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**Research Article**

# *In vitro* assessment of *Trichoderma asperellum* interaction with combination fungicides

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**Abstract**

This study was carried out to assess how well *Trichoderma asperellum*, a widely used biological control agent, performs in the presence of selected combination group fungicides. The experiment took place during 2024-2025 at the Department of Plant Pathology, Dr. Sharadchandra Pawar College of Agriculture, Baramati, using a Completely Randomized Design (CRD). Seven combination fungicides— Ridomil Gold (Metalaxyl M 4% + Mancozeb 64% WP), Folio Gold (Metalaxyl M 33% + Chlorothalonil 33.1% SC), Indofil Sprint (Carbendazim 25% + Mancozeb 50% WS), Amistar Top (Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC), Syngenta Gloit (Propiconazole 13.9% + Difenoconazole 13.9% EC), Dhanuka Lustre (Carbendazim 25% + Flusilazole 12.5% SE), Bayer Nativo (Tebuconazole 50% +Trifloxystrobin 25% WG) were tested at both full and half of their recommended doses using the poison food technique. Results showed that Metalaxyl M 4% + Mancozeb 64% WP was highly compatible with 22.22 per cent growth inhibition at half concentration and moderate compatibility with 53.70 per cent growth inhibition at recommended concentration with *T. asperellum,* while the remaining fungicides significantly suppressed the growth of the biocontrol agent with 83.52 to 94.44 per cent growth inhibition. These findings highlight the potential for combining *T. asperellum* with compatible fungicides like Metalaxyl M 4% + Mancozeb 64% WP in integrated disease management programs, while suggesting caution or avoidance for those with inhibitory effects.

**Keywords:** *Trichoderma asperellum*, Biological control, Compatibility, Fungicides, Integrated disease management

## Introduction

*Trichoderma* spp., particularly *Trichoderma asperellum*, is extensively utilized as biological control agents due to their rapid growth, abundant sporulation, strong mycoparasitic ability and capacity to induce plant defenses and promote plant growth. Species of *Trichoderma* aggressively parasitize host fungi (Barnett & Binder, 1973). During mycoparasitism, *Trichoderma* isolates coil around the hyphae of *Rhizoctonia solani*, causing depletion of cellular contents and eventual collapse (Chu & Wu, 1981). These species attach to pathogens such as *Sclerotium rolfsii* and *R. solani* through hyphal coils, hooks or appressoria and upon detachment, lysed areas and penetration points appear on the pathogen's hyphae (Elad *et al.,* 1983). In addition to parasitism, *Trichoderma* employs competitive interactions; for example, application to grape flowers enables rapid colonization of senescing tissues ahead of *Botrytis*, thereby reducing disease incidence in fruit (Harman *et al.,* 1996).

These characteristics position *T. asperellum* as a key agent in integrated disease management (IDM) programs aimed at suppressing soil-borne and certain foliar pathogens while lowering dependence on chemical fungicides. However, its field deployment often occurs within production systems where fungicides remain a routine input, making chemical–biological compatibility an essential consideration for effective and sustainable combined control strategies (Yao *et al.,* 2023). When applied to soil at planting, antagonistic biocontrol agents efficiently manage diseases under low to moderate pathogen pressure, but their performance declines under high disease intensity, requiring supplemental control tactics for adequate suppression. As modern plant protection increasingly favors integrated approaches, biological agents are valued for their reduced non-target and environmental impacts compared with synthetic pesticides (Cook & Baker, 1983). The combined use of chemical fungicides with biocontrol organisms has consequently gained attention for achieving additive or synergistic effects in managing soil-borne diseases (Bheemaraya *et al.,* 2012).

Integrating fungicides with biocontrol agents also supports residue reduction by enabling lower fungicide use. When used together, antagonists and both contact and systemic fungicides can lessen chemical inputs and minimize resistance development. Chemical fungicides perform effectively under environmental conditions unfavorable to biological agents, while established biocontrol organisms provide preventive protection by colonizing wounds or senescing tissues (Harman & Kubicek, 1998). Therefore, assessing *in vitro* compatibility is a critical preliminary step prior to recommending joint applications of *T. asperellum* with fungicides. Such evaluations clarify which fungicides allow the antagonistic fungus to remain active while still contributing protective chemical action (Kumar *et al.,* 2017).

