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***In-vitro* Evaluation of *Trichoderma* Species and Some Selected Botanical Extracts against *Alternaria helianthi* (Hansf) Causing *Alternaria* Blight of Sunflower**

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigates the *in vitro* efficacy of bioagents and selected botanicals in managing *Alternaria helianthi*, the pathogen responsible for Alternaria blight in sunflower (*Helianthus annuus* L.). Two bioagents, *Trichoderma harzianum* and *Trichoderma viride*, and six botanicals—*Azadirachta indica* (neem), *Lantana camara* (lantana), *Curcuma longa* (turmeric), *Aloe barbadensis* (aloe vera), *Withania somnifera* (ashwagandha), and *Mentha spicata* (pudina)—were evaluated using the dual culture and poison food technique, respectively. The results showed that *T. harzianum* exhibited the highest inhibition of mycelial growth at 76.32%, while neem extract was the

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most effective among botanicals, achieving a maximum inhibition of 75.03% at 15% concentration. These findings suggest the potential of bioagents and botanicals in eco-friendly management strategies for *Alternaria* blight in sunflower cultivation.

Keywords: *Trichoderma* species; botanical extracts; *Alternaria helianthin*; *Alternaria* blight; sunflower.

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oilseed crop from the Asteraceae family, native to North America. Its name comes from its sun-like appearance. Characterized by large, yellow flower heads, long taproots, and rough leaves, the plant's seeds develop from achenes and face the sun. Sunflowers are grown globally, and their products are widely commercialized for culinary use and livestock feed (Yegorov et al., 2019). India ranks 13th position in sunflower production with 0.4 m/ha cultivation area with production of 0.83 t/ha. Karnataka is the largest producer of sunflower in India which accounts for 0.24 m/ha area, 0.13 mt production with 0.34 t/ha production. Sunflower suffers from many diseases caused by fungi, bacteria, and viruses. Sunflower is the known host of more than 30 pathogens mostly fungi which under certain climatic condition may impair the normal physiology of the plant so that yield and oil quality are reduced significantly (Gulya et al., 1994). *Alternaria* leaf blight caused by *Alternaria helianthi* is the most devastating disease causing about 90% loss in the seed yield and 34% loss to the oil yield of the sunflower in India (Udayashankar et al., 2012) and Brand et al. (2020) reported that for each 10% increase in severity of *Alternaria* and *Septoria* spot on sunflower yield, there was a reduction of 663.3 kg /ha in sunflower yield for severities greater than 24 per cent.

2. MATERIALS AND METHODS

In this study, two *Trichoderma* species, *Trichoderma viride* and *Trichoderma harzianum*, were tested against the previously identified *Alternaria helianthi* using the dual culture technique (Dennis and Webster, 1971). The botanical extracts utilized included neem, lantana, turmeric, aloe vera, ashwagandha, and pudina, applied at concentrations of 5%, 10%, and 15%. These extracts were incorporated into PDA media and a 5 mm disc of *A. helianthi* was inoculated to evaluate their efficacy using the poison food technique. Plates were incubated at 27°C, and radial growth was measured on the 3rd, 5th, and 7th days post-inoculation. Data

from the dual culture technique were recorded at 24-hour intervals until the control plates were fully covered. Mycelial inhibition for both methods was calculated using the formula provided by Arora and Upadhyay (1978).

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

Where, I= Mycelium inhibition percentage, C= Mycelium growth (mm) in control and T= Mycelium growth (mm) in treatment. The collected data were analyzed using ANOVA and significant differences between treatments were determined at a 5% probability level.

3. RESULTS

3.1 *In vitro* Efficacy of Bioagents

The data illustrated in Table 1 showed that the effect of two bioagents, *Trichoderma viride* and *Trichoderma harzianum* on the mycelium growth (MG) and mycelium inhibition (MI) of *Alternaria helianthi* over five time intervals viz., 48, 72, 96, 120, and 144 hours after inoculation. The control group exhibited no inhibition, with mycelium growth reaching 74.48 mm at 144 hours. In contrast, *T. viride* showed reduced mycelium growth, measuring 10.92 mm at 48 hours and increasing to 21.87 mm by 144 hours, with mycelium inhibition percentages rising from 46.33% to 70.63% over the same period, resulting in an average mycelium inhibition of 61.90%. Meanwhile, *T. harzianum* displayed even more pronounced effects, with mycelium growth starting at 9.62 mm at 48 hours and reaching 17.63 mm at 144 hours, while mycelium inhibition improved from 52.72% to 76.32%, yielding an average mycelium inhibition of 68.58%. Overall, both bioagents effectively inhibited the growth of *Alternaria helianthi*, with *Trichoderma harzianum* demonstrating superior efficacy compared to *Trichoderma viride*.

