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***Trichoderma* root colonization dynamics and their correlation with phosphorus mobilization in tropical soils**

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Abstract

Somewhere between the soil particle and the root surface lies a biological battlefield where microorganisms compete for carbon and nutrients and *Trichoderma* species are among the most effective colonizers. This research investigated root colonization dynamics of four *Trichoderma* species (*T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*) and a consortium of all four on mung bean (*Vigna radiata* L.) in a tropical Fluvisol at Bangladesh Agricultural University, Mymensingh, during the 2022-2023 kharif season. *T. asperellum* achieved the highest individual colonization rate (56.8%), while the consortium reached 63.2%. Root colonization correlated strongly with rhizosphere acid phosphatase activity ($r = 0.87$) and phosphorus solubilization ($r = 0.81$). The consortium treatment increased available soil P by 34.1 mg kg⁻¹ and grain yield by 38.7% over the uninoculated control. These findings show that multi-species *Trichoderma* inoculation can serve as a biological alternative for phosphorus management in tropical legume systems.

Keywords: *Trichoderma*, root colonization, phosphorus mobilization, acid phosphatase, mung bean, tropical soil, biofertilizer, rhizosphere, consortium inoculation

Introduction

Picture a tropical soil where 90% of applied phosphorus is locked within hours into iron and aluminum complexes, effectively invisible to plant roots [1]. This is the reality for farmers across South and Southeast Asia, where phosphorus fixation in acidic Fluvisols wastes billions in fertilizer investment annually [2]. Biological agents that can unlock this fixed phosphorus offer an attractive alternative to simply applying more chemical fertilizer.

Trichoderma species are well known as biocontrol agents, but their role in nutrient mobilization particularly phosphorus solubilization through organic acid production and phosphatase enzyme secretion has gained attention only recently [3, 4]. These fungi colonize the root surface and outer cortical cells, forming an intimate association that allows them to channel solubilized nutrients directly to the host plant [5]. Different *Trichoderma* species vary in their colonization ability and P-solubilizing capacity, and whether combining multiple species in a consortium improves performance over single-species inoculation remains an open question [6, 7].

This research tracked root colonization dynamics of four *Trichoderma* species individually and as a consortium on mung bean in a tropical Fluvisol at Mymensingh, Bangladesh, and correlated colonization levels with rhizosphere phosphatase activity, P solubilization, and crop yield [8].

Extraction protocol

Trichoderma isolates were obtained from the culture collection of the Department of Microbiology, Bangladesh Agricultural University. Each species was grown on potato dextrose agar (PDA) at 28 °C for 7 days. Spore suspensions were prepared by flooding plates with sterile 0.1% Tween 80, scraping with a sterile glass rod, and filtering through four layers of cheesecloth. Spore concentration was adjusted to 1×10^8 conidia mL⁻¹ using a Neubauer haemocytometer. For the consortium treatment, equal volumes of each species suspension were mixed. Talc-based formulations were prepared by mixing spore suspensions with sterilized talc

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Powder (mesh size 325) at 10^8 CFU g^{-1} and carboxymethyl cellulose (0.5%) as adhesive. Formulations were air-dried to 8-10% moisture and stored at 4 °C until use [9, 10].

Quality control and calibration

Viability of each *Trichoderma* formulation was verified by serial dilution plating on *Trichoderma* Selective Medium (TSM) before field application; all formulations exceeded 5×10^7 CFU g^{-1} . Root colonization was quantified by the root segment method: 20 randomly selected 1-cm root segments per plant were surface-sterilized (1% NaOCl, 1 min), plated on TSM, and incubated at 28 °C for 5 days. Colonization percentage was calculated as (colonized segments / total segments) $\times$ 100. Species identity of re-isolated colonies was confirmed by ITS sequencing for 10% of samples. Acid phosphatase activity in the rhizosphere soil was measured by the p-nitrophenyl phosphate (pNPP) method at pH 6.5 [11, 12].

Materials and Methods

Materials

Mung bean variety BARI Mung-6 was grown in a randomized

block design with four replications and six treatments: uninoculated control, *T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*, and the four-species consortium. Plot size was 3 m $\times$ 3 m. Seeds were treated with talc formulation at 10 g kg^{-1} seed before sowing. A soil drench of spore suspension (100 mL per metre row at 10^8 conidia mL^{-1}) was applied at 15 DAS. The soil was a non-calcareous dark grey Fluvisol (pH 5.8, organic C 1.07%, available P 11.4 mg kg^{-1} , clay 34%) at the university research farm (24.7°N, 90.4°E) [13].

Methods

Root colonization was assessed at 20, 40, and 60 DAS using the root segment plating method. Rhizosphere soil was collected at 40 DAS for acid phosphatase, available P (Bray-1), and soluble organic acids (citric, oxalic, malic) by HPLC. Yield components (pods per plant, seeds per pod, 1000-seed weight) and grain yield were recorded at harvest from net plot area. Pearson correlations were computed among colonization, enzyme activity, P availability, and yield. Data were analyzed by ANOVA with DMRT at 5% using SPSS 26 [14].

