



Synergistic Role of *Trichoderma viride* with Other Phosphate Solubilizing Microorganisms for Enhancing Phosphate Solubilization in Wheat Cultivation of Manipur

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ABSTRACT

Background: The study investigates the role of *Trichoderma viride* (T.V) and other phosphate solubilizing microorganisms (PSM) in enhancing phosphorus solubilisation and other nutrient availability in wheat (*Triticum aestivum*) cultivation.

Methods: The present work was conducted at FEEDS experimental farm, Manipur on 15/12/ 2021 in Wheat (Shriram super 252). The experiment was carried out with six treatments(T), T1 @ *Trichoderma viride* (T.V), T2 @ *Azotobacter chroococcum* (A.C) + *Trichoderma viride* (T.V), T3 @ *Pseudomonas putida* (P.P) + *Trichoderma viride* (T.V), T4 @ *Azotobacter chroococcum* (A.C) + *Pseudomonas putida* (P.P) + *Trichoderma viride*, T5 @ *Azotobacter chroococcum* (A.C) and T6 @ *Azotobacter chroococcum* (A.C) + *Pseudomonas putida* (P.P) with four replications. Graded doses of nitrogen (N), phosphorus (P) and potassium (K) are applied in the ratio 100:50:50. Soil sample for PSM enumeration were collected at 0,30,65 and 95 days after sowing (DAS).

Result: The results demonstrated a significant increase in microbial populations, phosphorus solubilisation, nutrient availability and grain yield. Out of the six treatments, T3 which received T. V and P. P showed highest Carbon (c), P and Potassium(K) content and T6 showed highest Nitrogen(N) content in the soil. The mean of the highest total Phosphate solubilizing bacteria (PSB) population is observed in 30 DAS in T6 and the lowest total PSB population in T1 at 65 DAS. The mean of the highest total Phosphate solubilizing fungi (PSF) population is at 30 DAS in T1 and Lowest PSF population detected is in T6 at 0 DAS. The highest yield was observed in T4@ *Azotobacter chroococcum* (A.C) + *Pseudomonas putida* (P.P) + *Trichoderma viride* indicating a synergistic effect.

Key words: Biofertilizers, Grain yield, Phosphorus availability, *Trichoderma viride*, Wheat cultivation.

INTRODUCTION

Wheat (*Triticum aestivum*) is the major source of crop based human nutrition and a part of daily dietary need in one form or the other. The average wheat consumption is 318 grams per person each day, making up 83% of the overall cereal consumption (Iqbal *et al.*, 2022). Wheat grains contain 14.7% protein, 2.1% fat, 78.1% starch and 2.1% mineral matter (Peterson, 1965) and it beats every other single grain crop (including wheat, maize, etc.) (Erenstein *et al.*, 2022) in production and acreage and is grown across a broad range of climatic situations. P is a vital nutrient for plant growth, playing a critical role in various physiological processes such as energy transfer, photosynthesis and cell division. It also contributes to seed germination and root development, which are essential for optimal crop production and the percentage of protein in wheat can be influenced to a certain extent by the time of fertilizer application and fertilizer type (Iqbal *et al.*, 2022). However, phosphorus availability in soils is often limited due to its fixation into insoluble forms, making it inaccessible to plants (Alam *et al.*, 2022). One promising solution to overcome this challenge lies in the use of PSMS, which convert insoluble forms of P into soluble forms that plants can readily absorb (Tian *et al.*, 2021., Silva *et al.*, 2023). PSF and PSB play a crucial role in this process by secreting organic acids and enzymes that help release phosphorus

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from soil minerals (Vassileva *et al.*, 2022). T.V is a soil fungus known for its ability to decompose organic matter, solubilize phosphate and enhance nutrient availability in the soil. It acts as a natural biofertilizer, improving soil health and promoting plant growth (Mahato *et al.*, 2018 and Balakrishnan *et al.*, 2020). Moreover, T.V has been shown

to enhance root and shoot development, increase grain yields and improve plant resistance to diseases (Yao *et al.*, 2023). When used in combination with other PSMs, such *Azotobacter* and *Pseudomonas*, it can further boost phosphorus solubilization and nutrient cycling, leading to improved soil fertility and higher grain yield of wheat (Pan and Cai, 2023). T.V also protects the wheat plants from diseases (Chou *et al.*, 2020, Mau *et al.*, 2022, Yao *et al.*, 2023).

