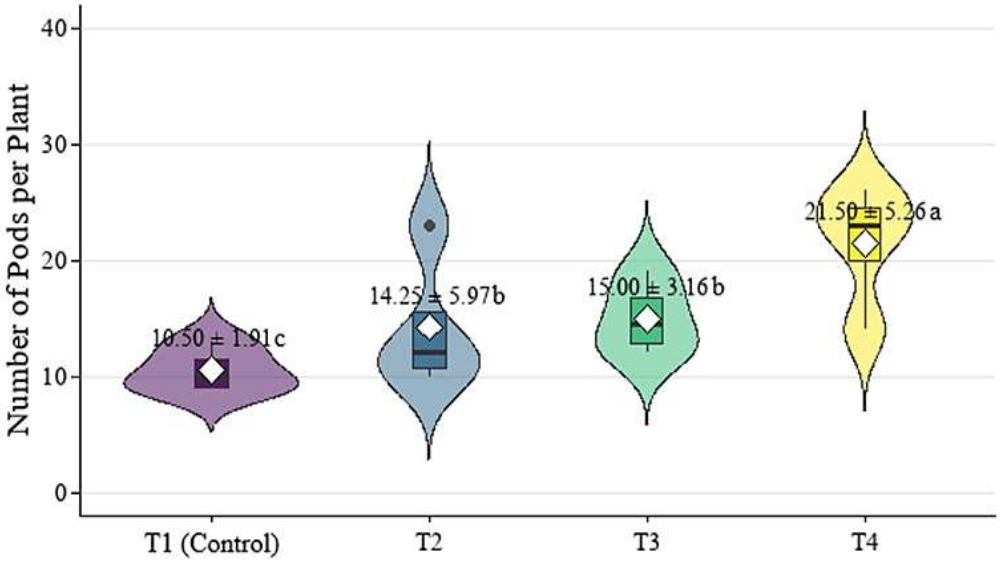

Figure 5. Number of pods per plant distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

Number of Seeds per Pod (NSpP)

Although T4 showed the highest mean (3.0 ± 0.00 seeds per pod), differences relative to T3 (2.75 ± 0.50) were not statistically significant ($p > 0.05$). Both treatments exceeded the control (2.0 ± 0.00) (Figure 6).

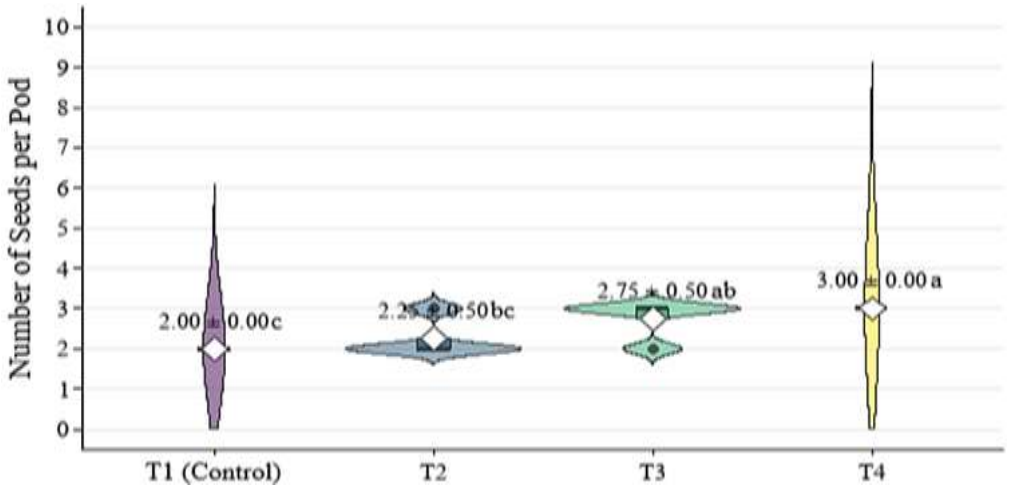

Figure 6. Number of seeds per pod distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

Root Weight (RW)

Root weight was highest in T4 (68.10 ± 15.33 g) and T2 (62.27 ± 22.05 g), both exceeding the control (35.50 ± 7.21 g) (Figure 7).

