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### Compatibility evaluation of *Trichoderma* species with plant extracts under *In-vitro* conditions

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#### ABSTRACT

The present study evaluated the compatibility of botanical insecticides with three *Trichoderma* species, *T. hamatum*, *T. harzianum* and *T. asperellum* to assess their suitability for integrated disease management. Seven botanicals marigold, karanj, lantana, parthenium, datura, periwinkle and neem were tested at 5% and 10% concentrations. All botanicals exhibited high compatibility with *T. hamatum* and *T. asperellum*, with mycelial growth ranging from 64.17 mm to 88.50 mm and growth inhibition below 30%. For *T. harzianum*, most botanicals at 5% concentration were compatible, while karanj, datura, neem and lantana at 10% showed moderate compatibility, with growth inhibition ranging from 33.15% to 44.26%. These findings suggest that botanical insecticides, particularly at lower concentrations, can be safely integrated with *Trichoderma* spp. without compromising their growth, offering a promising approach for sustainable crop protection.

#### Introduction

In recent years, growing concerns over the excessive and indiscriminate use of synthetic pesticides have encouraged the search for safer alternatives in plant disease management. Although chemical control has been effective to some extent, its continuous application often leads to resistance development in pathogens, a decline in beneficial soil microflora, and negative impacts on human health and the environment (Meena *et al.*, 2020; Hobbelen *et al.*, 2014). As a result, botanicals derived from medicinal and aromatic plants such as neem (*Azadirachta indica*), garlic (*Allium sativum*), tulsi (*Ocimum*

*sanctum*) and onion (*Allium cepa*) have emerged as eco-friendly options due to their antifungal and antibacterial properties.

*Trichoderma* species are widely recognized as effective biocontrol agents that suppress a broad range of soil-borne plant pathogens through mechanisms such as competition for nutrients and space, mycoparasitism, secretion of antifungal metabolites and induction of plant defense responses (Sood *et al.*, 2020; Vinale *et al.*, 2013). Beyond pathogen suppression, they also promote nutrient uptake, plant growth and yield improvement (Campos *et al.*, 2020). Because of these multifaceted roles, *Trichoderma* has been widely adopted in seed

treatment, soil application and bioformulations marketed as biopesticides and biofertilizers (Kumar et al., 2014). However, the combined use of *Trichoderma* with botanicals requires careful evaluation. Some plant extracts can be compatible and enhance its efficacy, while others may inhibit its growth and colonization, reducing its effectiveness as a biocontrol agent (Bagwan, 2010).

Since plant extracts are increasingly used in sustainable agriculture, understanding their compatibility with *Trichoderma* is essential for integrated disease management. The present study was therefore undertaken to evaluate the *In-vitro* compatibility of *Trichoderma hamatum*, *T. harzianum* and *T. asperellum* with selected plant extracts commonly used as botanicals in plant protection practices.

## Material and Methods

The study was conducted in the *Trichoderma* Laboratory, Agricultural Research Station, Ummedganj, Kota, Rajasthan, India during 2025. Compatibility tests were performed using the poisoned food technique (Nene and Thapliyal, 1993) in a Completely Randomized Design (CRD) with three replications. The treatments comprised T<sub>1</sub> = Marigold (*Tagetes erecta*), T<sub>2</sub> = Karanj (*Pongamia glabra*), T<sub>3</sub> = Lantana, (*Lantana camara*), T<sub>4</sub> = Parthenium (*Parthenium hysterophorus*), T<sub>5</sub> = Datura (*Datura stramonium*), T<sub>6</sub> = Periwinkle (*Vinca rosea*) and T<sub>7</sub> = Neem (*Azadirachta indica*) each at 5% and 10% along with T<sub>8</sub> = Control.

Potato Dextrose Agar (PDA) medium was sterilized and cooled to approximately 40°C before amending with the required concentrations of plant extracts. About 20 ml of amended PDA was poured into 9 cm sterilized Petri plates. Plates without botanicals served as controls. Nine insecticides were tested at recommended concentrations. A 5 mm mycelial disc from a 7-day-old culture of *Trichoderma hamatum*, *T. harzianum*, or *T. asperellum* was aseptically transferred to the center of each Petri plate. Plates were incubated at 25 ± 2°C and radial mycelial growth was measured after 7 days.

Mycelial growth inhibition was calculated using the formula advocated by Vincent (1947) as given below.

**Table 1.** Effect of plant extracts on *T. hamatum*

Plant extracts	Radial growth* (mm)		Per cent inhibition (%)**		Nature of compatibility
	5%	10%	5%	10%	
T <sub>1</sub> : Marigold ( <i>Tagetes erecta</i> )	85.00	79.00	5.56 (13.62)	12.22 (20.45)	H H
T <sub>2</sub> : Karanj ( <i>Pongamia glabra</i> )	71.67	64.17	20.37	28.70	H

$$\text{Percent growth inhibition (PGI)} = \frac{C-T}{C} \times 100$$

Where, I = percent inhibition, C = colony diameter in control, and T = colony diameter in treatment.