Azotobacter chroococcum have resulted in increase of macro and micro elements in leaves due to inoculation and manure (Ali *et al.*, 2025), Use of phosphate solubilizing bacteria and organic matter significantly had higher yield than control and their combination with phosphate fertilizer had significant effect on reducing phosphate fertilizer use. It also increased the plant dry matter by 24.7%. (Nosratabad and Shariati, 2015)

This study aims to show the synergistic effect of T.V with other PSM, increase in P solubilisation, more nutrient availability and its suitability for sustainable agriculture due to its huge potential to replace chemical fertilizers.

MATERIALS AND METHODS

The experiment was conducted on 15/12/2021 to study the effect of *Trichoderma viride* with PSMs in wheat cultivation. A popular hybrid wheat variety (Shriram super 252) is used as wheat production and quality could be enhanced through the development of new and improved varieties that are able to produce a superior yield and perform better under various agro-climatic stresses and conditions (Khalid *et al.*, 2023). The research was carried out at the experimental farm of FEEDS Group of Institution, College of Agricultural Sciences, Hengbung, Manipur. The soil is clayey in texture, reddish brown in colour with initial pH of 5.8, Electrical conductivity of 0.13 ds/m and Cation exchange capacity of 21.8 meq/100 g. The initial soil analysis at the experimental farm is recorded along with final reading in the end to observed the benefits of the treatments on soil nutrient availability. The experimental

design followed a randomized block design (RBD) with six treatments and four replications following micro plot technique. The 6 treatments include control (C), T1@ *Trichoderma viride*, T2@ *Azotobacter chroococcum* + *Trichoderma viride*, T3@ *Pseudomonas putida* + *Trichoderma viride*, T4@ *Azotobacter chroococcum* + *Pseudomonas putida* + *Trichoderma viride*, T5@ *Azotobacter chroococcum* alone and T6@ *Azotobacter chroococcum*+ *Pseudomonas putida*. These individual plot size is 10 sq.mt (size of the unit plot 2 m × 4.5 m) with 1m apart from each replication. The strain *Trichoderma viride* is collected from Microbiology lab Hengbung, prepared from Potato dextrose agar (PDA) with rose Bengal (Fig 1) as culture medium incubated for 7 days at 28°C. *Pseudomonas putida* and *Azotobacter chroococcum* biofertilizers are provided from the experimental farm. Except for control, full recommended graded doses of nitrogen (N), phosphorus (P) and potassium (K) at the ratio 100:50:50 were applied in the field. Soil samples were collected at 0, 30 (tillering stage), 65 (jointing stage) and 95 (spike emergence) days after sowing (DAS) for enumeration of PSM population and the plant height and spikelet height is also recorded at the same time (Table 4). The collected samples are cultured in pikovskaya medium (PM) for enumeration of PSMs at pH 5.6 containing glucose, tricalcium phosphate, ammonium sulphate, potassium chloride, magnesium, ferrous and manganese sulphate and yeast extract in 1 L sterilized distilled water. The cultured samples were incubated at 28±1°C for 5 days. Through serial dilutions, colony-forming unit(CFU) was estimated 1×10^3 CFU/g using pour plate method (Table 1). Day and night temperatures were maintained at 35°C and 23°C (Fig 2). Grain yield and biological yield are measured in kg ha⁻¹ and converted to t ha⁻¹ and Harvest Index is also calculated (Table 3). The data was analyzed by ANOVA (analysis of variance). The critical difference (CD) of the treatments was considered to be significant at the 5% level (P= 0.05).

