In Vitro evaluation of *Trichoderma* species and some selected botanical extracts against *Alternaria helianthi* (Hansf) causing Alternaria blight of sunflower

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

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 (World agricultural production, 2022). 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 helianthin 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.

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.

Results:

***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*.

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

Bio agents	48hrs.		72hrs.		96hrs.		120hrs.		144hrs		Avg.
	Mycelium growth (MG) in mm and mycelium inhibition (MI) in %										
	MG	MI	MG	MI	MG	MI	MG	MI	MG	MI	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
T.viride(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
T.harzianum (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		

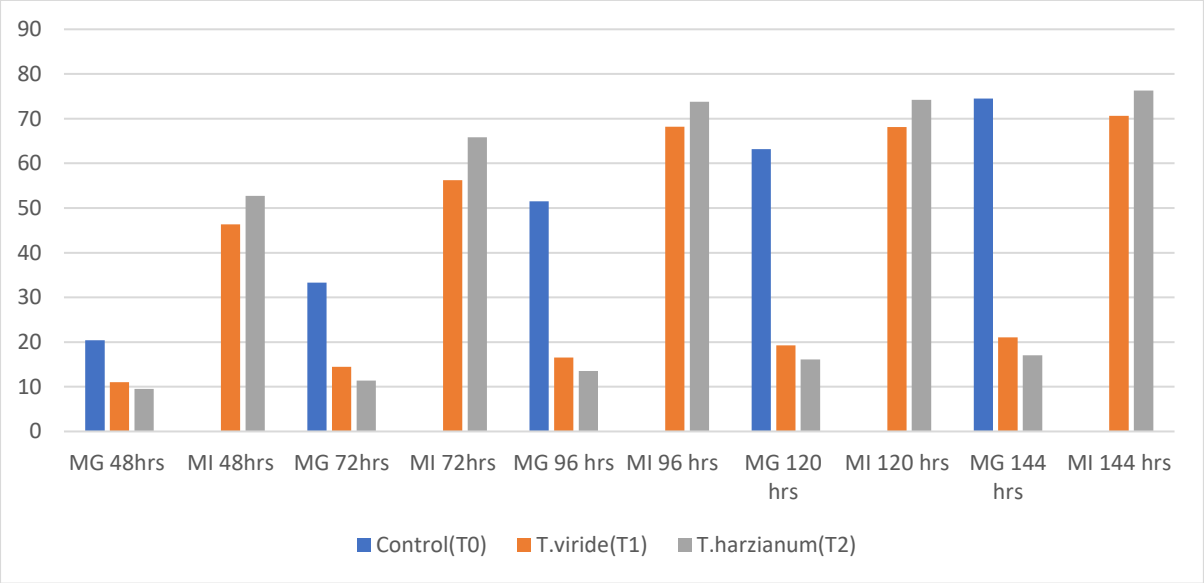

Fig 1. Comparison of mycelium growth (mm)and inhibition (%) among the treatment

Plate 1. A) *T. viride* B) *T. harzianum C*) control at144 hrs.

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 (T_0) 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 (T_1) 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 (T_2**)** 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 (T_3) and Aloe vera (T_4) 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 (T_5) had a radial growth of 25.28 mm (24.03% inhibition) at 3rd DAI, with increasing inhibition percentages over time. Pudina (T_6) exhibited minimal inhibition, with growth at 32.70 mm (1.74% inhibition) at 3rd DAI, while Hexaconazole at 0.2% (T_7 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*.

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

Treatment no	Treatments	Radial growth @3rd DAI	Inhibition% @ 3rd DAI	Radialgrowth@ 5th DAI	Inhibition%@ 5thDAI	Radial growth @7th DAI	Inhibition %@ 7th 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	