In this study, we examine the compatibility of combination-group fungicides with *T. asperellum* using standard *in vitro* assays and antagonism metrics, with the objective of identifying fungicide–*Trichoderma* combinations that preserve biological efficacy while enabling necessary chemical disease control.

## Material and Methods

### *Source of Trichoderma culture*

Rhizospheric soil was collected from the College of Agriculture, Baramati farm to obtain *Trichoderma asperellum*. The samples were subjected to serial dilution and subsequently cultured on Potato Dextrose Agar (PDA) and *Trichoderma* Selective Medium (TSM) following the protocol of Johnson and Curl (1972). Identification of *T. asperellum* was performed using both cultural traits and microscopic observations, focusing on colony characteristics, growth behaviour and the morphology of mycelia, conidiophores, phialides and conidia as outlined by Harman and Kubicek (2002). To ensure viability and purity, the fungal cultures were regularly sub-cultured and maintained on PDA and TSM slants under sterile conditions throughout the study.

### *Fungicides*

In the present investigation, seven combination fungicides— (Metalaxyl M 4% + Mancozeb 64% WP), (Metalaxyl M 33% + Chlorothalonil 33.1% SC), (Carbendazim 25% + Mancozeb 50% WS), (Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC), (Propiconazole 13.9% + Difenoconazole 13.9% EC), (Carbendazim 25% + Flusilazole 12.5% SE), (Tebuconazole 50% + Trifloxystrobin 25% WG) —were sourced from the Plant Pathology Division, Dr. Sharadchandra Pawar College of Agriculture, Baramati, and utilized for the study.

### *In vitro compatibility of T. asperellum with fungicides*

The compatibility of *T. asperellum* with various combination fungicides was assessed following the Standard Poisoned Food Technique described by Nene and Thapliyal (1993). Required quantities of each fungicide were calculated based on their active ingredient content to obtain the intended concentrations. The fungicides were then mixed separately into sterilized, cooled Potato Dextrose Agar (PDA). Approximately 20 ml of the amended medium was poured aseptically into sterile 90 mm Petri plates and allowed to solidify at room temperature. Each fungicide treatment and concentration was replicated three times. Once the medium had solidified, a 5 mm mycelial disc from a seven-day-old, actively growing culture of *T. asperellum* was placed at the center of each plate using a flame-sterilized cork borer under aseptic conditions. The plates were incubated at 28 ± 1 °C. PDA plates without fungicide, inoculated in the same manner, served as untreated controls for evaluating fungal growth in the absence of chemical inhibition.

Treatment details

Design: Completely Randomized Design (CRD)
Replication: Three
Treatment: Eight
Technique: Poison Food technique (Nene & Thapliyal, 1993)

Observations on the mycelial growth of *Trichoderma asperellum* were taken when the control plates showed fungal growth up to the margin of the Petri dish. Radial mycelial diameter was measured for each fungicide treatment to evaluate growth. The average values from three replications were computed and mycelial inhibition was expressed as a percentage relative to the untreated control. The percentage inhibition was calculated using the formula described by Vincent (1927):

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

Where,
I = Percent growth inhibition
C = Radial growth of fungus in control plate
T = Radial growth of fungus in treated plate

The range for compatibility based on inhibition percentage is given in Table 1 (Saha *et al.,* 2023).

### *Statistical analysis*

The experimental data were analyzed statistically using software designed for a Completely Randomized Design (CRD). Standard error (SE) and critical difference (CD) at the 5% probability level (P=0.05) were computed to compare the treatment means. The analytical procedures followed the guidelines outlined by Panse and Sukhatme (1967).

## Results and Discussion

As shown in Table 2 and Figures1 and 2, among the combination fungicides tested with *T. asperellum* at half of the recommended concentration, fungicide Metalaxyl M 4% + Mancozeb 64% WP resulted highly compatible with *T. asperellum* as its percent mycelial growth inhibition was 22.22 per cent while, fungicides Metalaxyl M 33% + Chlorothalonil 33.1% SC and Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC were found slightly compatible with mycelial growth inhibition of 83.52 and 85.92 per cent. However, the fungicides *viz.,* Carbendazim 25% + Mancozeb 50% WS, Propiconazole 13.9% + Difenoconazole 13.9% EC, Carbendazim 25% + Flusilazole 12.5% SE and Tebuconazole 50% + Trifloxystrobin 25% WG were found completely incompatible by causing complete mycelial growth inhibition *i.e.,* 94.44 per cent of *T. asperellum*.