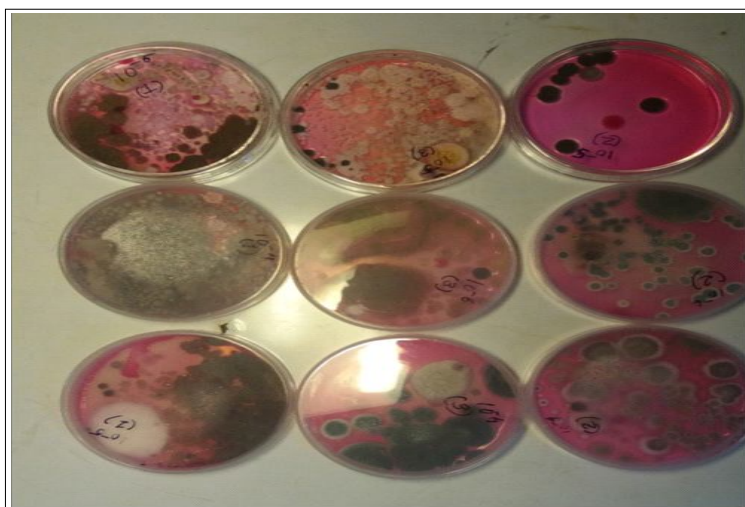


Fig 1: *Trichoderma viride* detected using PDA with rose Bengal at different dilutions.

Table 1: Enumeration of phosphate solubilizing microorganisms (n=4; mean $\pm$ SE).

Soil samples	Phosphate solubilizers					
	0 DAS		30 DAS		65 DAS	
	Bacteria (1 $\times$ 10 ³ cfu/g)	Fungi (1 $\times$ 10 ³ cfu/g)	Bacteria (1 $\times$ 10 ³ cfu/g)	Fungi (1 $\times$ 10 ³ cfu/g)	Bacteria (1 $\times$ 10 ³ cfu/g)	Fungi (1 $\times$ 10 ³ cfu/g)
T1 @ <i>Trichoderma viride</i> (T.V)	10.7 $\pm$ 0.007	9 $\pm$ 1.52	29 $\pm$ 0.012	29.7 $\pm$ 2.19	16.2 $\pm$ 0.001	20.7 $\pm$ 1.2
T2 @ <i>Azotobacter chroococcum</i> (A.C)	12.3 $\pm$ 0.009	8.7 $\pm$ 1.2	32.3 $\pm$ 0.013	28.3 $\pm$ 2.03	19.2 $\pm$ 0.001	20.3 $\pm$ 1.86
+ <i>Trichoderma viride</i> (T.V)						
T3 @ <i>Pseudomonas putida</i> (P.P)	13.6 $\pm$ 0.001	7.3 $\pm$ 2.33	34.3 $\pm$ 0.012	23.3 $\pm$ 1.86	19.8 $\pm$ 0.001	17.7 $\pm$ 1.76
+ <i>Trichoderma viride</i> (T.V)						
T4 @ <i>Azotobacter chroococcum</i> (A.C)	13.7 $\pm$ 0.001	7.7 $\pm$ 1.33	35 $\pm$ 0.015	24 $\pm$ 1.73	21.4 $\pm$ 0.002	19.3 $\pm$ 1.2
+ <i>Pseudomonas putida</i> (P.P)						
+ <i>Trichoderma virid e</i> (T.V).						
T5 @ <i>Azotobacter chroococcum</i> (A.C)	14.3 $\pm$ 0.003	3.7 $\pm$ 0.33	33.3 $\pm$ 0.013	14 $\pm$ 1=99.73	21.1 $\pm$ 0.001	11.7 $\pm$ 2.03
T6 @ <i>Azotobacter chroococcum</i> (A.C)+ <i>Pseudomonas putida</i> (P.P)	13.9 $\pm$ 0.001	2.3 $\pm$ 0.33	37.3 $\pm$ 0.009	9.7 $\pm$ 2.33	22.4 $\pm$ 0.002	8.7 $\pm$ 1.2
CD (0.05)	15 $\times$ 10 ³	4.214 $\times$ 10 ³	39 $\times$ 10 ³	6.13 $\times$ 10 ³	0.4 $\times$ 10 ³	4.872 $\times$ 10 ³
					0.6 $\times$ 10 ³	0.511 $\times$ 10 ³

