



Original Research Article

Seed bio-priming with *Trichoderma* spp. enhances germination and seedling vigour in *Oroxylum indicum*

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Abstract

Oroxylum indicum is an important medicinal plant. Due to its extensive use in many ayurvedic formulations, it is in high demand. Its natural population is declining due to over-exploitation and habitat destruction. The plant propagates mainly through seeds; however, low seed viability and poor germination rates pose serious obstacles to its regeneration. Additionally, fungal infections cause seed degradation that hinders effective multiplication. *Trichoderma* is well known for its ability to both control harmful fungi and stimulate plant development. This study used seven isolates of *Trichoderma* spp., including four isolates of *T. harzianum* and three isolates of *T. viride*, to investigate the effectiveness of seed bio-priming. Germination parameters and seedling growth indices were assessed, and the data were statistically analysed using ANOVA and Tukey HSD tests. The results demonstrated significant improvements in germination percentage, enhanced seed vigour index and increased seedling growth in all the bio-primed seeds compared to the control. Among all the isolates, *T. harzianum* isolate (ST1) consistently showed superior performance. The results affirm the potential of *Trichoderma*-based seed bio-priming as a suitable, eco-friendly and sustainable approach for large-scale propagation and conservation of *O. indicum*.

Keywords: Germination, *Oroxylum indicum*, Seed bio-priming, Seedling vigour, *Trichoderma*.

Introduction

Oroxylum indicum (L.) Benth. Ex-Kurz is a well-known medicinal plant species, commonly known as Indian trumpet tree or Shyonaka. Its bark, seeds, and roots possess medicinal values and are being used in various ayurvedic formulations. However, destructive and unsustainable harvesting, habitat loss, coupled with low seed germination rates, are leading to the rapid decline of this species in nature, making it endangered and possibly facing extinction soon (Samatha and Swamy, 2020; Pandey et al., 2018). Propagation through seeds is recommended as the most appropriate method for mass cultivation of *O. indicum* (Shankar and Tripathi, 2015). However, the light-weight seeds naturally dispersed and do not establish well in nature, it exhibits poor germination and are susceptible to fungal pathogens. It has also been reported that the seeds are infected with pathogenic fungi, rendering seeds prone to microbial deterioration (Pande and Gupta, 2011). The loss of seed viability due to infections by the fungal pathogens

such as *Aspergillus* spp. and *Penicillium* spp. is reported. *Fusarium solani* has been reported to cause seed decay and seed abortion (Mehrotra, 1990). Thus, it is important to implement eco-friendly and efficient methods for its propagation. The application of sustainable methods is essential to safeguard the long-term existence and accessibility of this valuable medicinal plant (Kumar et al., 2021).

Several studies have looked at the functions of plant growth-promoting microorganisms (PGPM), as their capacity to produce phytohormones that improve the absorption of vital nutrients, helping in plant development (Chen et al., 2019). Bio-inoculants are a promising tool for improving plant growth in an environmentally friendly manner (Raja et al., 2006). In rhizosphere soils, *Trichoderma* spp. is an important genus of plant growth-promoting fungi (PGPF) that are essential for protecting plants against soil-borne diseases and stimulating general plant development (Asghar et al., 2024). Interest in *Trichoderma*

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species is gaining popularity as they have been widely studied and acknowledged in the literature for their dual role in managing plant diseases and promoting plant growth (de Sousa et al., 2021).

Trichoderma spp. protects seeds from pathogens while inducing germination by producing principal biochemical compounds that facilitate a better microenvironment for germination and growth (Windham et al., 1986). For instance, seeds treated with *T. harzianum* showed maximum germination and possessed better root length along with increased seedling biomass, thereby enhancing production in the final crop stage (Pooja et al., 2003). Such traits make *Trichoderma* a highly useful bioinoculant and biocontrol agent for enhanced plant propagation. Given the limited research on this aspect of *O. indicum*, the present study was carried out on bioprimering of *O. indicum* seeds with *Trichoderma* to see its efficacy in overcoming the difficulties in seed germination and enhancing seedling growth.

Materials and Methods

Mature seed pods and rhizospheric soil of *O. indicum* were collected during the fruiting season (January 2022 to March 2022) from natural stands across Uttarakhand, Punjab and Haryana. Samples were processed at the Forest Pathology Laboratory, Forest Research Institute, Dehradun. *Trichoderma* spp. were isolated using the serial dilution method on potato dextrose agar (PDA) (Dhingra and Sinclair, 1995) and identified through morphological and microscopical characterizations (Rifai, 1969; Samuels, 1996).