At recommended concentration of combination fungicides tested with *T. asperellum*, fungicide Metalaxyl M 4% + Mancozeb 64% WP was moderately compatible with 53.70 per cent mycelial growth inhibition and fungicide

**Table 1:** Nature of compatibility based on inhibition percentage

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

**Table 2:** *In Vitro* effect of combination fungicides on *T. asperellum*

| Tr. No. | Treatments | Radial mycelial growth (mm)* | | % Growth inhibition | |
|---|---|---|---|---|---|
| | | Half of the recommended concentration | Recommended concentration | Half of the recommended concentration | Recommended concentration |
| $T_1$ | Metalaxyl M 4% + Mancozeb 64% WP | 70.00 | 41.67 | 22.22 | 53.70 |
| $T_2$ | Metalaxyl M 33% + Chlorothalonil 33.1% SC | 14.83 | 5.00 | 83.52 | 94.44 |
| $T_3$ | Carbendazim 25% + Mancozeb 50% WS | 5.00 | 5.00 | 94.44 | 94.44 |
| $T_4$ | Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC | 12.67 | 8.33 | 85.92 | 90.74 |
| $T_5$ | Propiconazole 13.9% + Difenoconazole 13.9% EC | 5.00 | 5.00 | 94.44 | 94.44 |
| $T_6$ | Carbendazim 25% + Flusilazole 12.5% SE | 5.00 | 5.00 | 94.44 | 94.44 |
| $T_7$ | Tebuconazole 50% + Trifloxystrobin 25% WG | 5.00 | 5.00 | 94.44 | 94.44 |
| $T_8$ | Control | 90.00 | 90.00 | 00.00 | 00.00 |
| | S.E.(m)± | 0.47 | 0.44 | - | - |
| | C.D. (5%) | 1.42 | 1.32 | - | - |

*Mean of three replications

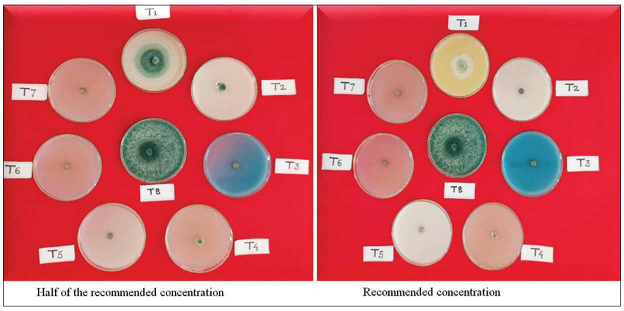


**Figure 1:** *In vitro* assessment of *T. asperellum* with combination fungicides at half of the recommended and recommended concentration