RESULTS AND DISCUSSION

The pH of the soil in these treatments remained acidic ranging 4.0-5.5, the sequence is as follows T6<T2<T4<T3 <T1<T5<C (Table 1). The secretions of the PSM made the soil more acidic. Wheat cultivation thrives best in neutral pH and acidic soil is not suitable unless liming is done but addition of biofertilizers resulted in high grain yield by making nutrients available (Ghimirey *et al.*, 2024). The OC content of the soil is increased with the application of the PSM (Kumar and Rai, 2020). The sequence of availability of OC is <T6<C<T2<T5<T4<T1<T3 (Table 2). It has been reported that soil O composition strongly influences soil microbial community composition (Ng *et al.*, 2013, Salles *et al.*, 2019). The increment in moisture content is due to the activities of the PSM in the soil where it improves the physical properties of the soil. *Trichoderma* can effectively increase the content of large aggregates in soil, increase the soil water holding capacity and improve soil physical properties (Zhu *et al.*, 2022). There is maximum moisture increase with *Trichoderma viride* (T1) followed by *Trichoderma viride* + *Pseudomonas putida* (T3) and least moisture content in Control (Table 2). The sequence of moisture content increases as T2<T6<T4<T5<T3 <T1<C (Table 2) and total N is as follows T6>T5>T4>T2>T3>T2>C (Table 2). The results of this study align well with previous research on the role of T.V in enhancing microbial population dynamics and phosphorus solubilisation and there is positive correlation between available P and PSM population (Fig 4). The sequence of available P increases as T6<T4<T3<T5<T2<T1<C (Table 2). Increase in microbial populations is observed, particularly in treatments involving T.V, confirms the findings of Gao *et al.* (2023), who demonstrated that *Trichoderma* species enhance microbial activity by promoting beneficial interactions in the soil.

The significant improvements in phosphorus availability are also consistent with earlier studies by Song *et al.* (2023), which highlighted the effectiveness of T.V in solubilizing inorganic phosphates through organic acid production. Highest root system and spike development is observed in T4 as compared to C (Fig 3a and Fig 3b).

The mean of the highest total PSB population of 37.3 $\times$ 10³ CFU/g in T6@ *Azotobacter chroococcum* (A.C) + *Pseudomonas putida* (P. P) is recorded at 30 DAS (Table 1) The lowest total PSB population of 10.7 $\times$ 10³ CFU/g was noticed in T1@*Trichoderma viride* (T.V) at 65 DAS (Table 1). The sequences of the reading for total PSB population at 0 DAS as follow T5>T6>T4>T3>T2>T1, at 30 DAS the total PSB is as follow T6>T4>T3>T5>T2>T1, at 65 DAS the total PSB is as follow T6>T4>T5>T3>T2>T1 and at 95 DAS the sequence is as follows T6>T4>T3>T5>T2>T1 (Table 1).

The mean of the highest total PSF population of 29.7 $\times$ 10³ CFU/g in T1@ *Trichoderma viride* (T.V) is recorded at 30 DAS (Table 1). Lowest PSF population detected was 2.3 $\times$ 10³ cfu/g in T6@ *Azotobacter chroococcum* (A.C) + *Pseudomonas putida* (P.P) at 0 DAS (Table 1).

The highest grain yield was recorded highest in T4 and the lowest value was in T1 and control. The grain yield could be ranked as T4>T2>T3>T6>T5>T1>C (Table 3).

From the given data and observed experiment in fields, best germination was recorded in T4. *Trichoderma* spp. have been proved by various research to increase growth and yield of the plants due to nutrient availability. The yield data indicated that crop improved significantly with the application of T.V and other PSMs (Table 3). The correlation between crop yield and harvest index is positive (Fig 5). Treatment T4, which included the combination of A.C, P.P and T.V, resulted in the highest grain yield, with a 25% increase compared to the control (C). Periodic growth of plant height and spikelet height is recorded and T4 receiving T.V with A.C and P.P shows highest development (Table 4). Previous findings in wheat cultivations are increasing grain (36.2 %) and shoot yield (37.8 %) in rainfed

wheat (Khosravi and Mahmoudi, 2013) and increase of yield and various growth indices (Shams El-deem *et al.*, 2020). The PSM population and the grain yield also shows positive correlation (Fig 6) and the positive correlation of PSM population with available P and also grain yield supports the synergistic role of PSM with T.V in wheat cultivation for phosphate solubilization and better yield.

Interpretation of results in the context of previous studies on T.V and PSM interactions

The results of this study align well with previous research on the role of T.V in enhancing microbial population dynamics and phosphorus solubilization. The observed increase in microbial populations, particularly in treatments involving T.V, confirms the findings of Gao *et al.* (2023), who demonstrated that *Trichoderma* species enhance microbial activity by promoting beneficial interactions in

Table 2: Chemical analysis of the soil under different treatments.