3.2 Efficacy of Botanical Extracts

The data presented in Table 2 revealed the mean radial growth and inhibition percentages of *Alternaria helianthi* at 3rd days after inoculation

(DAI), 5th DAI and 7th DAI across various treatments at 5% concentration. The untreated control (T₀) exhibited a radial growth of 33.28 mm at 3rd DAI, increasing to 60.32 mm at 5th DAI, and 80.76 mm at 7th DAI. Among the treatments, Neem extract (T₁) showed the highest inhibition, with radial growth of 23.4 mm (29.68% inhibition) at 3rd DAI, 35.58 mm (41.01% inhibition) at 5th DAI and 45.10 mm (44.15% inhibition) at 7th DAI. Lantana (T₂) had moderate effects, with growth at 30.38 mm (8.71% inhibition) at 3rd DAI, 52.22 mm (13.42% inhibition) at 5th DAI and 62.58 mm (22.51% inhibition) at 7th DAI. Turmeric (T₃) and Aloe vera (T₄) also demonstrated some inhibition, with growth measurements of 31.58 mm (5.10% inhibition) and 28.72 mm (13.70%

inhibition) at 3rd DAI, respectively. Ashwagandha (T₅) had a radial growth of 25.28 mm (24.03% inhibition) at 3rd DAI, with increasing inhibition percentages over time. Pudina (T₆) exhibited minimal inhibition, with growth at 32.70 mm (1.74% inhibition) at 3rd DAI, while Hexaconazole at 0.2% (T₇ treated check) was the most effective treatment, showing only 8.85 mm growth (72.58% inhibition) at 3rd DAI, 10.65 mm (82.34% inhibition) at 5th DAI and 12.39 mm (84.69% inhibition) at 7th DAI. The critical difference (C.D.) values of 0.56, 0.74, and 0.57 indicate statistical significance among the treatments. Overall, among the botanicals used Neem and Ashwagandha was the most effective in inhibiting the mycelium growth of *Alternaria helianthi*.

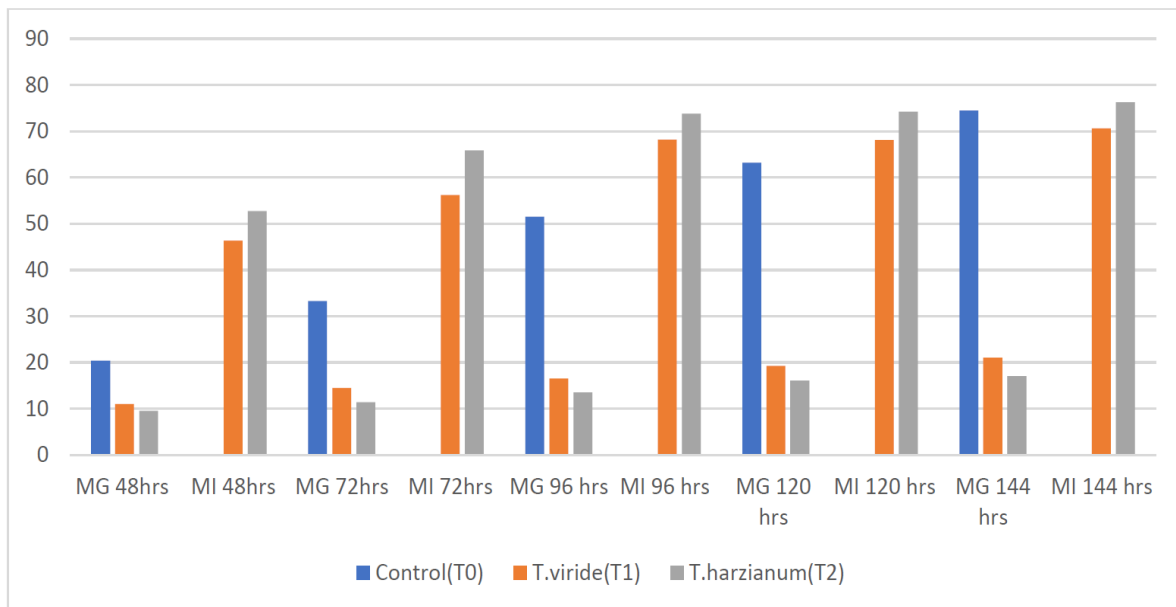


Fig. 1. Comparison of mycelium growth (mm) and inhibition (%) among the treatment

