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Seed bio-priming with *Trichoderma harzianum* isolates and its Rhizosphere Colonization in Wheat

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Authors' contributions

This work was carried out in collaboration among all authors. Author SVK designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript and managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: In this study, the different isolates of *Trichoderma harzianum* have abilities like antifungal, insecticidal, soil- remediation, plant growth promoting and rhizosphere colonizing properties which assist its biocontrol action were investigated *in vitro*.
Study Design: CRD (Completely Randomized Design).
Place and Duration of Study: An experiment was conducted at Plant Pathology Section, College of Agriculture, Nagpur during 2023-2024.

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Methodology: A seven days sporulated culture of *Trichoderma harzianum* isolates (ATH, BTH, CTH, DAG, Thdept) and wheat seeds were collected from Plant Pathology Section, College of Agriculture, Nagpur. The best isolate was identified by using biopriming technique on wheat seeds and the root colonization was also observed.
Results: Among all the isolates, the best treatment having highest germination per cent, root and shoot length and SVI is T_5 (Thdept) i.e. 72.50%, 23.60 cm, 15.97 cm, 2854.50 respectively followed by T_2 (BTH). These results showed that two isolates of *Trichoderma harzianum* could be used as effective biocontrol for promoting the growth of wheat crop *in vitro*.
Conclusion: The growth and yield will increase by adding *Trichoderma* spp. in soil and make it most resistant wheat crop against different fungus. So by identifying the best isolate or combination of isolates will improve the health and quality of the product.

Keywords: Trichoderma harzianum; Triticum aestivum; bio-priming; rhizosphere colonization.

1. INTRODUCTION

Trichoderma, a biological fungus widely used for plant pest control, mainly exists in the soil, air, plant surface, and other ecological environments and can effectively control a variety of plant diseases (Haouhach et al., 2020). *Trichoderma spp.* has been known to invade other fungi for more than six decades. They're also well-known among scientists as powerful biological control agents (Naseby et al., 2000; Sharma et al., 2014). Biological control agents through seed treatment gets introduced into the soil environment which provides an opportunity for them to colonize the roots. Seed treatments do not guarantee protection against root diseases (Kraft & Papavizas, 1983; Liu & Vaughan, 1965 Merriman & Russell, 1990), if these biocontrol agents are poor root surface colonizers (Mendez-Castro & Alexander, 1983) or incapable of being transported by the root through the soil profile (Madsen & Alexander, 1982). Biopriming is a process of biological seed treatment that refers to a combination of seed hydration and seed inoculation with beneficial organisms to protect seed. The technique helps seeds to evenly germinate even under adverse soil conditions (Singh et al., 2003).

2. MATERIALS AND METHODS

The study was carried out on **"**Seed bio-priming of with *Trichoderma harzianum* isolates and its rhizosphere colonization in wheat**"** during year 2023-24 at Plant Pathology Section, College of Agriculture, Nagpur. Five isolates were obtained from department which were procured from different ICAR institutes or universities and these isolates were utilized in studying the effect on crop physiological characteristics.

2.1 Biopriming of Seed

Wheat seeds were soaked in water for overnight. Mix the formulation of biocontrol agent (*Trichoderma harzianum* isolates with the pre-soak seeds at the rate of 10-15 g/kg of seeds) (Rawat et al., 2011). Mix well and incubate the seeds under room temperature and high humidity. Sterilized soil in petri plate was taken and kept bioprimed seeds on soil and sprayed autoclaved water. Incubate the seeds under room temperature. Observations were taken using stereobinocular microscope.

2.2 Root Imprinting

The roots were washed with tap water for 5 min to remove adhering soil particles. They were surface sterilized by dipping in 75% ethanol for 2 min and then rinsing three times with sterile water, then placed in a 5% Sodium hypochlorite solution and shaking was performed for 5 min, followed by rinsing with 3 times in sterilized distilled water. After careful drying, the surface sterilized roots were cut into 1 cm sections and transferred to the *Trichoderma* specific medium (TSM) with four per thousand penicillin streptomycin (100X) to isolate the endophytic fungi. Roots were placed in petri plates, and the plates were maintained at 28ºC for incubation (Dole, 2023).

