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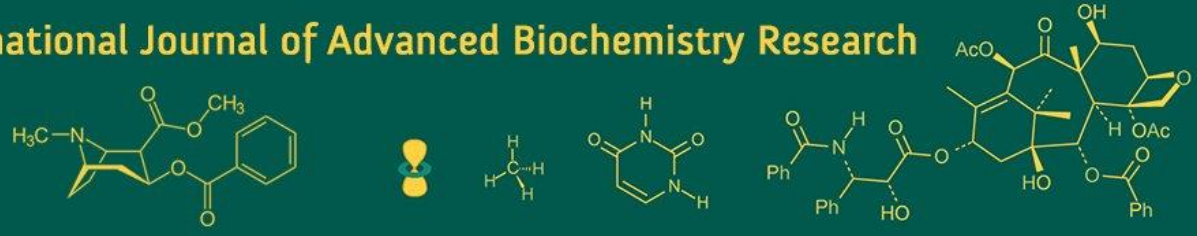

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Compatibility analysis of *Trichoderma koningii* with selected systemic fungicides under laboratory conditions

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Abstract
The compatibility of *Trichoderma koningii*, a widely used biocontrol agent, with selected systemic fungicides was evaluated to support integrated disease management strategies. The study was conducted during 2024-2025 at the Department of Plant Pathology, Dr. Sharadchandra Pawar College of Agriculture, Baramati, following a Completely Randomized Design (CRD). Seven systemic fungicides—Fosetyl-Al 80% WP, Tebuconazole 25.9% EC, Fluxapyroxad 333 g/L FS, Difenoconazole 25% EC, Kresoxim-methyl 44.3% SC, Propiconazole 25% EC, and Hexaconazole 5% EC—were tested using the poisoned food technique at both full and half of the recommended doses. The results demonstrated that Fluxapyroxad and Kresoxim-methyl were highly compatible with *T. koningii*, allowing over 90% radial growth even at the full recommended dose. Fosetyl-Al showed moderate compatibility, with 63.33% and 68.89% growth inhibition at half and full doses, respectively. In contrast, Tebuconazole, Difenoconazole, Propiconazole and Hexaconazole exhibited strong inhibitory effects, with 90-94.44% suppression, indicating incompatibility with the biocontrol fungus. The results indicate that *T. koningii* can be successfully used in combination with compatible fungicides like Fluxapyroxad and Kresoxim-methyl to improve crop protection, whereas fungicides that strongly inhibit its growth should be used with caution or avoided. This study highlights the need to evaluate fungicide compatibility to maximize the effectiveness of biocontrol agents and promote sustainable disease management.

Keywords: *Trichoderma koningii*, biological control, compatibility, fungicides, integrated disease management

1. Introduction
Biological control has become a cornerstone of sustainable agriculture, providing an environmentally safe alternative to synthetic pesticides. Among beneficial microorganisms, fungi of the genus *Trichoderma* are particularly notable due to their diverse antagonistic strategies, including mycoparasitism, nutrient and space competition, production of antimicrobial compounds and induction of plant defense mechanisms (Mukherjee *et al.*, 2022; Contreras-Cornejo *et al.*, 2016) [24, 7]. These filamentous fungi are widely distributed across agricultural soils, decomposing plant residues, forest litter and organic waste environments. Their ecological success is attributed to rapid growth, efficient substrate utilization, opportunistic behavior and prolific production of spores and bioactive metabolites, enabling effective rhizosphere colonization and pathogen suppression (Alexander, 1961; Singh *et al.*, 2009) [2, 35]. Research over the past decades has demonstrated that *Trichoderma* not only suppresses pathogens but also enhances plant growth, nutrient uptake and soil health (Harman *et al.*, 2004; Regragui & Lahlou, 2012) [15, 28]. Different species exhibit multiple antagonistic mechanisms, including enzymatic degradation of pathogen cell walls, antibiosis, nutrient competition and environmental modification, contributing to improved plant physiology and the establishment of a favorable rhizosphere microbial community.
Among *Trichoderma* species, *Trichoderma koningii* is recognized as an effective biocontrol agent for managing fungal diseases and is often suggested as an alternative to chemical fungicides (Samuels *et al.*, 2006; Oyesola *et al.*, 2024). It exerts its biocontrol potential