Treatments	pH	Organic carbon (g/kg)	Moisture content (%)	Available phosphorus (kg/ha)	Available potassium (kg/ha)	Total nitrogen (mg/Kg)
C	5.5	14.01	45	11.80	115	0.10
T1	5.0	15.50	54	13.45	133.34	0.27
T2	4.4	14.40	46	19.24	163.34	0.83
T3	4.6	15.70	53	28.13	230	0.57
T4	4.5	15.10	49	32.33	201	0.93
T5	5.2	14.83	51	21.24	219	1.07
T6	4.0	12.70	48	35.15	240	1.13
Mean	4.74	14.61	49.43	23.06	185.95	0.7
Range	4-5.5	12.70-15.70	45-53	11.80-35.13	115-240	0.10-1.13
CD (5% Level of significance)	0.35	0.56	5.15	4.06	86.79	0.48
Standard deviation	0.52	1.03	3.41	9.07	49.15	0.4
Initial	5.8	13	44	10.098	110	0.11

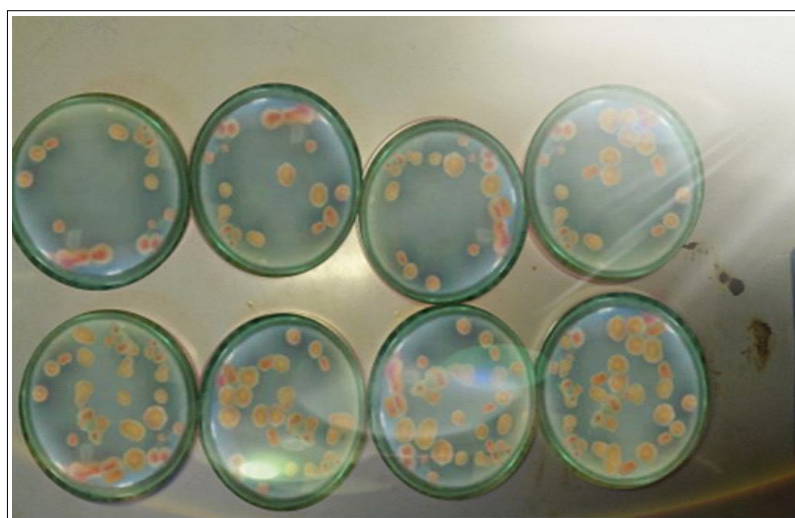


Fig 2: Maximum PSM enumeration of T6 at 30 DAS (lower petri dish of the four replications) and at 65 DAS (upper petri dish of the four replications).

the soil. The significant improvements in phosphorus availability (up to 30% in treatment T4) are also consistent with earlier studies by Song *et al.* (2023), which highlighted the effectiveness of T.V in solubilizing inorganic phosphates through organic acid production.

Furthermore, the increased grain observed in this study corroborate previous research showing that the application of T.V results in improved crop performance due to better nutrient uptake and enhanced plant growth (Elhaissofi *et al.*, 2022). The present study provides

additional evidence of T.V beneficial role in wheat cultivation, particularly when combined with other PSMs like *Azotobacter* and *Pseudomonas*.

Analysis of synergistic effects between T.V and other PSMs on phosphorus availability and plant growth

The combined application of T.V with other PSMs, such as A.C and P.P, showed a clear synergistic effect on phosphorus solubilization and plant growth. In treatments that combined these microorganisms (T4), phosphorus availability and

Table 3: Yield of wheat crop.

Treatment	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield	Harvest index (%)
C	4.5	5.9	10.4	43.26
T ₁	6.4	7.9	14.3	44.75
T ₂	9.6	11.0	20.6	46.60
T ₃	8.2	9.5	17.6	46.59
T ₄	10.2	11.5	21.27	47
T ₅	6.9	8.1	15	46
T ₆	8.1	9.3	17.6	46.02
SE(d)	1.95	1.92	3.78	1.31
CD 5%	1.99			



Fig 3(a): Comparison of root system of wheat between C and T4.



Fig 3(b): Physical comparison of spike of wheat between C and T4.

microbial population increased significantly compared to treatments where each microorganism was applied individually. This synergistic interaction is likely due to the complementary mechanisms of phosphorus solubilization employed by T.V and the other PSMs. While *Trichoderma* produces organic acids and enzymes to break down insoluble phosphorus, bacteria like *Pseudomonas* and

Azotobacter contribute additional solubilizing compounds and nitrogen fixation, further boosting nutrient availability (Elhaissofi *et al.*, 2022).