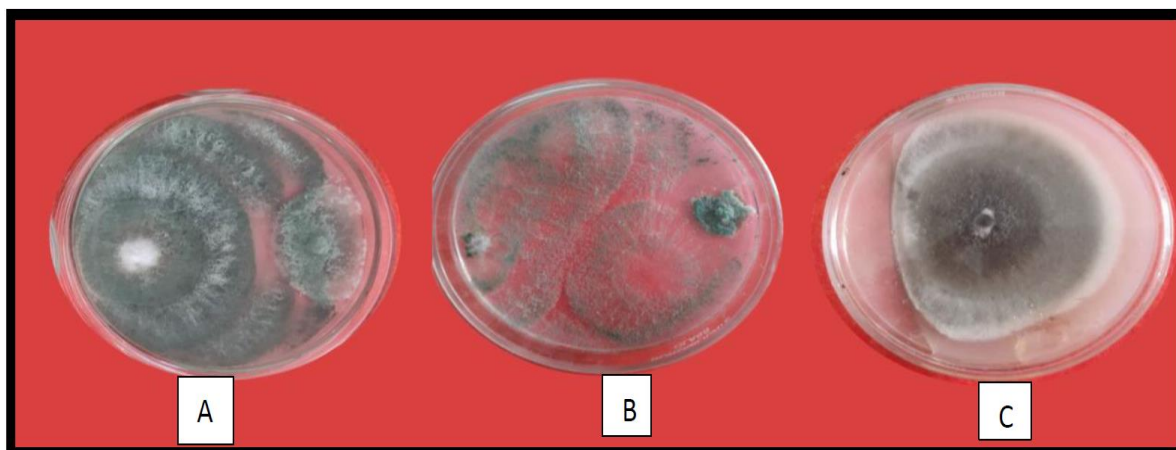


Plate 1. A) *T. viride* B) *T. harzianum* C) control at 144 hrs

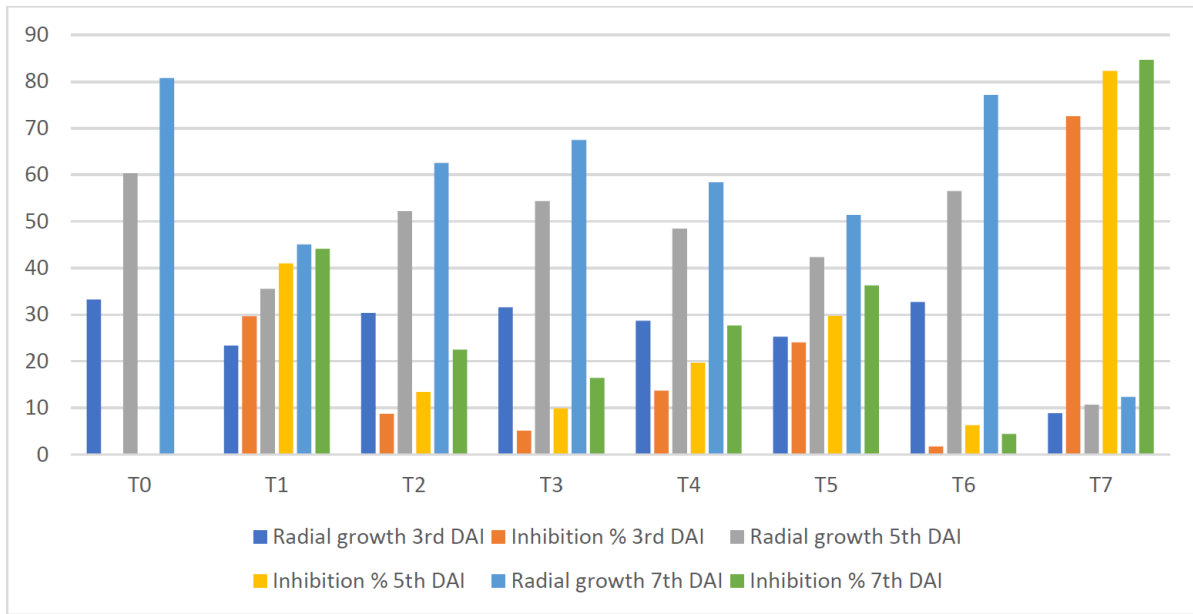


Fig. 2. Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 5% concentration

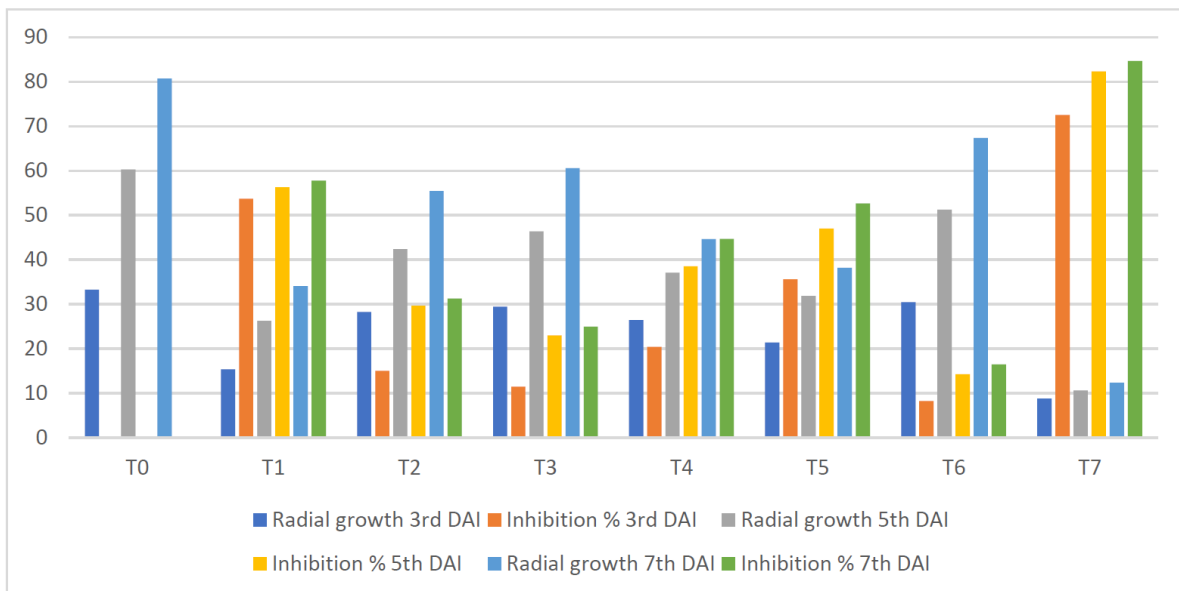


Fig. 3. Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 10% concentration

The data showed in Table 3 revealed the mean radial growth and inhibition percentages of *Alternaria helianthi* across various treatments at 10% concentration. The untreated control (T₀) had a radial growth of 33.28 mm at 3rd DAI, increasing to 80.76 mm by 7th DAI. Neem (T₁) was effective, with growth reduced to 15.4 mm (53.72% inhibition) at 3rd DAI and 34.08 mm (57.80% inhibition) at 7th DAI. Lantana (T₂) showed moderate inhibition, with growth at 28.28 mm (15.02% inhibition) at 3rd DAI and 55.50 mm