Chart 1. Media used in present study and its composition

TSM (Trichoderma Specific Media)

$MgSO_4.7H2O$	0.2g
KH_2PO_4	1.5g
KCl	0.14g
NH_4NO_3	1g
Glucose	3g
Agar	20g
Rose Bengal	0.15g
Distilled water	1 litre

3. RESULTS AND DISCUSSION

The seeds of wheat were treated with *Trichoderma harzianum* isolates simultaneously

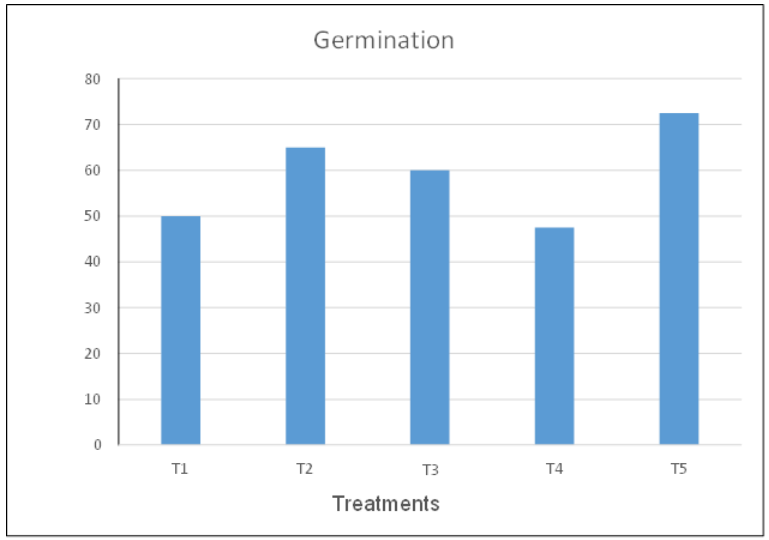

Fig. 1. Germination Percentage of wheat seeds by *Trichoderma harzianum* isolates bio-priming

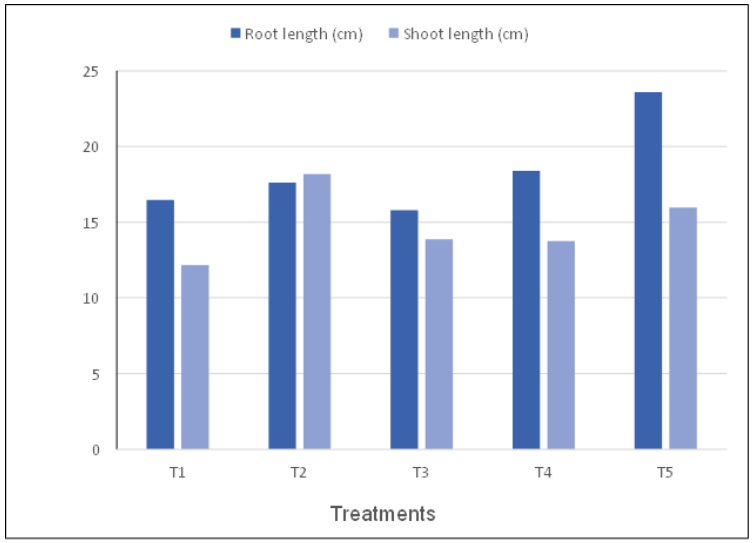

Fig. 2. Root-shoot length of wheat seedling by bio-priming of *Trichoderma harzianum* isolates