through mycoparasitism, competition, antibiosis via secondary metabolites and induction of systemic resistance in plants. In addition, *T. koningii* promotes plant growth and nutrient availability by solubilizing phosphorus and iron through organic acid and siderophore production **(**Gutiérrez-Moreno *et al.,* 2025) [12] and synthesizes antibiotics such as gliotoxin and harzianic acid, which inhibit the growth of soilborne pathogens like *Gaeumannomyces graminis* var. *tritici* (Simon *et al.,* 1988) [33].

Despite promising results under laboratory and greenhouse conditions, *Trichoderma*-based biocontrol agents often fail to achieve consistent efficacy in field settings. A major factor is the incompatibility between fungal bioagents and commonly applied synthetic fungicides. For successful integration into disease management programs, a biocontrol agent must remain physiologically active and competitive in the presence of these chemicals (Desai *et al.,* 2002; Khandelwal *et al.,* 2012) [8, 17]. Fungicides vary in their toxicity depending on their active ingredients, formulations, dosages and the sensitivity of specific *Trichoderma* isolates (Abeysinghe *et al.,* 2019) [1]. Therefore, assessing fungicide compatibility for individual isolates is critical before recommending them for field use (Mukherjee *et al.,* 2020) [23].

While compatibility studies are available for species such as *T. harzianum* and *T. asperellum*, there is limited information on the response of *T. koningii* to systemic fungicides. Given its strong antagonistic activity, production of cell-wall-degrading enzymes and antifungal secondary metabolites, *T. koningii* is a promising candidate for managing soil-borne and foliar pathogens (Zhang *et al.,* 2018) [39]. However, its practical field application requires a careful evaluation of fungicide interactions to avoid potential inhibition.

Therefore, the present study, entitled Compatibility Analysis of *Trichoderma koningii* with Selected Systemic Fungicides Under Laboratory Conditions was undertaken to evaluate the tolerance and compatibility of *T. koningii* with selected systemic fungicides. The findings are expected to guide the selection of compatible fungicide-bioagent combinations, support integrated disease management strategies and enhance the bioefficacy and commercial use of *T. koningii* under field conditions.

2. Materials and Methods

2.1 Source of *Trichoderma* culture

Rhizospheric soil was collected from various crops at the College of Agriculture, Baramati farm to isolate *Trichoderma koningii*. The isolation of *Trichoderma koningii* was performed using the serial dilution plate technique as described by Johnson and Curl (1972). Soil samples were cultured on Potato Dextrose Agar (PDA) and *Trichoderma* Selective Medium (TSM) to promote fungal growth while minimizing contamination. Emerging fungal colonies were initially screened based on characteristic *Trichoderma* morphology, including rapid growth, colony texture and pigmentation. Species-level identification of *T. koningii* was carried out by examining both macroscopic and microscopic features, focusing on colony morphology, growth patterns and the structure of conidiophores, phialides and conidia, following the taxonomic keys of Samuels *et al.* (2012) [30] and Kubicek *et al.* (2008) [18]. To ensure the purity of cultures, each isolate was purified using the hyphal tip method (Tuite, 1969) [37]. Confirmed *T. koningii* isolates were preserved throughout the study by periodic sub-culturing on PDA and TSM slants under aseptic conditions, ensuring their viability, morphological stability and consistent biocontrol potential.