The enhanced plant growth metrics, including increases in root length, shoot length and biomass in treatments T2, T3 and T4, demonstrate the beneficial effects of combining these biofertilizers (Aseel *et al.*, 2023). This combination

Table 4: Periodic record of plant height and spikelet height.

Sl. no	30 DAS	65 DAS		95 DAS	
	Plant height	Plant height	Spikelet height	Plant height	Spikelet height
C	24.84	77.33	8.00	79.67	8.17
T1	24.67	84.33	11.33	85.67	11.67
T2	31.34	87.67	13.00	89.34	13.33
T3	30.34	87	12.67	88	13.00
T4	31.67	88.33	13.33	89.67	13.67
T5	28.34	82	12.00	85.34	12.34
T6	29.34	85.66	12.33	87	12.67
SE (d)	2.66	3.46	1.50	2.98	1.52
CD (5%)	1.89	2.28	1.73	2.98	1.19

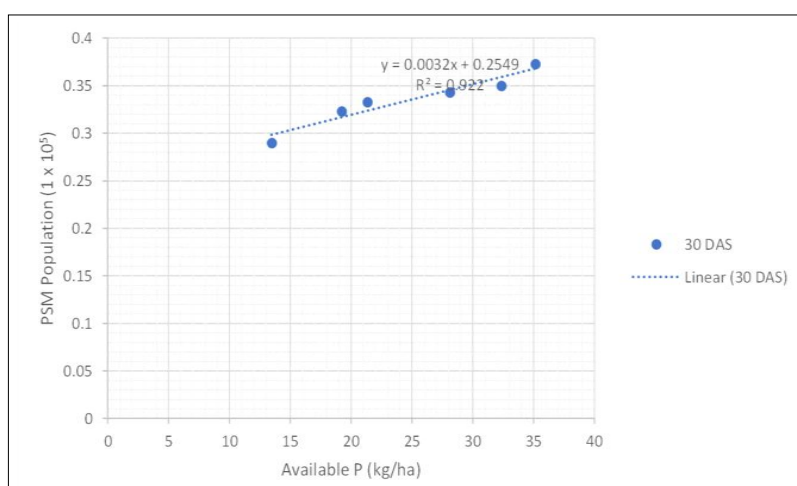


Fig 4: Correlation of available phosphorus and PSB population (30DAS).

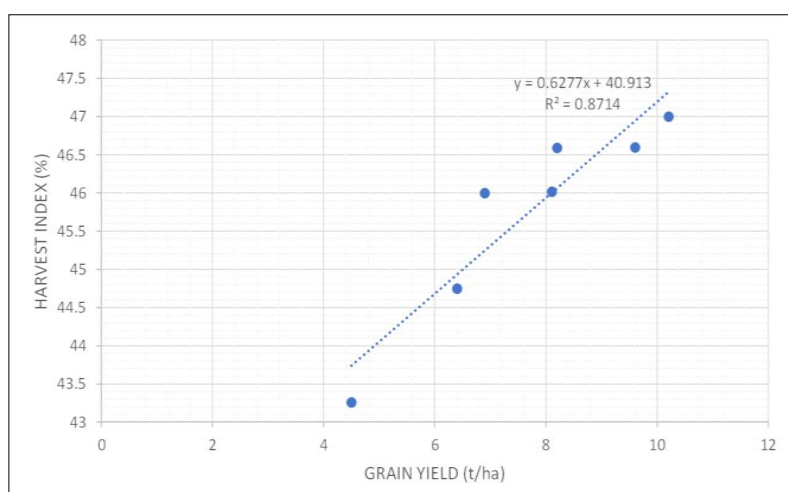


Fig 5: Correlation of crop yield and harvest index.