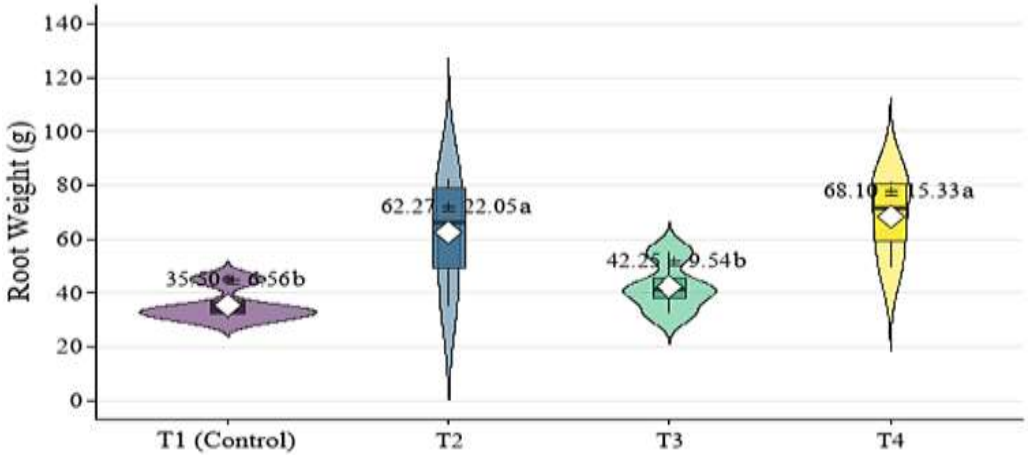

Figure 7. Root weight distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

Number of Stems per Plant (NSP)

The highest NSP value was observed in T4 (7.75 ± 1.26 stems $plant^{-1}$) (Figure 8).

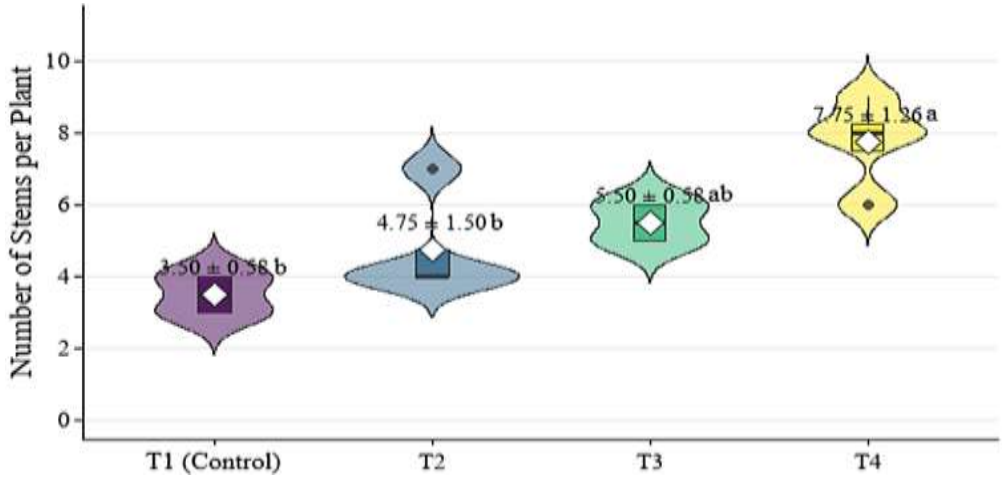

Figure 8. Number of stems per plant distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

Yield

Treatment T4 produced the highest pod yield (29.25 ± 9.22 t ha^{-1}) (Figure 9).