(31.27% inhibition) at 7th DAI. Turmeric (T₃) had growth of 29.46 mm (11.47% inhibition) at 3rd DAI and 60.58 mm (24.98% inhibition) at 7th DAI. Aloe vera (T₄) showed 26.48 mm (20.43% inhibition) at 3rd DAI, while Ashwagandha (T₅) had 21.42 mm (35.63% inhibition) at 3rd DAI and 38.20 mm (52.69% inhibition) at 7th DAI. The critical difference (C.D.) values indicate significant differences among treatments. Overall, Neem and Ashwagandha were the most effective treatments.

Table 1. Mycelium growth (mm) and inhibition (%) of *Alternaria helianthi* by *Trichoderma viride* and *Trichoderma harzianum*

Bio agents	48 hrs.		72 hrs.		96 hrs.		120 hrs.		144 hrs		Avg.	
	Mycelium growth (MG) in mm and mycelium inhibition (MI) in %											
	MG	MI	MG	MI	MG	MI	MG	MI	MG	MI		
Control (T ₀)	20.35 ^a	00.00	33.35 ^a	00.00	51.40 ^a	00.00	63.28 ^a	00.00	74.48 ^a	00.00	00.00	
<i>T. viride</i> (T ₁)	10.92 ^b	46.33	14.60 ^b	56.22	16.34 ^b	68.21	20.17 ^b	68.12	21.87 ^b	70.63	61.90	
<i>T. harzianum</i> (T ₂)	9.62 ^c	52.72	11.39 ^c	65.84	13.48 ^c	73.77	16.16 ^c	74.25	17.63 ^c	76.32	68.58	
C.D. (5%)	0.47		0.49		0.49		0.77		0.64			