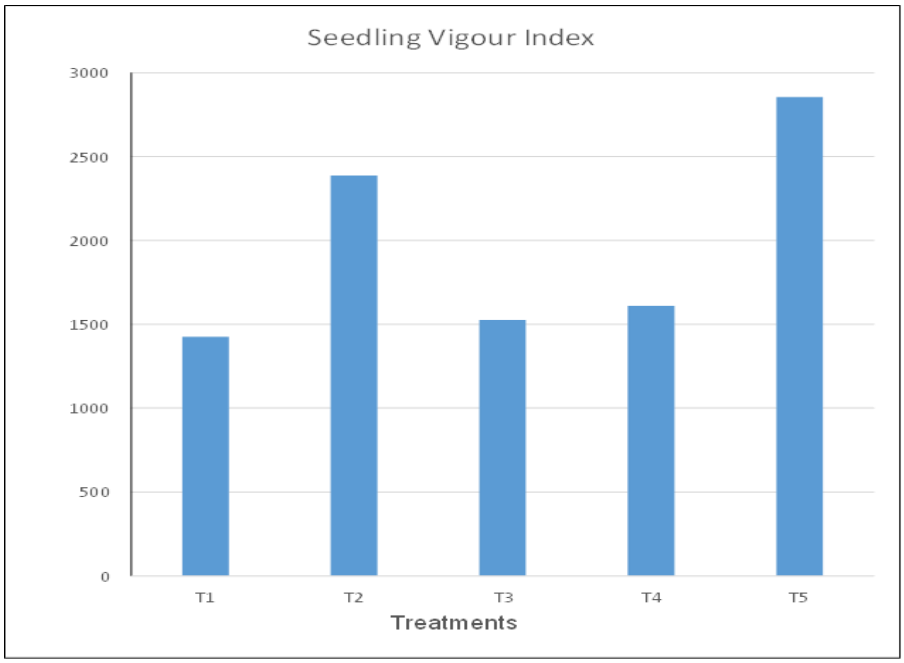

Fig. 3. Seedling Vigour Index of wheat seedling by bio-priming of *Trichoderma harzianum* isolates

with untreated seeds. The outcomes are shown under

3.1 Bio-Priming of Wheat with Isolates of *Trichoderma harzianum*

The data revealed in Table.1 and depicted in Figs.1,2,3,4 represent that *Trichoderma harzianum* isolates enhance the root, shoot elongation. Highest root enlongation was shown by T_5 (23.6 cm) followed by T_4 (18.4 cm), T_2 (17.62 cm), T_1 (16.47 cm), T_3 (15.8 cm). Similarly, highest shoot elongation was shown by T_2 (18.17 cm), T_5 (15.97 cm), T_3 (13.87 cm), T_4 (13.75 cm), T_1 (12.17 cm) whereas highest germination and also vigour index showed by T_5 (72.5% and 2854.5) followed by T_2 (65% and 2387.25), T_3 (60% and 1526.25), T_4 (47.5% and 1609.5). Among all isolates of *Trichoderma* T_1 (50% and 1425) showed least germination and seedling vigour index. The result below agrees with (Rawat et al., 2011) alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum L*.) by seed biopriming with salinity tolerant isolates of *Trichoderma harzianum*. Plants obtained from bioprimed seeds showed higher shoot and root length. These results are in agreement with those of other workers (Howell, 2003; Benitez et al., 2004), who reported that *Trichoderma* strains produce plant growth hormones like cytokinin-like molecules, e.g. zeatin and gibberellin GA3 or GA3-related. It had been concluded by their experimentation that seed bioprimed with *T. harzianum* isolate Th- 14 increased the ability of germination by (1.87%), root length (1.61 cm) and shoot length (1.45 cm) as compared with the control.

3.2 Colonization of *Trichoderma harzianum* Isolates in Soil

Colonization permits the bioprotectant to take possession of the substrate before either competitive microflora or plant- pathogenic fungi. Solid matrix priming (SMP) is a seed-treatment process (Harman & Taylor, 1988) in this process, seeds are first coated with the bioprotectant in a slurry procedure and immediately transferred to an organic matrix (coal or a lignaceous shale). At the physiological level, the positive effects of priming are due to specific metabolic changes induced in the seed when water up-take starts (Dell'Aquila & Bewley, 1989). As a consequence of rehydration, main cellular processes are triggered. Pre-germinative metabolism, i.e., transition from the quiescent state of dry seeds to the active proliferating state of germinating seeds/seedlings (Wei et al., 2013). We speculate that similar processes at physiological level of the introduced fungal spores (*Trichoderma harzianum* isolates) might also get initiated with the rehydration of quiescent state of dry seeds.