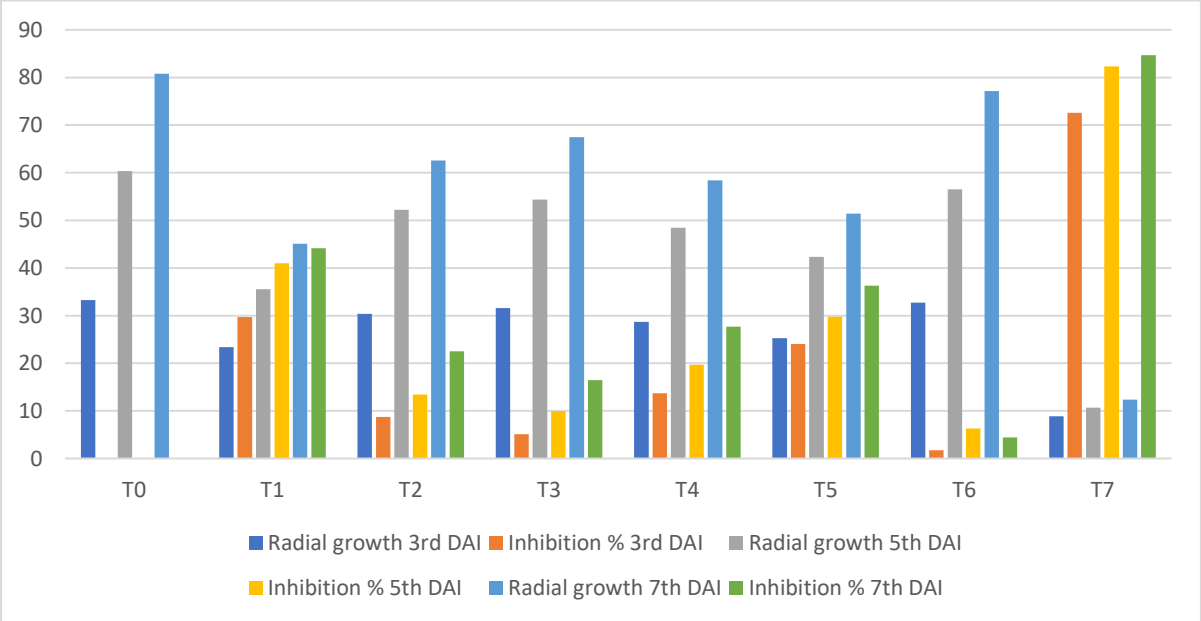

Fig 2 Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 5% concentration

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@3rd DAI	Inhibition %@3rd DAI	Radial growth@ 5th DAI	Inhibition %@ 5th DAI	Radial growth @7th DAI	Inhibition %@ 7th 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	

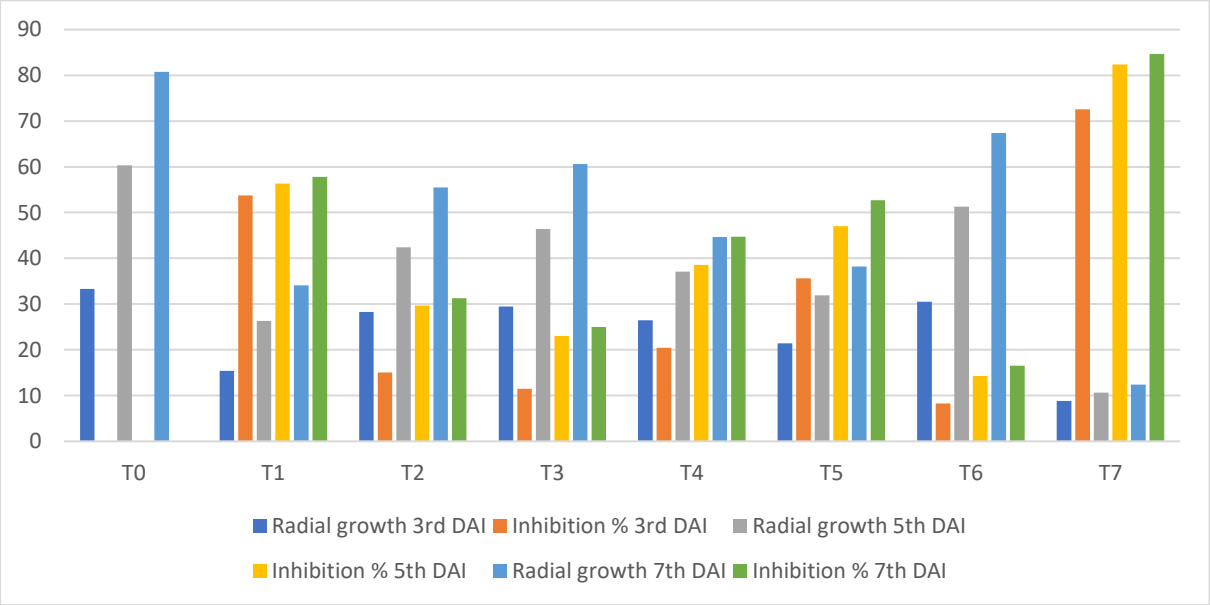

Fig 3. Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 10% concentration

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 (T_0) had a radial growth of 33.28 mm at 3rd DAI, increasing to 80.76 mm by 7th DAI. Neem (T_1**)** 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 (T_2) 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 (T_3) had growth of 29.46 mm (11.47% inhibition) at 3rd DAI and 60.58 mm (24.98% inhibition) at 7th DAI. Aloe vera (T_4) showed 26.48 mm (20.43% inhibition) at 3rd DAI, while Ashwagandha (T_5) 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 4. **Effect of selected plant leaf extracts on the radial growth (mm) and inhibition percent at 15% concentration**

Mean of the five replications							
Treatment no	Treatments	Radial growth @ 3rd DAI	Inhibition% @ 3rd DAI	Radial growth @ 5th DAI	Inhibition %@ 5th DAI	Radial growth@ 7th DAI	Inhibition %@ 7th 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	