2.2 Fungicides

Seven systemic fungicides—Fosetyl-Al 80% WP, Tebuconazole 25.9% EC, Fluxapyroxad 333 g/L FS, Difenoconazole 25% EC, Kresoxim-methyl 44.3% SC, Propiconazole 25% EC and Hexaconazole 5% EC—were procured from the Plant Pathology Department, Dr. Sharadchandra Pawar College of Agriculture, Baramati and used in the study.

2.3 *In vitro* compatibility of *Trichoderma koningii* with fungicides

The compatibility of *Trichoderma koningii* with selected systemic fungicides was evaluated using the Standard Poisoned Food Technique as described by Nene and Thapliyal (1993) [25]. Fungicide concentrations were calculated based on their active ingredient content and incorporated into sterilized, cooled Potato Dextrose Agar (PDA). About 20 ml of the amended medium was poured into 90 mm sterile Petri plates and allowed to solidify. Three replicates were maintained for each fungicide and concentration.

A 5 mm mycelial disc from a 7-day-old actively growing *T. koningii* culture was placed at the center of each plate under aseptic conditions. Plates were incubated at 28 ± 1 °C, while uninoculated PDA plates without fungicide served as controls. Radial mycelial growth was measured once the control plates reached the edge of the Petri dish. Percentage inhibition of growth for each treatment was calculated relative to the control using Vincent's formula (1927) [38].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent growth inhibition

C = Radial growth of fungus in control plate

T = Radial growth of fungus in treated plate

The compatibility categories were determined based on the percentage of mycelial growth inhibition-Saha *et al.* (2023) [29]

Inhibition	Nature of compatibility
0-30%	Highly compatible
31-60%	Moderately compatible
61-90%	Slightly compatible
91-100%	Non-compatible

Experimental Design and Statistical Analyses

The experiment was conducted in Completely Randomized Design (CRD) with 8 treatments and three replications. Technique used for conducting this experiment was Poison Food technique (Nene & Thapliyal, 1993) [25] The data gathered in this study was analyzed by using the appropriate statistical methods as explained by Panse and Sukhatme (1967) [27]. The standard error (SE) and critical difference (CD) at the 5% significance level (P = 0.05) were calculated to compare treatment means.

Results and Discussion

As presented in Table 1, Plate 1 and Figure 1, the compatibility of *Trichoderma koningii* with different systemic fungicides at half of the recommended concentration varied considerably. Fosetyl-Al 80% WP was found to be slightly compatible, exhibiting 63.33% mycelial growth inhibition. Fluxapyroxad 333 g/L FS and Kresoxim-methyl 44.3% SC were highly compatible, causing only 4.81% and 23.89% inhibition, respectively. In contrast, Tebuconazole 25.9% EC, Propiconazole 25% EC, and Hexaconazole 5% EC were completely incompatible, resulting in 94.44% mycelial growth inhibition, statistically similar among them. Difenoconazole 25% EC was also incompatible, showing 90.60% inhibition.

When tested at their recommended concentrations, Fluxapyroxad 333 g/L FS remained highly compatible with 22.22% inhibition, while Kresoxim-methyl 44.3% SC showed moderate compatibility with 33.70% inhibition. Fosetyl-Al 80% WP was slightly compatible (68.89% inhibition) and Difenoconazole 25% EC remained incompatible (90.37% inhibition). Tebuconazole 25.9% EC, Propiconazole 25% EC and Hexaconazole 5% EC continued to be completely incompatible at recommended doses, exhibiting 94.44% inhibition.