The nature of compatibility is classified based on the percentage of inhibition: treatments showing 0–30% inhibition are considered highly compatible, those with 30.1–60% inhibition are moderately compatible, 60.1–90% inhibition indicates slight compatibility and values exceeding 90% are regarded as non-compatible (Saha et al., 2023). Data on mycelial growth and inhibition percentage were subjected to arcsine transformation before analysis of variance (ANOVA). Treatment means were compared at a 5% significance level.

## Results and Discussion

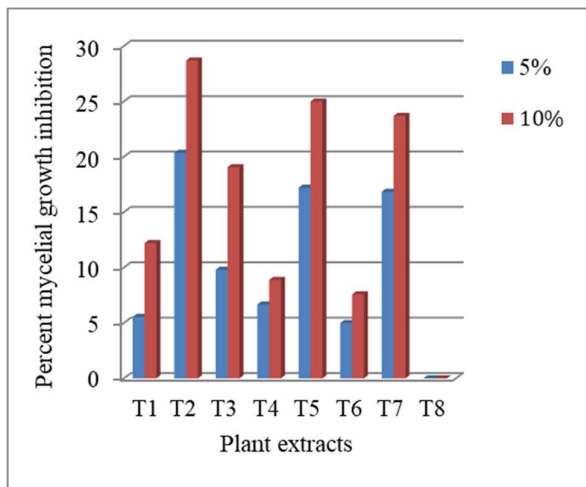
### Effect of plant extracts on *Trichoderma hamatum*

The *In-vitro* study revealed that all tested plant extracts exhibited high compatibility with *T. hamatum*, as indicated by low levels of mycelial growth inhibition (<30%). The radial growth of the fungus ranged from 64.17 mm to 85.50 mm, confirming that none of the botanicals significantly suppressed fungal growth. Among the treatments, periwinkle (*Catharanthus roseus*) and marigold (*Tagetes erecto*) extracts showed the least inhibitory effect, recording minimal inhibition of 5.00-12.22%, followed by parthenium (*Parthenium hysterophorus*) with inhibition below 10% even at higher concentration. This suggests excellent compatibility with *T. hamatum*, moderate inhibition was observed with extracts of lantana (*Lantana camara*), datura (*Datura stramonium*) and neem (*Azadirachta indica*), where inhibition ranged between 16-25%. The highest inhibition was recorded in karanj (*Porigamia pinnata*) extract (28.70% at 10%), but it still remained within the highly compatible category (Table 1, Fig.1). Dubey et al. (2007) observed that botanicals like neem and lantana showed minimal inhibition on *Trichoderma* while effectively suppressing pathogens. Singh (2012) also emphasized that botanical extracts can be combined with biocontrol agents due to their selective toxicity.

T <sub>3</sub> : Lantana ( <i>Lantana camera</i> )	81.17	72.83	(26.82)	(32.39)	H
T <sub>4</sub> : Parthenium ( <i>Parthenium hysetrophorus</i> )	84.00	82.00	(18.24)	(25.89)	H
T <sub>5</sub> : Datura ( <i>Datura stramonium</i> )	74.50	67.50	(14.93)	(17.32)	H
T <sub>6</sub> : Periwinkle ( <i>Vinca rosea</i> )	85.50	83.17	(24.51)	(29.99)	H
T <sub>7</sub> : Neem ( <i>Azadirachta indica</i> )	74.83	68.67	(12.88)	(15.98)	H
T <sub>8</sub> : Control	90.00	90.00	(24.23)	(29.13)	H
T <sub>8</sub> : Control	90.00	90.00	0.00	0.00	-
SEm±	0.37	0.33	(0.00)	(0.00)	-
CD (5%)	1.11	0.99	0.55	0.45	-
			1.66	1.34	-

\*Average of three replications; \*\*Figures in parentheses are Arc sine transformed

H: Highly compatible, M: Moderately compatible, S: Slightly compatible N: Non-Compatible



**Fig. 1.** *In-vitro* evaluation of per cent mycelial growth inhibition of *T. hamatum* against plant extracts

**Effect of plant extracts on *Trichoderma harzianum***

The *In-vitro* evaluation demonstrated that *T. harzianum* exhibited high compatibility with most plant extracts at lower concentration (5%), where marigold, karanj, lantana, parthenium, datura,

periwinkle and neem supported substantial mycelial growth (81.17-69.83 mm) with less than 30% inhibition, indicating their suitability for combined use (Table 2, Fig. 2). However, at higher concentration (10%), certain extracts such as karanj, lantana, datura and neem exhibited moderate compatibility, reducing fungal growth (60.17-50.17 mm) and increasing inhibition (33.15-44.26%), suggesting a dose-dependent effect of plant metabolites on fungal growth.