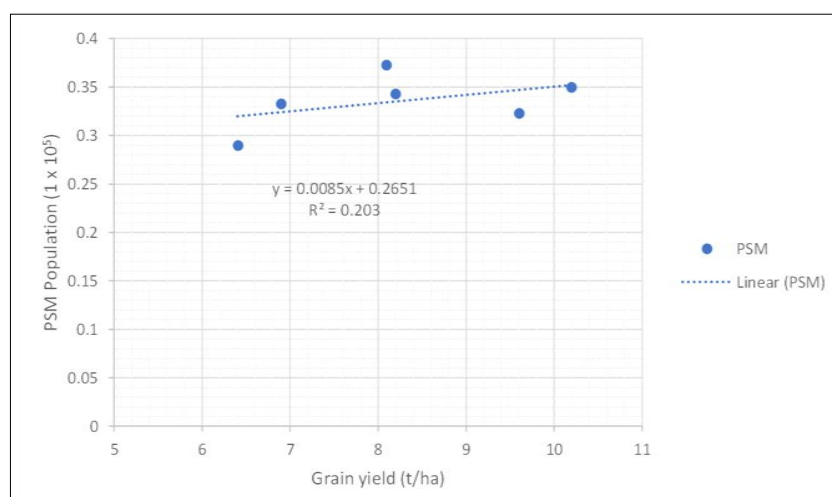


Fig 6: Correlation of PSB population (30 DAS) and grain yield.

not only improved nutrient uptake but also stimulated root development (Fig 3a), leading to better overall plant health. Previous studies, such as those by Mehetre and Mukherjee, 2015, Saleemi *et al*, 2017 reported similar findings where *Trichoderma* enhanced root growth and nutrient absorption, confirming the synergistic potential of these PSMs when applied together.

How T.V improves soil health and promotes sustainable wheat cultivation

One of the most significant benefits of using T.V in wheat cultivation is its contribution to soil health. In addition to solubilizing phosphate, *Trichoderma* enhances the overall microbial diversity and activity in the soil, as observed in the increased microbial populations in treatments involving T.V (Poveda and Eugui, 2022). The fungus's ability to suppress soil-borne pathogens through competitive exclusion and production of antifungal metabolites helps create a more balanced and healthy soil environment, reducing the need for chemical pesticides (Malik *et al.*, 2024, Meena *et al*, 2021, Joshi and Gururaj, 2025, Parmar and Gohel, 2025).

Furthermore, the improvements in soil nutrient levels such as increased nitrogen and potassium in addition to phosphorus demonstrate that T.V plays a role in nutrient cycling (Table 2), further enhancing soil fertility (Bononi *et al.*, 2020, Chen *et al*, 2021). These effects not only increase grain yields but also reduce the dependence on synthetic fertilizers, which is crucial for promoting sustainable and environmentally friendly agricultural practices (Srivastava, 2015, Song *et al.*, 2023).

The integration of T.V into wheat cultivation systems offers a sustainable solution to many of the challenges associated with intensive farming, such as soil degradation and nutrient depletion. By promoting natural nutrient cycling and enhancing plant growth, T.V can help improve wheat productivity while minimizing the environmental impact of chemical inputs, aligning with the goals of sustainable agriculture (Mathews *et al.*, 2010 and Vassileva *et al.*, 2022).

CONCLUSION

This study highlights the significant role of T.V in enhancing phosphorus solubilization and improving grain yield of wheat. The application of T.V, both alone and in combination with other PSMs like A.C and P.P resulted in substantial increases in microbial population, phosphorus availability and plant growth parameters. The highest yields were observed in treatments combining T.V with other PSMs, confirming the synergistic effect of these biofertilizers in promoting nutrient cycling and plant health. These findings align with previous research demonstrating the multiple benefits of T.V as a biofertilizer that not only improves soil fertility but also enhances crop productivity in a sustainable manner. The use of T.V in wheat cultivation offers an eco-friendly alternative to chemical fertilizers, reducing the environmental impact of intensive farming. Wheat thrives best in neutral range pH to slightly acidic soil but the addition of T.V with PSMs and utilization of high yielding variety in this study in acidic soil condition yet resulted in good yield and therefore further research is recommended to investigate its performance across varying soil types, pH, moisture levels and environmental conditions. In addition, studies focusing on the long-term effects of repeated *Trichoderma* application on soil health and crop performance would provide valuable insights into its potential for broader agricultural adoption. Further research is recommended to optimize its use under varying soil conditions.

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Disclaimer

The experiment, views and results conveyed and shown in this article is an original work. The authors take full responsibility

for the accuracy of the information provided, but will not be held responsible for losses that may result from the utilization of this work.

Conflict of interest

The author declares that there is no conflict of interest for the publication of this article and preparation of the manuscript.

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