Results

Table 1: *Trichoderma* root colonization, acid phosphatase activity, and phosphorus status at 40 DAS

Treatment	Root col. (%)	Acid phos. (μg pNP $g^{-1} h^{-1}$)	Available P (mg kg^{-1})	Citric acid (μg g^{-1})	Oxalic acid (μg g^{-1})	P sol. (mg kg^{-1})
Control	12.4	87.3	11.4	4.2	2.8	8.7
<i>T. harzianum</i>	48.7	143.7	28.6	12.7	8.4	21.3
<i>T. viride</i>	42.3	131.4	24.8	10.4	7.1	18.7
<i>T. asperellum</i>	56.8	167.2	36.7	16.8	11.3	28.4
<i>T. koningii</i>	38.1	118.6	21.3	8.7	5.9	16.2
Consortium	63.2	184.3	45.5	19.4	13.7	34.1
SE(m) $\pm$	2.87	6.42	1.83	0.94	0.67	1.47
CD (5%)	8.84	19.8	5.64	2.90	2.06	4.53

The consortium treatment achieved the highest root colonization (63.2%), acid phosphatase activity (184.3 μg pNP $g^{-1} h^{-1}$), and P solubilization (34.1 mg kg^{-1}) (Table 1). Among individual

species, *T. asperellum* was the most effective colonizer and P solubilizer. All inoculated treatments significantly outperformed the uninoculated control for every parameter measured

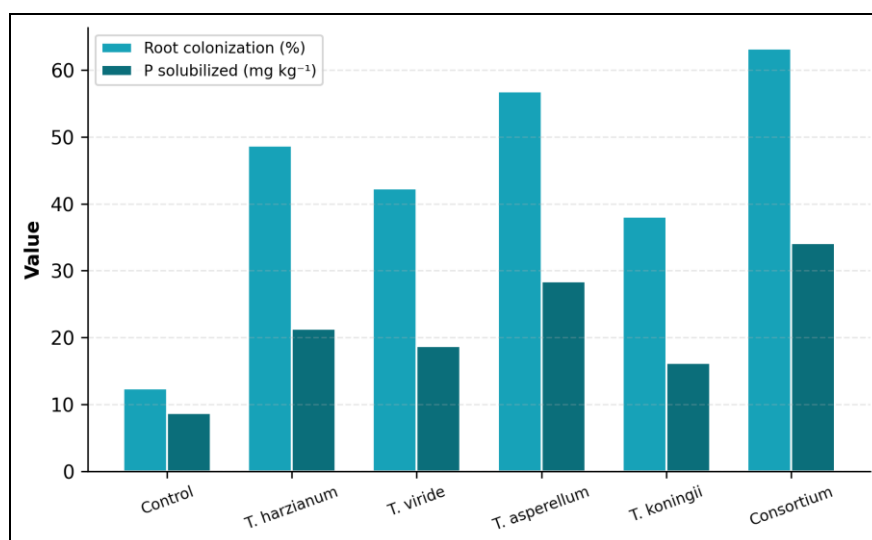


Fig 1: Root colonization percentage and phosphorus solubilized by four *Trichoderma* species and their consortium at 40 days after sowing



Fig 2: Correlation network showing relationships among *Trichoderma* root colonization, rhizosphere enzyme activity, phosphorus status, and plant growth parameters. Line thickness represents correlation strength

Comprehensive interpretation

The bar chart (Figure 1) clearly separates the consortium from all single-species treatments for both colonization and P solubilization. The correlation network (Figure 2) reveals that root colonization is the central hub connecting hyphal density ($r = 0.91$), acid phosphatase ($r = 0.74$), and P solubilization ($r = 0.81$), confirming that colonization intensity drives the downstream nutrient mobilization cascade.

Discussion

The consortium's superior performance likely reflects niche complementarity different *Trichoderma* species colonize different root zones and produce different organic acid profiles, collectively solubilizing more P than any single species alone [6, 7]. *T. asperellum*'s dominance among individual species is consistent with its known aggressive colonization strategy and high production of both citric and oxalic acids [4]. The strong correlation between colonization and acid phosphatase ($r = 0.87$) confirms that enzyme production is proportional to fungal biomass in the rhizosphere [3].

The 34.1 mg kg^{-1} increase in P solubilization by the consortium is biologically meaningful equivalent to roughly $15 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, which could replace about 30% of the recommended chemical P fertilizer for mung bean [1, 2]. The 38.7% grain yield increase in the consortium treatment was driven primarily by higher pods per plant and seeds per pod, consistent with P-responsive yield components in legumes [5].

A limitation was the single-season and single-soil-type scope. *Trichoderma* colonization efficiency varies with soil pH, organic matter, and native microbial competition [8]. Multi-location trials across different Fluvisol variants and pH ranges are needed before scaling these recommendations to farmers [15].

Conclusion

A four-species *Trichoderma* consortium achieved 63.2% root colonization and increased phosphorus solubilization by 34.1 mg kg^{-1} in a tropical Fluvisol, translating to a 38.7% grain yield increase in mung bean over the uninoculated control. Among individual species, *T. asperellum* was the most effective

colonizer and P mobilizer. The tight correlation between colonization intensity and phosphatase activity confirms that establishing a strong fungal presence on roots is the key to unlocking fixed soil phosphorus.

For smallholder farmers in Bangladesh and similar tropical environments, seed treatment plus soil drenching with a *Trichoderma* consortium offers a low-cost biological strategy to reduce dependence on chemical P fertilizer. Validating these results across multiple soil types, cropping seasons, and legume species is the essential next step for extension programs.

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