Among all treatments, periwinkle and marigold extracts showed minimal inhibitory effect even at higher concentrations, whereas neem (10%) recorded the highest inhibition (44.26%), though still within a tolerable range. Therefore, the findings indicate that most botanicals, particularly at lower concentrations, are compatible with *T. horzionum* and can be effectively integrated into eco-friendly disease management strategies. Similar observations have been reported by Dubey *et al.* (2007) and Sharma *et al.* (2012), who documented that botanicals such as neem and lantana exert minimal inhibitory effects on *Trichoderma*, supporting their combined application in integrated disease management programs.

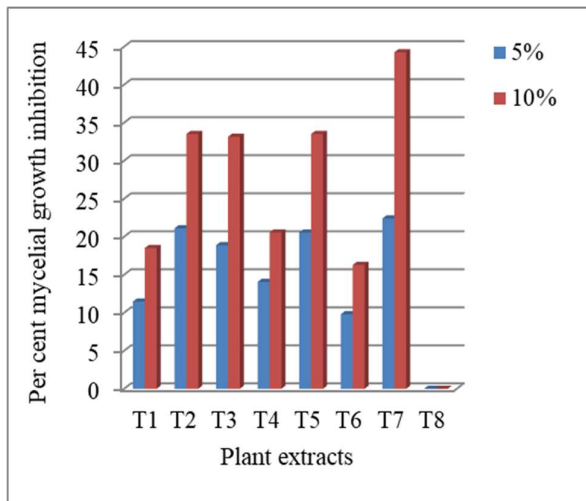
**Table 2.** Compatibility of plant extracts with *T. harzianum*

Plant extracts	Radial growth* (mm)		Per cent inhibition (%)**		Nature of compatibility
	5%	10%	5%	10%	
T <sub>1</sub> : Marigold ( <i>Tagetes erecta</i> )	79.67	73.33	11.48 (19.77)	18.52 (25.48)	H
T <sub>2</sub> : Karanj ( <i>Pongamia glabra</i> )	71.00	59.83	21.11 (27.34)	33.52 (35.37)	H
T <sub>3</sub> : Lantana ( <i>Lantana camera</i> )	73.00	60.17	18.89 (25.74)	33.15 (35.15)	M

T <sub>4</sub> : Parthenium ( <i>Parthenium hysetrophorus</i> )	77.33	71.50	14.07 (22.02)	20.56 (26.94)	M
T <sub>5</sub> : Datura ( <i>Datura stramonium</i> )	71.50	59.83	20.56 (26.94)	33.52 (35.37)	H
T <sub>6</sub> : Periwinkle ( <i>Vinca rosea</i> )	81.17	75.33	9.81 (18.16)	16.30 (23.79)	H
T <sub>7</sub> : Neem ( <i>Azadirachta indica</i> )	69.83	50.17	22.41 (28.25)	44.26 (41.70)	H
T <sub>8</sub> : Control	90.00	90.00	-	-	M
SEm±	0.58	0.46	0.79	0.55	-
CD (5%)	1.75	1.37	2.38	1.66	-

\*Average of three replications; \*\*Figures in parentheses are Arc sine transformed

H: Highly compatible, M: Moderately compatible, S: Slightly compatible N: Non compatible



**Fig. 2.** In-vitro evaluation of per cent mycelial growth inhibition of *T. harzianum* against plant extracts

### Effect of plant extracts on *Trichoderma asperellum*

The *In-vitro* evaluation indicated that *T. asperellum* exhibited high compatibility with all tested plant extracts, as reflected by substantial mycelial growth (67.33-88.50 mm) and low per cent inhibition (<30%) across both 5% and 10% concentrations (Table 3, Fig. 3). Among the treatments, periwinkle and marigold extracts showed the least inhibitory effect, recording minimal inhibition of 1.67-10.74% and 3.15-10.37%, respectively, followed by lantana and parthenium. Even at higher concentration (10%), extracts of karanj, datura and neem caused only mild inhibition (18.70-25.19%), confirming their compatibility. Comparative evaluation among three species revealed that *T. asperellum* was the most tolerant, followed by *T. hamatum*, while *T. harzianum* showed relatively

higher sensitivity at increased concentrations. The results clearly suggest that these botanicals can be safely integrated with *T. asperellum* in eco-friendly disease management programs. Similar findings were reported by Dubey *et al.* (2007) and Sharma *et al.* (2012). They also observed that various botanicals extracts has minimal inhibitory effects on *Trichoderma*, supporting their combined use in integrated disease management strategies.