Table 2. Effect of selected plant leaf extracts on the radial growth (mm) and inhibition percent at 5% concentration

Treatment no	Treatments	Radial growth @3 rd DAI	Inhibition% @ 3 rd DAI	Radial growth @ 5 th DAI	Inhibition % @ 5 th DAI	Radial growth @7 th DAI	Inhibition % @ 7 th DAI
T ₀	Untreated check	33.28	0.00	60.32	0.00	80.76	0.00
T ₁	Neem	23.4	29.68	35.58	41.01	45.10	44.15
T ₂	Lantana	30.38	8.71	52.22	13.42	62.58	22.51
T ₃	Turmeric	31.58	5.10	54.34	9.91	67.46	16.46
T ₄	Aloe vera	28.72	13.70	48.42	19.72	58.40	27.68
T ₅	Ashwagandha	25.28	24.03	42.36	29.77	51.44	36.30
T ₆	Pudina	32.70	1.74	56.50	6.33	77.18	4.43
T ₇	Hexaconazole @0.2%	8.85	72.58	10.65	82.34	12.39	84.69
C.D. (5%)		0.56		0.74		0.57	

Table 3. Effect of selected plant leaf extracts on the radial growth (mm) of *Alternaria helianthi* and inhibition percent at 10 % concentration

Treatment no	Treatments	Radial growth@3 rd DAI	Inhibition %@3 rd DAI	Radial growth@ 5 th DAI	Inhibition %@ 5 th DAI	Radial growth @7 th DAI	Inhibition %@ 7 th DAI
T ₀	Untreated check	33.28	0.00	60.32	0.00	80.76	0.00
T ₁	Neem	15.4	53.72	26.34	56.33	34.08	57.80
T ₂	Lantana	28.28	15.02	42.42	29.67	55.50	31.27
T ₃	Turmeric	29.46	11.47	46.42	23.04	60.58	24.98
T ₄	Aloe vera	26.48	20.43	37.06	38.56	44.66	44.70
T ₅	Ashwagandha	21.42	35.63	31.94	47.04	38.20	52.69
T ₆	Pudina	30.54	8.23	51.30	14.25	67.40	16.54
T ₇	Hexaconazole@0.2%	8.85	72.58	10.65	82.34	12.39	84.69
C.D. (5%)		0.41		0.57		0.58	

Table 4. Effect of selected plant leaf extracts on the radial growth (mm) and inhibition percent at 15% concentration

Treatment no	Treatments	Mean of the five replications					
		Radial growth @ 3 rd DAI	Inhibition % @ 3 rd DAI	Radial growth @ 5 th DAI	Inhibition %@ 5 th DAI	Radial growth@ 7 th DAI	Inhibition %@ 7 th DAI
T ₀	Untreated check	33.28	0.00	60.32	0.00	80.76	0.00
T ₁	Neem	8.82	73.49	15.00	75.13	20.16	75.03
T ₂	Lantana	24.46	26.50	31.26	48.17	36.32	55.02
T ₃	Turmeric	26.28	21.03	35.28	41.51	41.54	48.56
T ₄	Aloe vera	22.42	32.63	27.22	54.87	30.52	62.20
T ₅	Ashwagandha	15.42	53.66	21.68	65.05	24.18	70.05
T ₆	Pudina	28.38	14.72	38.30	36.50	51.40	36.35
T ₇	Hexaconazole @ 0.2%	8.85	72.58	10.65	82.34	12.39	84.69
C.D. (5%)		0.51		0.46		0.53	