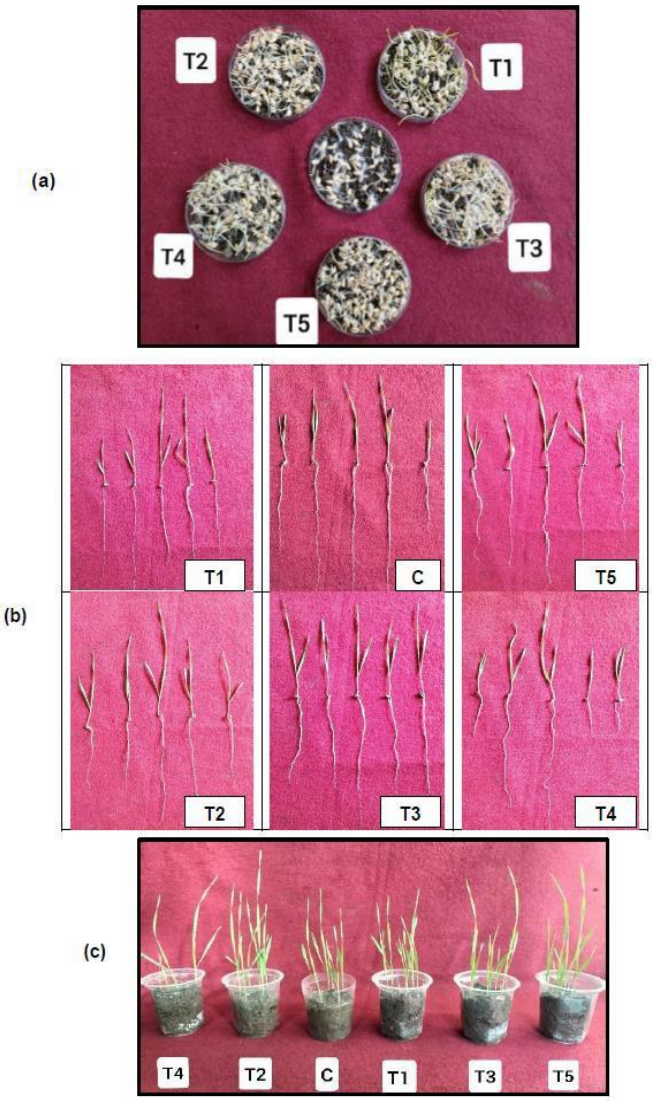

Fig. 4. (a) represents germination of wheat bioprimed by different isolates of *T. harzianum*, (b) represents root- shoot length of wheat, (c) represents pots of wheat bioprimed by different isolates of *T. harzianum*

Table 1. Effect of *Trichoderma harzianum* isolates on seedling vigour by biopriming of wheat seeds

Treatment No.	Germination of seed out of 10 seeds	Germination (%)	Root Length (cm)	Shoot length (cm)	SVI
T_1	5.0	50	16.47	12.17	1425
T_2	6.5	65	17.62	18.17	2387.25
T_3	6.0	60	15.80	13.87	1526.25
T_4	4.75	47.50	18.40	13.75	1609.50
T_5	7.25	72.50	23.60	15.97	2854.50
T_6 - Control	4.5	45	16.47	12.17	1425
F test	Sig	-	Sig	Sig	Sig
SE (±m)	0.49	-	2.56	1.81	326.84
CD (P = 0.05)	1.46	-	7.62	5.38	971.10

Table 2. Mycelial growth of *Trichoderma harzianum* isolates on TSM by root imprinting

Trichoderma harzianum isolates	Appearance
T_1	Dark greenish sporulation with white mycelium web outside which covers 7.2 cm diameter.
T_2	Pale greenish sporulation was observed which cover 4.73 cm of diameter.
T_3	White web like mycelial network and no sporulation was observed that cover 4.36 cm diameter.
T_4	No mycelial web and sporulation were observed.
T_5	Dark greenish sporulation in the form of layers was observed which covers 4.15 cm diameter.