Dual culture assays *in-vitro* were used to evaluate antagonism against various seed-associated pathogens of *O. indicum*. Seven promising isolates (four *T. harzianum*: ST1, ST2, ST3, ST4 and three *T. viride*: ST5, ST6, ST7), with high colony inhibition percentage, were selected. Talc-based bio-formulations of all selected *Trichoderma* isolates were prepared following the method of Jeyarajan & Nakkeeran (2000) using sterilized soapstone powder mixed with conidial suspension to achieve 2×10^7 cfu/g, with 1% CMC as an adhesive.

The experiment was conducted in May 2022. Seeds were surface-sterilized and bio-primed with the respective *Trichoderma* isolates formulations before sowing in sterilized nursery potting mixture (soil: sand: FYM, 1:1:1) with untreated seeds (control group). Watering was done daily to maintain adequate moisture.

Seed germination testing was conducted according to ISTA (2022). Germination parameters, including germination percentage (GP), germination speed index (GSI), mean germination time (MGT), mean daily germination (MDG), peak value (PV) and germination value (GV) were recorded as per Czabator (1962). Seed vigour index (SVI) was calculated following Abdul-Baki and Anderson (1973). Seedling growth traits (shoot

height, root length, total seedling length and collar diameter) were measured at 45 days.

The experiment followed a completely randomized design (CRD) with seven treatments (ST1–ST7) and a control, each with four replications. Data were analysed using one-way ANOVA followed by Tukey's HSD in R (v4.4.3) at $p < 0.05$.

Results and Discussion

The results of this study showed that seed bio-priming treatments with *Trichoderma* significantly improved seed germination and seedling development in *O. indicum*. Germination began on the 5th day after the experiment was set up in the nursery. Germination percentage was significantly higher across the treatments compared to the control. ST1 recorded the highest germination percentage (95.83%). ST4, ST6, ST3, ST7, and ST2 also performed well, showing high germination percentages comparable to ST1, whereas the control exhibited the lowest germination percentage (50%).

The germination speed index (GSI) also showed significant improvement across all treatments. The highest GSI was observed in ST1 (1.4), compared to the control (0.58), emphasizing the positive effect of seed bio-priming in accelerating germination speed. Mean germination time (MGT) displayed a statistically significant reduction in treated seeds, with ST1 showing the lowest MGT (8.88 days), indicating faster emergence. Similar trends were observed for mean daily germination (MDG) and germination value (GV), both of which were significantly higher in treated groups, particularly in ST1 and ST3, compared to the control. The peak value (PV) of germination was elevated in treatment ST1 (0.93), highlighting a higher proportion of seeds germinating within a shorter timeframe.

The notable enhancement in germination percentage and other germination parameters in treated seeds suggests that *Trichoderma* may hasten metabolic activity in the seeds. These findings are consistent with previous studies on other species treated with *Trichoderma*. According to earlier reports, *Trichoderma* bio-priming enhanced seed performance and seedling growth in *T. cacao* by activating crucial metabolic processes (de Sousa et al., 2021).

In terms of seedling development, bio-primed treatments significantly improved shoot height and root length. ST1 and ST3 exhibited superior seedling length (18.6 cm), and the longest root length was observed in ST1 (8.07 cm), compared to the control, which showed a total seedling length of 8.32 cm. Collar diameter also varied significantly among all the treatments, with ST1 showing the maximum diameter (3.62 mm). Significant differences were also observed in seed vigour index (SVI), with ST1 recording the highest SVI (1771.67), followed by ST3 (1558.12) and ST6 (1500.83). The control group exhibited stunted growth, with the lowest SVI (416.46).

This result is supported by evidence that interaction with *Trichoderma* promotes cell division, expansion, and differentiation, positively impacting root growth, encouraging root branching, and enhancing overall plant development (Contreras-Cornejo et al., 2022). Zheng and Shetty (2000) observed that *Trichoderma* spp. triggered the synthesis of phenolic compounds during seed germination, which promoted seed vigour. Similarly, Cai et al. (2013) noted that a secondary metabolite called harzianolide, synthesized by *Trichoderma* spp., positively influenced early plant development by promoting root elongation.