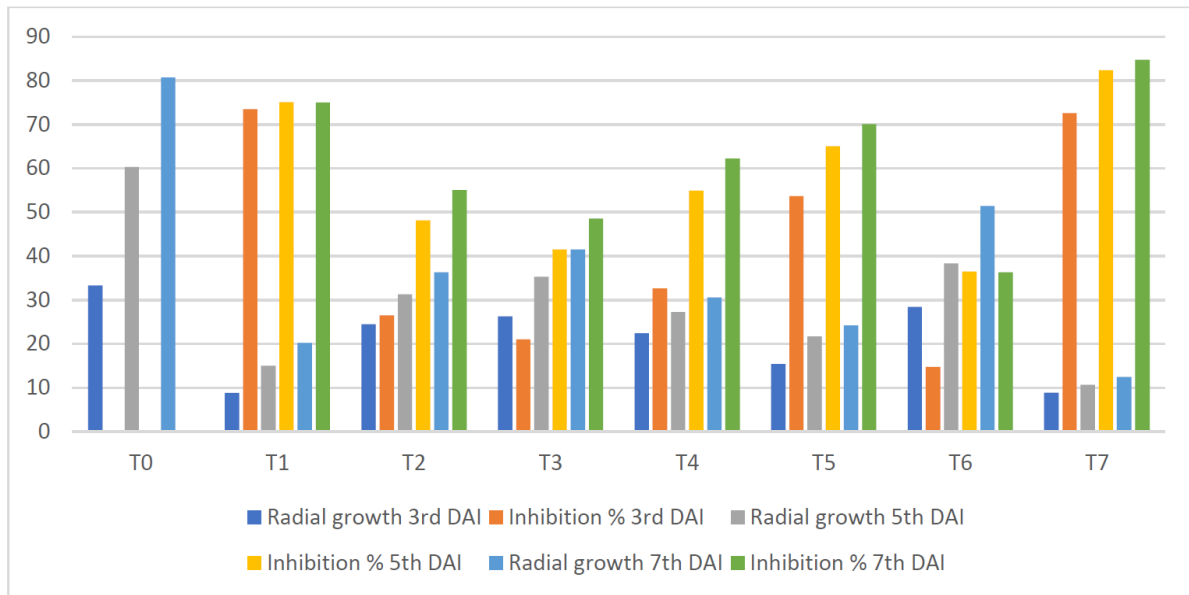


Fig. 4. Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 15% concentration

The data presented in the Table 4 showed the mean radial growth and inhibition percentages of *Alternaria helianthi* across various treatments at 15% concentration. The untreated control (T₀) showed radial growth of 33.28 mm at 3rd DAI, increasing to 80.76 mm by 7th DAI. Neem (T₁) was the most effective treatment, with growth reduced to 8.82 mm (73.49% inhibition) at 3rd DAI, 15.00 mm (75.13% inhibition) at 5th DAI, and 20.16 mm (75.03% inhibition) at 7th DAI. Lantana (T₂) demonstrated moderate inhibition, with growth at 24.46 mm (26.50% inhibition) at 3rd DAI and 36.32 mm (55.02% inhibition) at 7th

DAI. Turmeric (T₃) had radial growth of 26.28 mm (21.03% inhibition) at 3rd DAI and 41.54 mm (48.56% inhibition) at 7th DAI. Aloe vera (T₄) showed 22.42 mm (32.63% inhibition) at 3rd DAI, while Ashwagandha (T₅) had 15.42 mm (53.66% inhibition) at 3rd DAI and 24.18 mm (70.05% inhibition) at 7th DAI. Pudina (T₆) exhibited lower inhibition, with growth of 28.38 mm (14.72% inhibition) at 3rd DAI. The critical difference (C.D.) values of 0.51, 0.46, and 0.53 indicate significant differences among treatments. Overall, Neem was the most effective treatment, followed by Ashwagandha and Aloe vera.

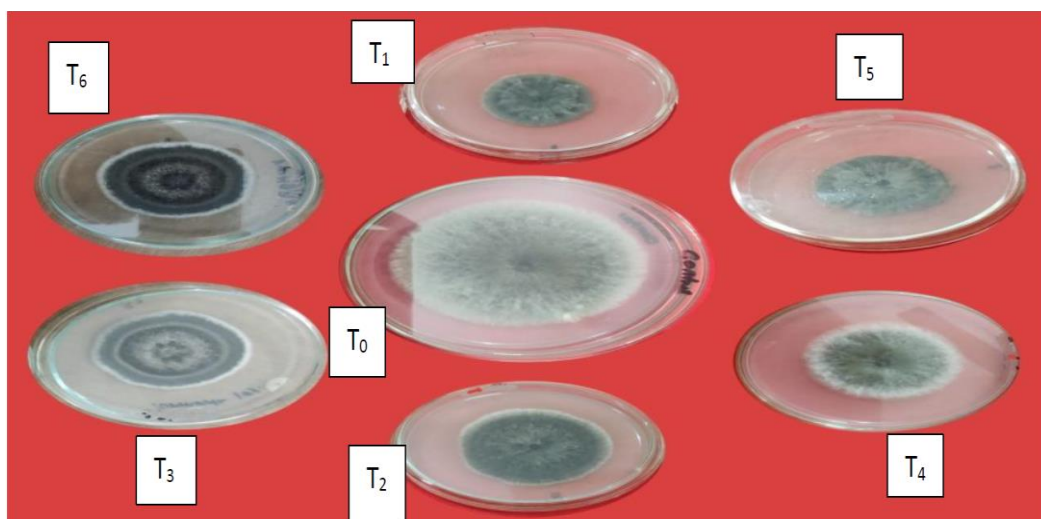


Plate 2. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 5% concentration on 7th DAI