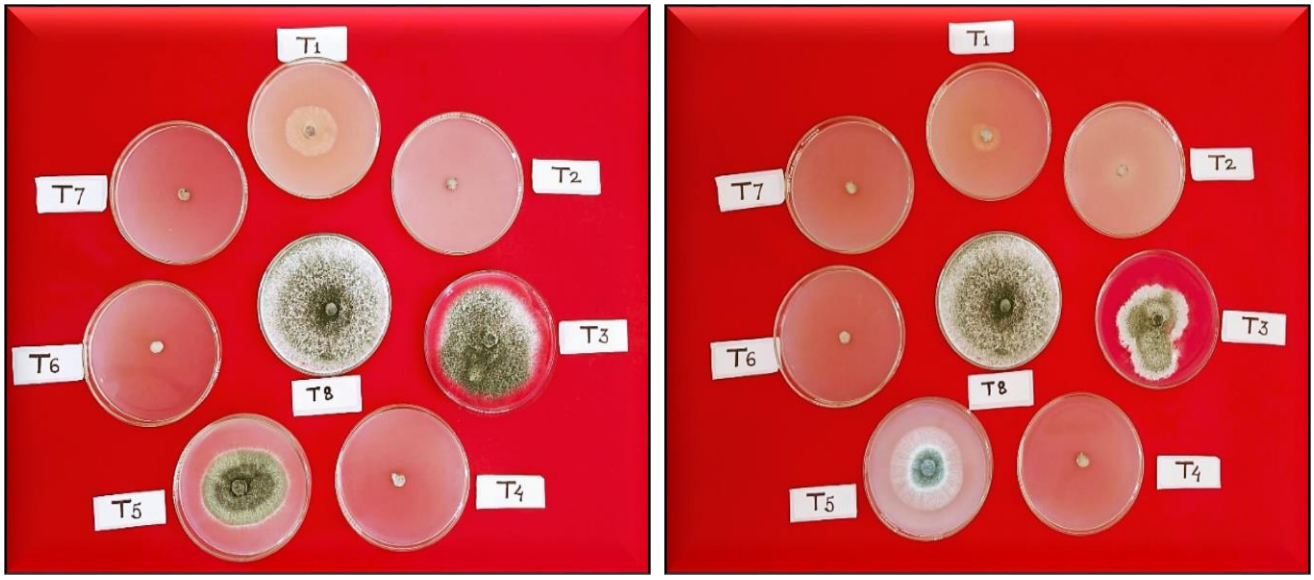
These findings align with previous reports. Haritha (2003) [14] observed the incompatibility of Hexaconazole 5% EC with *Trichoderma* species, which was corroborated by Manandhar *et al.* (2020) [21]. Haque *et al.* (2023) [13] reported non-compatibility of Propiconazole 25% EC with several *Trichoderma* species, while Bagwan (2010) [4] found *T. viride* and *T. harzianum* highly sensitive to Tebuconazole and Propiconazole. Saha *et al.* (2023) [29] identified Kresoxim-methyl 44.3% as safe for *T. asperelloides*, and Dinkwar *et al.* (2023) [9] reported high compatibility of Fluxapyroxad 333 g/L FS with various *Trichoderma* isolates. Arain *et al.* (2022) [3] found no growth of *T. harzianum* with Difenoconazole 25% EC and Propiconazole 25% EC. Similarly, Bharadwaz *et al.* (2023) [6] reported total incompatibility of Tebuconazole, Propiconazole, and Hexaconazole with *T. viride*. Comparable results have also been documented by Sharma *et al.* (2016) [32], Sonavane and Venkataravanappa (2017) [36], Dutta *et al.* (2017) [10], Gunda *et al.* (2018) [11], Meena Ravindra *et al.* (2018) [22], Kumar *et al.* (2019) [19], Maheshwary *et al.* (2020) [20], Singh *et al.* (2021) [34] and Bai *et al.* (2022) [5].

Tables

Table 1: *In-vitro* effect of systemic fungicides on the growth of *Trichoderma koningii*.