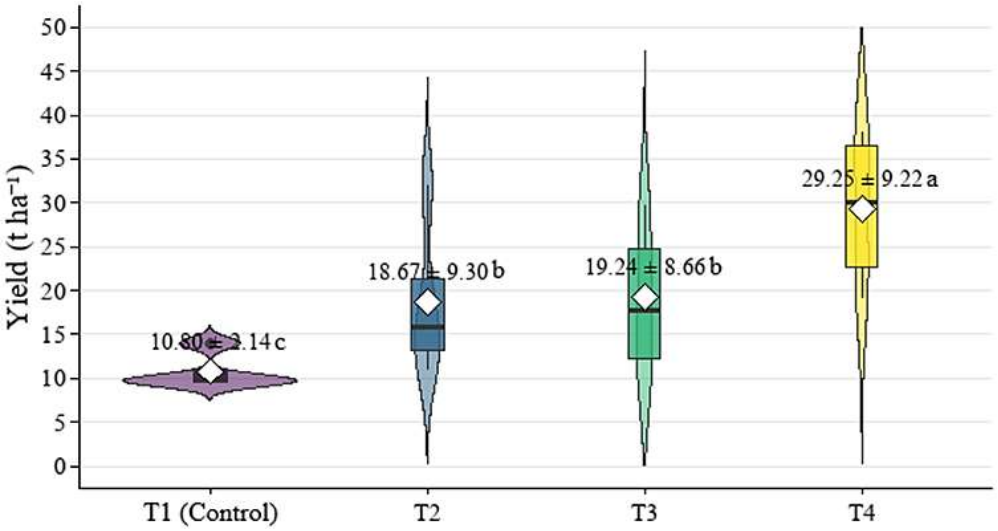

Figure 9. Yield distribution across treatments. Values represent mean ± SD. Different letters indicate significant differences ($p \leq 0.05$). T1: untreated control, T2: *Trichoderma* sp. (10^9 spores g^{-1}), T3: *Bacillus subtilis* (10^9 spores g^{-1}), T4: *Trichoderma* sp. (10^9 spores g^{-1}) + *Bacillus subtilis* (10^9 spores g^{-1}).

DISCUSSION

The detection of both pathogenic and beneficial microorganisms in the initial trap assessment confirms a baseline condition of disease pressure in the experimental area. The subsequent predominance of *Trichoderma* spp. and *B. subtilis* in treated plots demonstrates effective rhizosphere colonization following seed inoculation, which is consistent with previous reports describing the establishment of beneficial inoculants under field conditions (Carrión et al., 2019; De Corato, 2020).

The absence of *Phytophthora* spp. at the end of the cycle and the reduced colonization of Fusarium spp. and *Rhizoctonia* spp. suggest that the microbial consortium exerted suppressive effects. This observation aligns with studies reporting the antagonistic activity of *Trichoderma-Bacillus* consortia against soil-borne pathogens, particularly Oomycetes, through mechanisms such as antibiosis and competition (Abdullah et al., 2021; Poveda, 2022). Moreover, these genera are widely recognized for their ability to suppress pathogens via mycoparasitism and secretion of antimicrobial compounds (Harman, 2006; Attia et al., 2025). Similar outcomes have been reported in microbial consortium systems, where combined inoculation enhances pathogen suppression and yield performance (Wang et al., 2019).

The observed reduction in pathogen colonization further supports the concept that rhizosphere inoculation can shift microbial communities toward disease-suppressive states, improving plant health (Ali et al., 2024; Kredics et al., 2024). This shift is often associated with enhanced microbial competition and niche occupation, limiting pathogen establishment.

Despite these microbiological changes, vegetative growth variables (plant height, leaf length, and pod length) were not significantly affected by treatments. This suggests that these traits were predominantly governed by genetic factors and environmental conditions rather than microbial inoculation (Alori et al., 2017). Additionally, the lack of response may indicate limited stimulation of hormonal pathways associated with stem elongation, such as gibberellin biosynthesis (Egamberdieva et al., 2017).

From a physiological standpoint, it is plausible that plant resources were preferentially allocated toward reproductive development rather than vegetative expansion. This interpretation is consistent with plant resource allocation theory, in which trade-offs favor yield-related traits under improved nutrient acquisition or stress reduction (López-Bucio et al., 2015; Buckley & Avila-Sakar, 2013).

In contrast, reproductive traits responded strongly to microbial inoculation. The significant increase in the number of pods per plant under T4 highlights a synergistic interaction between *Trichoderma* spp. and B. subtilis. This synergy can be explained by complementary mechanisms: *Trichoderma* enhances root development and pathogen suppression, while B. subtilis promotes plant growth through phytohormone production and improved nutrient uptake (Tahir et al., 2017; Aloo et al., 2022; Singh & Pujari, 2022). Furthermore, microbial consortia are known to exert stronger biostimulant effects than single inoculants due to functional complementarity (Senkovs et al., 2021).