Seeds bio-primed with *Trichoderma* spp. developed a healthier and more elongated root system, which likely contributed to improved seedling development. This observation is corroborated by Singh et al. (2020), who reported that increased root length enhances water and nutrient absorption, provides stronger anchorage, and

aids successful seedling establishment. These findings suggest that bio-priming with *Trichoderma* significantly enhances seed germination and seedling growth in *O. indicum*. The observed improvements may be attributed to the synergistic effects of pathogen suppression, hormonal stimulation, and enhanced nutrient uptake facilitated by *Trichoderma* (Contreras-Cornejo et al., 2024).

Overall, bio-priming with all *Trichoderma* isolates substantially improved germination and seedling growth in *O. indicum* compared to the control. The daily seed germination percentages for different treatments are shown in Figure 1. Among the treatments, ST1 (*T. harzianum*) consistently performed superior across nearly all parameters, making it the most effective treatment, followed by ST3 (*T. harzianum*) and ST6 (*T. viride*) (Figure 2). Key metrics related to seed germination are summarized in Table 1, and seedling growth metrics in Table 2.

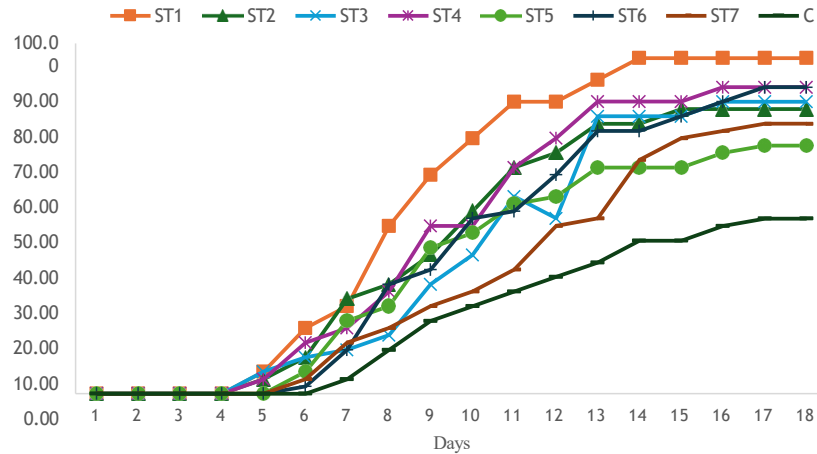


Figure 1: Daily seed germination percentage of different seed treatments ST1, ST2, ST3, ST4 (*T. harzianum*), ST5, ST6, ST7 (*T. viride*), and control (C)

Table 1: Effect of *Trichoderma* treatments on *O. indicum* seed germination

Treatments	GP (%)	GSI	PV	MGT	MDG	GV
ST1	95.83 ± 4.17 ^a	1.4 ± 0.05 ^a	0.93 ± 0.02 ^a	8.88 ± 0.14 ^d	0.64 ± 0.03 ^a	0.6 ± 0.03 ^a
ST2	81.25 ± 6.25 ^a	1.12 ± 0.07 ^{ab}	0.75 ± 0.03 ^{ab}	9.4 ± 0.15 ^{cd}	0.54 ± 0.04 ^a	0.41 ± 0.05 ^{ab}
ST3	83.33 ± 7.61 ^a	1.08 ± 0.08 ^{ab}	0.73 ± 0.07 ^b	10.17 ± 0.18 ^{abcd}	0.56 ± 0.05 ^a	0.42 ± 0.07 ^{ab}
ST4	87.5 ± 7.22 ^a	1.18 ± 0.13 ^{ab}	0.78 ± 0.04 ^{ab}	9.75 ± 0.23 ^{bcd}	0.58 ± 0.05 ^a	0.46 ± 0.06 ^{ab}
ST5	70.83 ± 2.41 ^{ab}	0.94 ± 0.04 ^b	0.66 ± 0.03 ^b	9.69 ± 0.76 ^{bcd}	0.47 ± 0.02 ^{ab}	0.31 ± 0.01 ^{bc}
ST6	87.5 ± 2.41 ^a	1.07 ± 0.02 ^{ab}	0.7 ± 0 ^b	10.61 ± 0.14 ^{abc}	0.58 ± 0.02 ^a	0.41 ± 0.01 ^{ab}
ST7	77.08 ± 7.89 ^a	0.89 ± 0.08 ^{bc}	0.61 ± 0.05 ^b	11.27 ± 0.16 ^a	0.51 ± 0.05 ^a	0.32 ± 0.06 ^{bc}
Control	50 ± 3.4 ^b	0.58 ± 0.03 ^c	0.39 ± 0.03 ^c	11.16 ± 0.19 ^{ab}	0.33 ± 0.02 ^b	0.13 ± 0.02 ^c
F- value	6.187	10.949	16.226	7.319	6.187	8.491
p-value	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
CV (%)	6.57	6.22	5.31	2.45	6.57	11.03