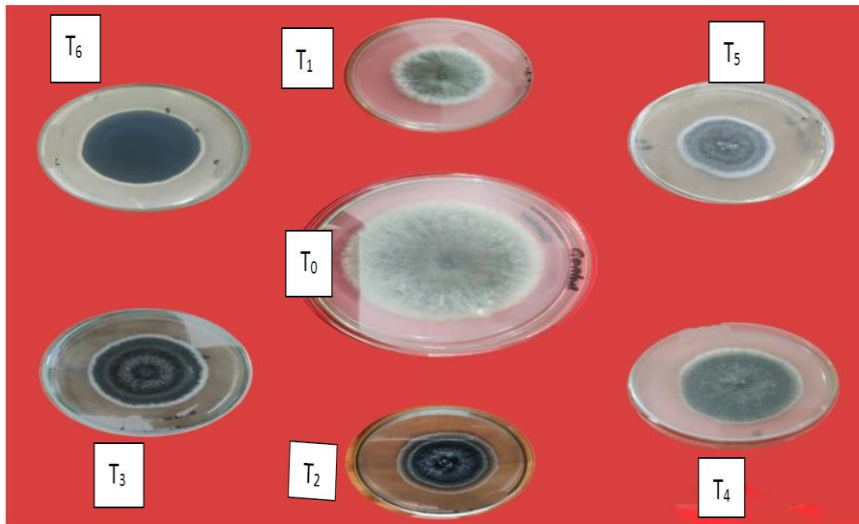


Plate 3. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 10% concentration on 7th DAI

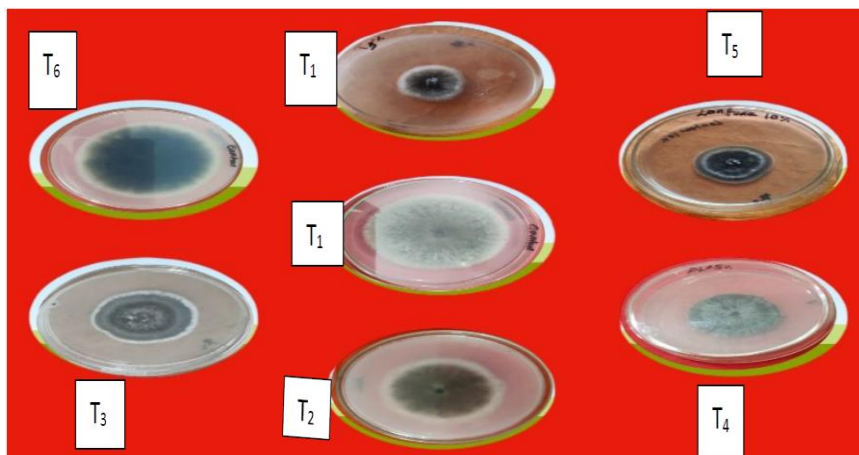


Plate 4. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 15% concentration on 7th DAI

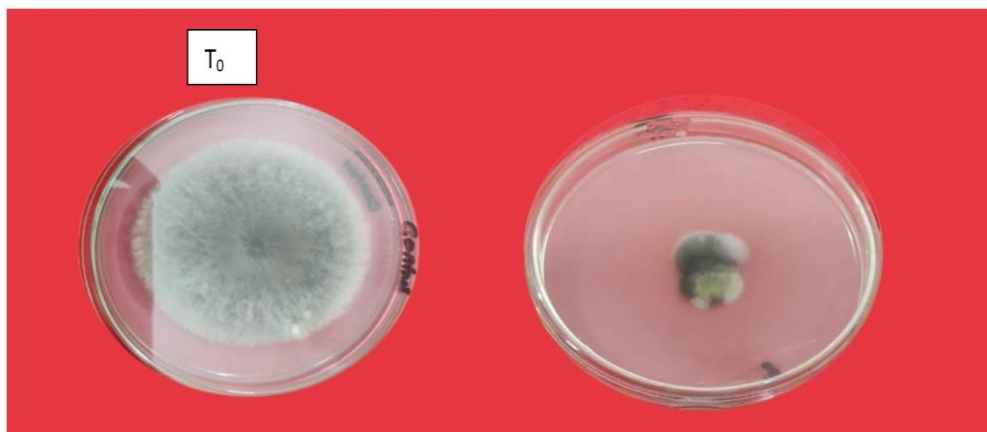


Plate 5. Mycelial growth of *Alternaria helianthi* on PDA media with Hexaconazole 0.2% (Treated check) on 7th DAI