The lack of statistical difference between T3 and T4 for seeds per pod suggests that seed filling may be primarily influenced by bacterial activity. Bacillus subtilis is well known for enhancing nitrogen and phosphorus availability, thereby improving nutrient-use efficiency and assimilate partitioning to grains (Pérez-Montaño et al., 2014; Liu et al., 2021; Rana et al., 2020). This reinforces the hypothesis that bacterial-mediated processes dominate during the grain-filling phase.

The increases observed in root biomass further support the role of both microorganisms in enhancing belowground development. *Trichoderma* spp. promote root branching and elongation through auxin-related pathways and secondary metabolites (Illescas et al., 2021; Chen et al., 2024), while Bacillus spp. enhance nutrient solubilization and uptake (Iqbal et al., 2024; Jensen et al., 2024). These effects collectively improve plant access to soil resources, which is critical for sustaining productivity.

Similarly, the higher number of stems per plant under combined inoculation reflects enhanced hormonal regulation. Both microorganisms influence the production of auxins, cytokinins, and gibberellins, which regulate branching and plant architecture (Hashem et al., 2019; Illescas et al., 2021; Galbieri et al., 2023; Shi et al., 2023). The superior performance of the consortium indicates a synergistic hormonal balance that favors the development of additional reproductive sites.

The yield increase observed in T4 is consistent with the integration of multiple beneficial mechanisms. These include improved nutrient availability through phosphate solubilization, siderophore production, and biological nitrogen fixation (Pérez-Montaño et al., 2014; Kour et al., 2020; Saha et al., 2016), as well as pathogen suppression through ecological competition (Mendoza-Mendoza et al., 2018). Such combined effects have been widely reported in systems using *Trichoderma*-Bacillus consortia (Silva et al., 2024).

In addition, both microorganisms play a key role in inducing systemic resistance (ISR), activating plant defense pathways and enhancing resilience to biotic stress (Martínez-Medina et al., 2013). This process involves complex signaling networks mediated by jasmonic acid, ethylene, and salicylic acid pathways, leading to primed defense responses (Salwan et al., 2022). Recent advances further demonstrate that *Trichoderma* can modulate plant immunity through molecular signaling and microbiome interactions, reinforcing its multifunctional role in crop systems (Shafi et al., 2026).

Beyond immediate crop responses, microbial inoculants contribute to long-term soil health. Their persistence in soil allows continued interaction with plant roots, promoting organic matter decomposition and nutrient cycling (Brotman et al., 2013; Kour et al., 2020). Additionally, the production of exopolysaccharides improves soil aggregation, porosity, and water retention (Saha et al., 2016), which are essential for sustainable agricultural systems.

Overall, the results demonstrate that the combined application of *Trichoderma* spp. and Bacillus subtilis effectively enhances reproductive performance and yield while contributing to disease suppression and soil quality improvement. These findings reinforce the potential of microbial consortia as a key component of sustainable intensification strategies in modern agriculture.

CONCLUSIONS

The initial presence of *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* spp. was confirmed, while *Trichoderma* spp. and *B. subtilis* were detected at the end of the cycle, with *Phytophthora* absent, indicating effective establishment and pathogen suppression.

Combined inoculation (*Trichoderma* + *B. subtilis*) enhanced agronomic performance (~ 21 pods plant^{-1}, 3 seeds pod^{-1}, ~ 68 g root weight, ~ 29.25 t ha^{-1} pod yield), representing a sustainable strategy to increase faba-bean yield under the study conditions. Benefits likely result from complementary microbial effects, such as *Trichoderma* spp. enhancing root growth and inducing systemic resistance, whereas *B. subtilis* mobilizing nutrients, producing phytohormones, and reducing pathogen pressure.

This combination shows potential to intensify production in low-fertility, disease-prone soils, reducing chemical input reliance. However, future research should focus on determining the mechanisms of action of the microorganisms and its efficacy under different agronomic management and faba-bean varieties.

AI USAGE

The authors used artificial intelligence tools solely to improve the linguistic quality of the manuscript, including grammar, spelling, and readability. No AI tools were used to generate scientific content, analyze data, or draw conclusions. All scientific content, interpretations, and conclusions are entirely the responsibility of the authors.

COMPETING INTERESTS

The authors have no competing of interests to declare.

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