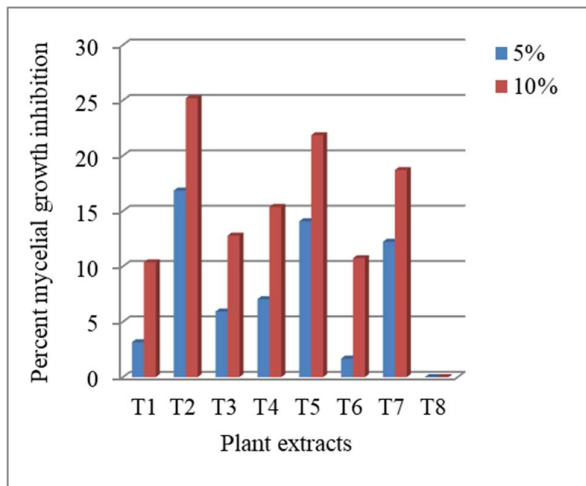
These findings indicate that plant extracts, particularly at lower doses, can be integrated with *Trichoderma* spp. without affecting their growth, thus holding promise for eco-friendly disease management strategies. Marigold and periwinkle are known to release root exudates that are generally supportive of microbial growth, including *Trichoderma* spp. These exudates are non-toxic and do not interfere with the fungal metabolism, unlike certain other plant species that may release inhibitory substances. Although direct studies on these specific plants are limited, existing research indicates that their chemical profiles create a favorable environment for mutual interaction and colonization by *Trichoderma* (Bharti *et al.*, 2024). Neem extract showed mycelial growth inhibition may be because of it contain azadirachtin that disrupts cellular integrity and enzyme pathways, leading to halted spore germination and hyphal growth (Almeida *et al.*, 2017). These findings are in confirmatory with the earlier reports of several workers (Bhagwan, 2010; Wavare, 2015; Maheshwari, 2014; Tapwal *et al.*, 2012). In an integrated disease management framework, *T. asperellum* can work synergistically with compatible fungicides to reduce pathogen load while minimizing chemical fungicide dependence (Kumari *et al.*, 2025).

**Table 3.** Effect of plant extracts on *T. asperellum*

Plant extracts	Radial growth* (mm)		Per cent inhibition (%)**		Nature of compatibility
	5%	10%	5%	10%	
T <sub>1</sub> : Marigold ( <i>Tagetes erecta</i> )	87.17	80.67	3.15 (10.16)	10.37 (18.76)	H
T <sub>2</sub> : Karanj ( <i>Pongamia glabra</i> )	74.83	67.33	16.85 (24.23)	25.19 (30.12)	H
T <sub>3</sub> : Lantana ( <i>Lantana camera</i> )	84.67	78.50	5.93 (14.03)	12.78 (20.94)	H
T <sub>4</sub> : Parthenium ( <i>Parthenium hysetrophorus</i> )	83.67	76.17	7.04 (15.37)	15.37 (23.07)	H
T <sub>5</sub> : Datura ( <i>Datura stramonium</i> )	77.33	70.33	14.07 (21.99)	21.85 (27.87)	H
T <sub>6</sub> : Periwinkle ( <i>Vinca rosea</i> )	88.50	80.33	1.67 (6.95)	10.74 (19.11)	H
T <sub>7</sub> : Neem ( <i>Azadirachta indica</i> )	79.00	73.17	12.22 (20.45)	18.70 (25.62)	H
T <sub>8</sub> : Control	90.00	90.00	0.00 (0.00)	0.00 (0.00)	-
SEm±	0.49	0.36	0.93	0.49	-
CD (5%)	1.48	1.09	2.79	1.48	-

\*Average of three replications; \*\*Figures in parentheses are Arc sine transformed

H: Highly compatible, M: Moderately compatible, S: Slightly compatible N: Non compatible



**Fig. 3.** In-vitro evaluation of per cent mycelial growth inhibition of *T. asperellum* against plant extracts

**Conclusion**

The present study demonstrated that most plant extracts were compatible with *T. hamatum*, *T. harzianum* and *T. asperellum*, particularly at lower concentration (5%). Marigold, parthenium and periwinkle showed high compatibility at both concentrations, while karanj, lantana, datura and

neem were highly compatible mainly at 5% concentration. The radial growth and low inhibition (<30%) indicated that these botanicals did not adversely affect the growth of *Trichoderma* spp. Among the three species, *T. asperellum* exhibited maximum tolerance, followed by *T. hamatum*, whereas *T. harzianum* was comparatively more sensitive at higher concentration (10%). The study highlights that plant extracts, when used at appropriate concentrations, can be safely combined with *Trichoderma* spp. This compatibility supports their integration in eco-friendly and sustainable disease management strategies, reducing dependency on chemical fungicides while enhancing biological control efficiency.

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**Conflict of Interest**

The authors have no conflict of interest to declare.

## Data Sharing

All relevant data are included in the manuscript.

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