Tr. No.	Treatments	Radial mycelial growth (mm)*		% Growth Inhibition	
		Half of the recommended concentration	Recommended concentration	Half of the recommended concentration	Recommended concentration
T_1	Fosetyl Al 80% WP	33.00	28.00	63.33	68.89
T_2	Tebuconazole 25.9% EC	5.00	5.00	94.44	94.44
T_3	Fluxapyroxad 333g/I FS	85.67	70.00	4.81	22.22
T_4	Difenaconazole 25% EC	11.83	8.50	90.60	90.37
T_5	Kresoxim methyl 44.3% SC	68.50	59.67	23.89	33.70
T_6	Propiconazole 25% EC	5.00	5.00	94.44	94.44
T_7	Hexaconazole 5% EC	5.00	5.00	94. 44	94.44
T_8	Control	90.00	90.00	00.00	00.00
	S.E.(m) ±	0.61	0.64	-	-
	C.D. (5%)	1.85	1.94	-	-

*Mean of three replications



Half of the recommended concentration Recommended concentration

Plate 1: Compatibility assay of *Trichoderma koningii* showing differential mycelial inhibition under varying fungicide treatments

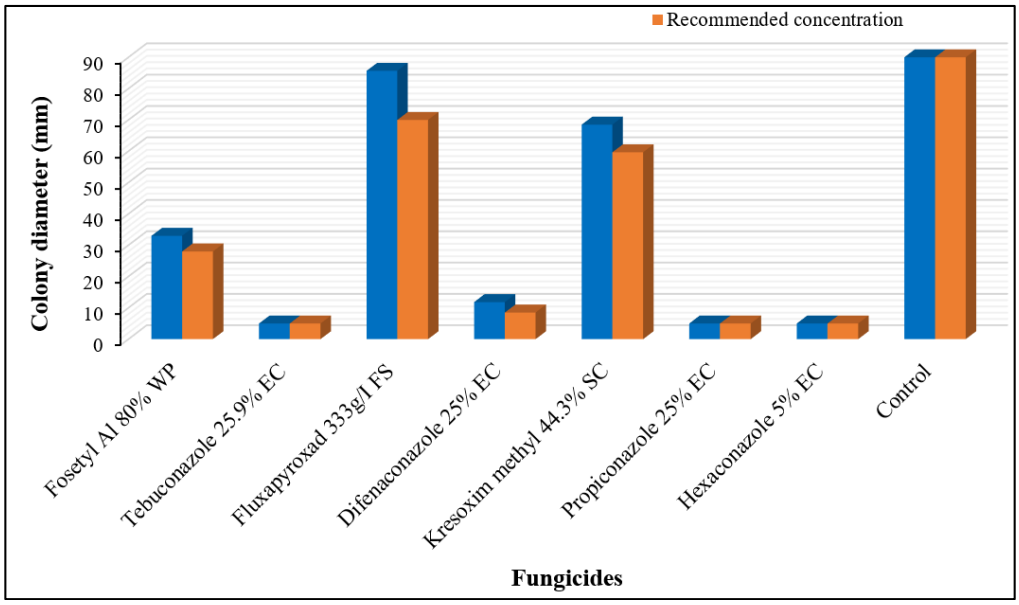


Fig 1: Comparative colony growth of *T. koningii* Under Different Fungicidal Treatments

Conclusion

Compatibility analysis of *Trichoderma koningii* with selected systemic fungicides under laboratory conditions revealed considerable variation in fungal tolerance. At half of the recommended concentrations, Fluxapyroxad 333 g/L FS and Kresoxim-methyl 44.3% SC were highly compatible with *T. koningii*, showing minimal inhibition of mycelial growth i.e., 4.81 and 23.89% respectively. When applied at the recommended concentrations, Kresoxim-methyl 44.3% SC exhibited moderate compatibility, indicating a slight increase in inhibitory effect (33.70%) with higher doses. In contrast, Fosetyl-Al 80% WP was slightly compatible at both half and full recommended concentrations with 63.33 and 68.89 % growth inhibition, demonstrating moderate suppression of fungal growth while still allowing *T. koningii* to survive and retain its activity. These results suggest that the degree of compatibility of systemic fungicides with *T. koningii* is both fungicide-specific and concentration-dependent, highlighting the importance of selecting appropriate fungicides when integrating chemical and biological disease management strategies.

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