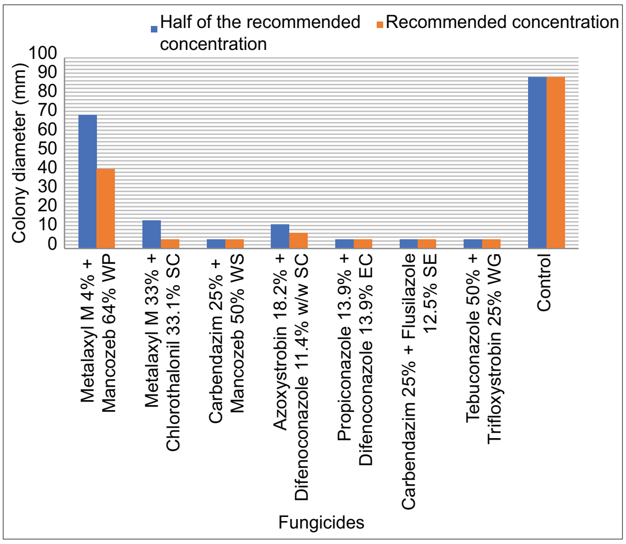


**Figure 2:** Colony growth of *T. asperellum* under different fungicidal treatment

Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC resulted non compatible with 90.74 per cent mycelial growth inhibition. However, fungicides *viz.,* Metalaxyl M 33% + Chlorothalonil 33.1% SC, Carbendazim 25% + Mancozeb 50% WS, Propiconazole 13.9% + Difenoconazole 13.9% EC, Carbendazim 25% + Flusilazole 12.5% SE and Tebuconazole 50% + Trifloxystrobin 25% WG were proved to be completely incompatible by causing maximum mycelial growth inhibition 94.44 per cent of *T. asperellum*. The results showed from the present study are in conformity with Maheshwary *et al.* (2020) were he proved high compatibility of *T. asperellum* with fungicide Metalaxyl M 4% + Mancozeb 64% WP while, fungicides Carbendazim 25% + Mancozeb 50% WS, Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC and Tebuconazole 50% + Trifloxystrobin 25% WG were incompatible with *T. asperellum*. Veena *et al.* (2022) proved that fungicide Metalaxyl M 4% + Mancozeb 64% WP, highly compatible with *T. asperellum* while Metalaxyl M 33% + Chlorothalonil 33.1% SC reported non-compatible at different concentrations. Kumar *et al.* (2017) reported Metalaxyl M 4% + Mancozeb 64% WP highly compatible and Carbendazim 25% + Mancozeb 50% WS completely inhibit the growth of *T. asperellum*. Present studies are also in conformity with earlier reports of different scientists *viz.*, Kumar *et al.* (2018); Manandhar *et al.* (2020); Kumar *et al.* (2021); Bai *et al.* (2022), Bharadwaz *et al.* (2023) and Poudel *et al.* (2024).

## Conclusions

The *in vitro* assessment of the compatibility of *T. asperellum* with seven combination fungicides revealed distinct differences in their interactions. Metalaxyl M 4% + Mancozeb 64% WP demonstrated high compatibility with 22.22 per cent growth inhibition at half the recommended dose and moderate compatibility with 53.70 per cent growth inhibition at the full recommended dose. Metalaxyl M 33% + Chlorothalonil 33.1% SC and Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC showed slight compatibility with 83.52 and 85.92 per cent growth inhibition respectively when tested at half of the recommended concentration.

In contrast, the remaining fungicidal combinations—Metalaxyl M 33% + Chlorothalonil 33.1% SC, Carbendazim 25% + Mancozeb 50% WS, Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC, Propiconazole 13.9% + Difenoconazole 13.9% EC, Carbendazim 25% + Flusilazole 12.5% SE, and Tebuconazole 50% + Trifloxystrobin 25% WG—were entirely incompatible, causing 91–100% mycelial growth inhibition at both tested concentrations. These findings emphasize the need for judicious selection of fungicides in integrated disease management programs to ensure that chemical applications do not hinder the beneficial activity of *T. asperellum*.

**Authors' contribution**

Tejashri T. Khore: Conceived the research idea, conducted the experiments, recorded observations, performed data analysis and prepared the initial draft of the manuscript. Chandrashekhar V. Shende: Provided guidance throughout the research work, supervised experimental design and analysis and critically reviewed and refined the manuscript. Shital S. Dhawan: Offered technical support in methodology and statistical analysis and assisted in interpreting results and improving the scientific content of the manuscript.

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## References

Bai, A. T., Vibha, J. B., Nair, R., & Upadhyay, A. (2022). Compatibility of *Trichoderma viride* with commonly used fungicides in management of early blight pathogen of tomato. *The Pharma Innovation Journal, 11*(1), 1734-1736.

Barnett, H. L., & Binder, F. L. (1973). The fungal host-parasite relationship. *Annual Review of Phytopathology, 11*, 273-292. https://doi.org/10.1146/annurev.py.11.090173.001421

Bharadwaz, P., Nath, B. C., Chetia, R., Saikia, S., Bora, P., & Bhattacharyya, P. N. (2023). *In vitro* studies on the compatibility of *Trichoderma viride* with commonly used agrochemicals in the vegetable cropping system. *Pest Management in Horticultural Ecosystems, 29*(1), 136-143.

Bheemaraya, Patil, M., Ramesh, Vendan, K. T., Amaresh, Y. S., & Rao, K. (2012). Compatibility of *Trichoderma* spp. With commonly used fungicides, insecticides and plant extracts. *Indian Journal of Plant Protection, 40*(2), 118-122.