* Germination Percentage (GP), Germination Speed Index (GSI), Peak Value (PV), Mean Germination Time (MGT), Mean Daily Germination (MDG), and Germination Value (GV)

Table 2: Effect of *Trichoderma* treatments on *O. indicum* seedling growth

Treatments	Shoot height (cm)	Root length (cm)	Total seedling length (cm)	Collar diameter (mm)	Seed vigour index
ST1	10.35 ± 0.55 ^a	8.22 ± 0.44 ^a	18.58 ± 0.84 ^a	3.62 ± 0.1 ^a	1771.67 ± 52.78 ^a
ST2	8.62 ± 0.5 ^{ab}	5.25 ± 0.46 ^{bc}	13.88 ± 0.87 ^{bc}	2.67 ± 0.25 ^{bc}	1143.12 ± 166.5 ^{bc}
ST3	11.07 ± 1.14 ^a	7.53 ± 0.28 ^a	18.6 ± 0.87 ^a	2.3 ± 0.21 ^{cd}	1558.12 ± 185.4 ^{ab}
ST4	10.95 ± 0.8 ^a	4.18 ± 0.85 ^c	15.12 ± 1.64 ^{abc}	3.34 ± 0.04 ^{ab}	1320.62 ± 171.9 ^{abc}
ST5	7.15 ± 0.48 ^{bc}	4.35 ± 0.15 ^c	11.5 ± 0.56 ^{cd}	3.28 ± 0.15 ^{ab}	817.29 ± 62.29 ^{cd}
ST6	10.62 ± 0.39 ^a	6.53 ± 0.32 ^{ab}	17.15 ± 0.09 ^{ab}	2.52 ± 0.31 ^{bc}	1500.83 ± 44.31 ^{ab}
ST7	6.72 ± 0.12 ^{bc}	4.75 ± 0.1 ^{bc}	11.47 ± 0.19 ^{cd}	2 ± 0.09 ^{cd}	881.46 ± 80.24 ^{cd}
Control	4.42 ± 0.55 ^c	3.9 ± 0.12 ^c	8.32 ± 0.53 ^d	1.65 ± 0.1 ^d	416.46 ± 42.13 ^d
F- value	14.883	15.8	19.978	15.407	14.963
p-value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
CV (%)	6.68	6.47	4.97	6.18	9.03

**Figure 2:** Morphological variation in 45-day-old *O. indicum* seedlings under treatments ST1, ST3 (*T. harzianum*), ST6 (*T. viride*), and control (C)

Conclusion

Seed bio-priming with *Trichoderma* spp. significantly enhanced germination, seedling vigour, and root development in *O. indicum*, confirming its strong potential as a reliable plant growth promoter. Among the tested isolates, *T. harzianum* (ST1) consistently outperformed others, highlighting its value as a sustainable and eco-friendly approach for enhancing the propagation and conservation of this medicinally important species. These findings provide a promising basis for integrating *Trichoderma*-based bio-priming into propagation and conservation programs, with future field evaluations expected to further standardize and expand its practical applications. To further strengthen these findings, incorporating biochemical profiling is recommended, as it may help elucidate the underlying mechanisms through which *Trichoderma* enhances seedling vigour.

Author Contributions

S U: Conceptualization, Investigation, Methodology, Writing-original draft, Formal Analysis, Data interpretation, Writing- Reviewing and Editing; **A N S:** Conceptualization, Visualization, Investigation, Methodology, Supervision, Validation, Writing-

Reviewing and Editing; **A P:** Conceptualization, Visualization, Methodology

Conflict of Interest

The authors declare no conflict of interest.

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