4. DISCUSSION

The findings of this study underscore the significant biocontrol potential of *Trichoderma* species, particularly *T. harzianum*, against *Alternaria helianthi*. The observed inhibition rates of 76.32% for *T. harzianum* and 70.63% for *T. viride* at 144 hours indicate not only effectiveness but also the sustainability of these biocontrol agents. This aligns with previous research that highlights the role of extracellular enzymes, such as chitinase and glucanase, in the antagonistic mechanisms employed by *Trichoderma*, which effectively disrupt fungal cell walls (Harman et al., 2004). The gradual increase in inhibition over time reinforces the idea that these agents can provide long-term control of fungal pathogens.

In comparison to the *Trichoderma* treatments, the chemical fungicide Hexaconazole at 0.2% consistently demonstrated superior efficacy, achieving inhibition rates of 72.58%, 82.34%, and 84.69% at 3, 5, and 7 days after inoculation, respectively. These results confirm the broad-spectrum efficacy of Hexaconazole, attributed to its mode of action that inhibits ergosterol biosynthesis, an essential component of fungal cell membranes (Dhingra and Sinclair, 1995, Sharma et al., 2018). While chemical fungicides are effective, their use raises concerns about environmental sustainability and the potential for developing resistance among fungal populations, highlighting the need for integrated pest management strategies that include both biological and chemical controls.

Among the plant-based treatments, Neem (*Azadirachta indica*) emerged as particularly effective, especially at higher concentrations, with inhibition rates of 29.68%, 53.72%, and 73.49% at 5%, 10%, and 15%, respectively. The efficacy of Neem is attributed to its bioactive compounds, such as azadirachtin, which interfere with fungal growth and spore germination (Bharati et al., 2010). This finding supports the increasing interest in using botanical extracts as environmentally friendly alternatives to synthetic fungicides.

Similarly, Ashwagandha (*Withania somnifera*) demonstrated notable antifungal activity, with inhibition rates ranging from 24.03% to 53.66%. Its antifungal properties are linked to its steroidal lactones and alkaloids, which disrupt fungal cellular processes (Sinha et al., 2010, Singh and Kapoor, 2015). The increasing inhibition

percentages with higher concentrations highlight its potential as an effective natural fungicide, complementing the use of *Trichoderma* species in pest management.

While Aloe vera exhibited moderate efficacy (13.70% to 44.70%), Turmeric (*Curcuma longa*) and Lantana (*Lantana camara*) showed lower inhibition rates of 5.10% to 24.98% and 8.71% to 31.27%, respectively. These results indicate that, while these plant extracts contain antifungal compounds, their effectiveness can be influenced by concentration and the specific fungal target (Rajasekaran et al., 2006, Choi et al., 2004). In contrast, Pudina (*Mentha sp.*) consistently showed the least effectiveness, with inhibition rates ranging from 1.74% to 16.54%, suggesting that its antifungal compounds are less potent against *Alternaria helianthi* compared to the other treatments (Choi et al., 2004).

Overall, the results of this study contribute significantly to the understanding of biological control in managing fungal pathogens. The integration of *Trichoderma* species and effective plant extracts like Neem and Ashwagandha into pest management strategies presents a promising approach to sustainable agriculture. This research not only reinforces the efficacy of biocontrol agents but also highlights the need for further exploration into the synergistic effects of combining biological and chemical treatments for optimal disease management in crops.

5. CONCLUSIONS

The study evaluated the antifungal efficacy of two bio-agents, *Trichoderma viride* and *Trichoderma harzianum*, and six plant extracts against *Alternaria helianthi in vitro*. *T. harzianum* consistently outperformed *T. viride* in inhibiting mycelial growth, achieving up to 76.32% inhibition at 144 hours. Among the plant extracts, neem showed the highest antifungal efficacy across all tested concentrations, with up to 75.03% inhibition by the 7thDAI. Other plant extracts, including ashwagandha, aloe vera, lantana, turmeric, and pudina, also reduced mycelial growth but to a lesser extent. Hexaconazole, the chemical control, demonstrated the highest overall inhibition at 84.69%. These results highlight the potential of both bio-agents and plant extracts as effective and eco-friendly alternatives for managing fungal diseases in agriculture.

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 writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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