Chu, F. F., & Wu, W. S. (1981). Biological and chemical control of *Rhizoctonia solani* by pea seed treatment. National Taiwan University*, 21*, 19-28.

Cook, R. I., & Baker, K. F. (1983). *The nature and practices of biological control of plant pathogens*. APS Books.

Elad, Y., Chet, I., Boyle, P., & Henis, Y. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*-scanning electron microscopy and fluorescence microscopy. *Phytopathology, 73*(1), 85-88.

Harman, G. E., & Kubicek, C. P. (1998). *Trichoderma* and *gliocladium* in biological control: an overview. In G. E. Harman & C. P. Kubicek (Eds.), *Trichoderma and Gliocladium: Enzymes, biological control and commercial applications* (Vol. 2, pp. 131-151). CRC Press.

Harman, G. E., & Kubicek, C. P. (2002). *Trichoderma and Gliocladium: Basic biology, taxonomy and genetics*. (1st ed.). CRC Press. https://doi.org/10.1201/9781482295320

Harman, G. E., Latorre, B., Agosin, E., San Martin, R., Riegel, D. G., Nielsen, P. A., Tronsmo, A., & Pearson, R. C. (1996). Biological and Integrated Control of *Botrytis* Bunch Rot of Grape Using *Trichoderma* spp. *Biological Control*, **7**(3), 259-266. https://doi.org/10.1006/bcon.1996.0092

Johnson, L. F., & Curl, E. A. (1972). *Methods for research on the ecology of soil-borne plant pathogens*. Burgess Publishing Company.

Kumar, R., Singh, S. K., Yadav, S., Kumar, R., Choubey, A. K., & Kumari, A. (2018). Compatibility of *Trichoderma viride* with different fungicide and organic cake. *Journal of Pharmacognosy and Phytochemistry, 7*(2), 2398-2401.

Kumar, S., Kumar, A., Kumar, R., & Rajesha, G. (2021). Study on compatibility of *Trichoderma viride* with different fungicides. *The Indian Journal of Agricultural Sciences, 91*(12), 1788-1792. https://doi.org/10.56093/ijas.v91i12.120808

Kumar, T. V., Veena, S. S., Karthikeyan, S., & Sreekumar, J. (2017). Compatibility of *Trichoderma asperellum* with fungicides, insecticides, inorganic fertilizers and bio-pesticides. *Journal of Root Crops, 43*(2), 68-75.

Maheshwary, N. P., Gangadhara Naik, B., Chittaragi, A., Naik, S. K., Satish, K. M., & Nandish, M. S. (2020). Compatibility of *Trichoderma asperellum* with fungicides. *The Pharma Innovation Journal, 9*(8), 136-140.

Manandhar, S., Timila, R. D., Karkee, A., Gupt, S. K., & Baidya, S. (2020). Compatibility study of *Trichoderma* isolates with chemical fungicides. *Journal of Agriculture and Environment, 21*, 9-18.

Nene, Y. L., & Thapliyal, P. N. (1993). *Fungicides in plant disease control*. (3rd ed.). IBH Publishing Company.

Panse, V. G., & Sukhatme, P. V. (1967). *Statistical analysis for agricultural workers*. ICAR.

Poudel, S., Pun, L. B., Paudel, R., Neupane, S., Pokharel, S., & Khanal, P. (2024). Compatibility study of *Trichoderma* sp. with Chemical Fungicides Commonly Used by Nepalese Farmers, Under *In-Vitro* Condition. *International Journal of Applied Biology, 8*(1), 51-61.

Saha, S., Pharate, S., Thosar, R. U., & Chavan, V. (2023). Compatibility of *Trichoderma asperelloides* with Fungicides Controlling Downy Mildew and Powdery Mildew in Grapes. *Grape Insight, 1*(1), 32-36.

Veena, S. S., Baby, A., & Karthikeyan, S. (2022). Study on compatibility of *Trichoderma asperellum* and fungicides for the development of environment friendly and cost-effective disease management strategies. *Journal of Root Crops, 48*(1 & 2), 35-40.

Vincent, J. M. (1927). Distoration of fungal hyphae in the presence of certain inhibitors. *Nature, 159*, 180. https://doi.org/10.1038/159850b0

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J., & Chen, J. (2023). *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology, 14*, 1160551. https://doi.org/10.3389/fmicb.2023.1160551