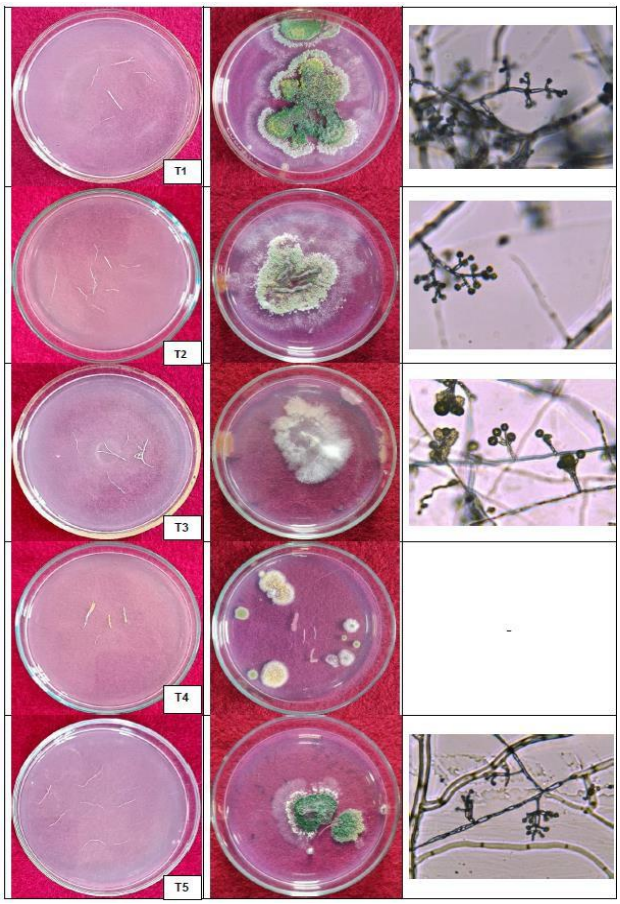

Fig. 5. Imprinting of wheat roots bioprimed by different isolates of *T. harzianum* and philiades observed in compound microscope (100X magnification)

during the germination process; the spore generally swells and forms a germ-tube and eventually a mature thallus. That as a result of pre-germinative metabolism the fungal spores (introduced on the surface of the wheat seeds), germinates and develops into a web of mycelium and seed surface colonization initiates. Table 2 and Fig. 5 determines the colonization in roots due to *Trichoderma* isolates they are imprinted. The data revealed in Fig. 6 represents that germination and colonization of *T. harzianum* isolates on the soil just near the bio-primed seeds. The roots are cutted into small bits of 1 Germination is transformation of a relatively inactive spore to the highly active vegetative thallus. This process is characterized by the absorption of water and by increases in respiration, changes in enzymic activity (Gottlieb & Caltrider, 1963) and biosynthesis of many cell components. Morphological changes also occur

Fig. 6. Germination and Colonization of *T. harzianum* isolates on the soil just near the bio-primed seeds

cm size and placed on TSM (*Trichoderma* Selective Media) and observations were taken after 3-4 days. The above description is similar with (Dole, 2023) as sampled roots of the plant derived by the seed treatment with *Trichoderma virens* BARC G2 mutant from several spots. All the sampled were surface sterilized and were placed in TSM media. Growth of *T. virens* BARC G2 mutant was observed originating from the surface sterilized roots. It was observed significant differences in the root growth (root volume/ root length, secondary roots etc) in rice and wheat plants derived after seed treatment with *Trichoderma virens* BARC G2 mutant (Hornby, 1990).

4. CONCLUSION

The present study evident that few isolates of *T. harzianum* was significantly effective over the other isolates. Among all the treatments, seed biopriming in wheat crop, the best treatment having highest SVI is Thdept (T_5) followed by T_2 (BTH). When the roots of wheat were placed on TSM, the morphological parameters were shown by all the isolates except T_4 (DAG) i.e. mycelial growth rate, colony appearance, shape of conidia and conidiophore and branching pattern of phialides. The present research, offers a new approach to alleviate the growth of seedling effectively through seed biopriming with *Trichoderma* isolates. It could be concluded that seed biopriming with *T. harzianum* isolate Thdept (T_5) increased the ability of wheat to grow successfully.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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