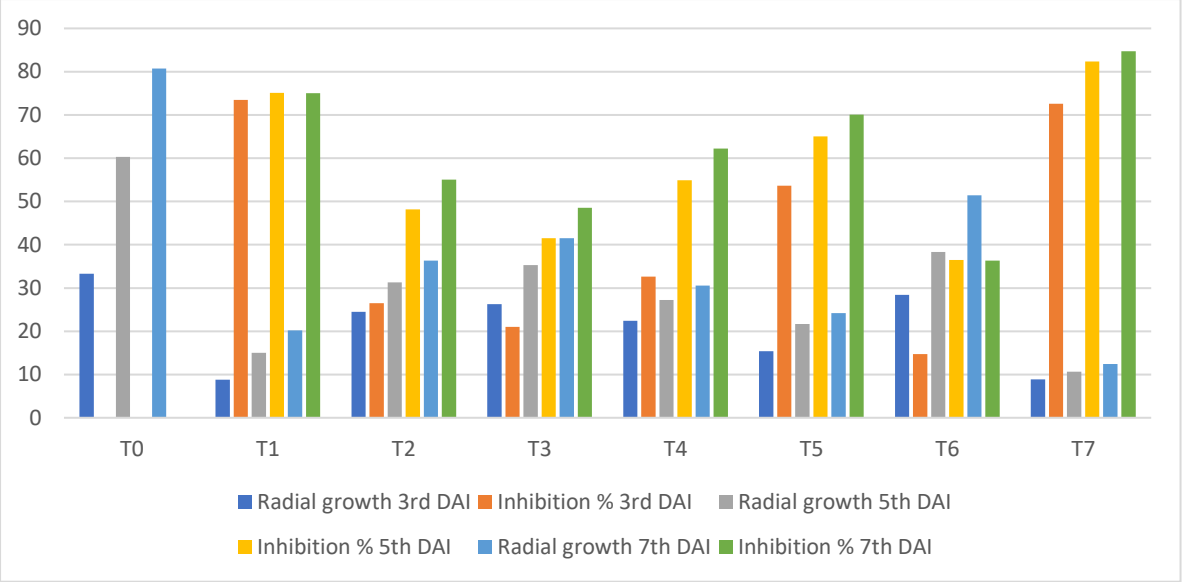

Fig 4 Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 15% concentration

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 (T_0) showed radial growth of 33.28 mm at 3rd DAI, increasing to 80.76 mm by 7th DAI. Neem (T_1) 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 (T_2) 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 (T_3) 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 (T_4) showed 22.42 mm (32.63% inhibition) at 3rd DAI, while Ashwagandha (T_5) had 15.42 mm (53.66% inhibition) at 3rd DAI and 24.18 mm (70.05% inhibition) at 7th DAI. Pudina (T_6) 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.

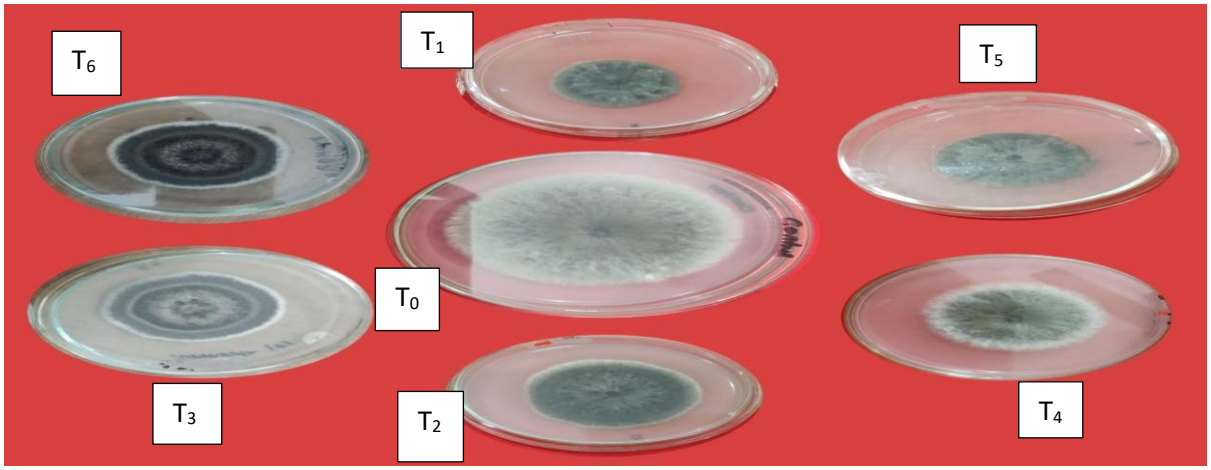

Plate 2. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 5% concentration on 7Th DAI.

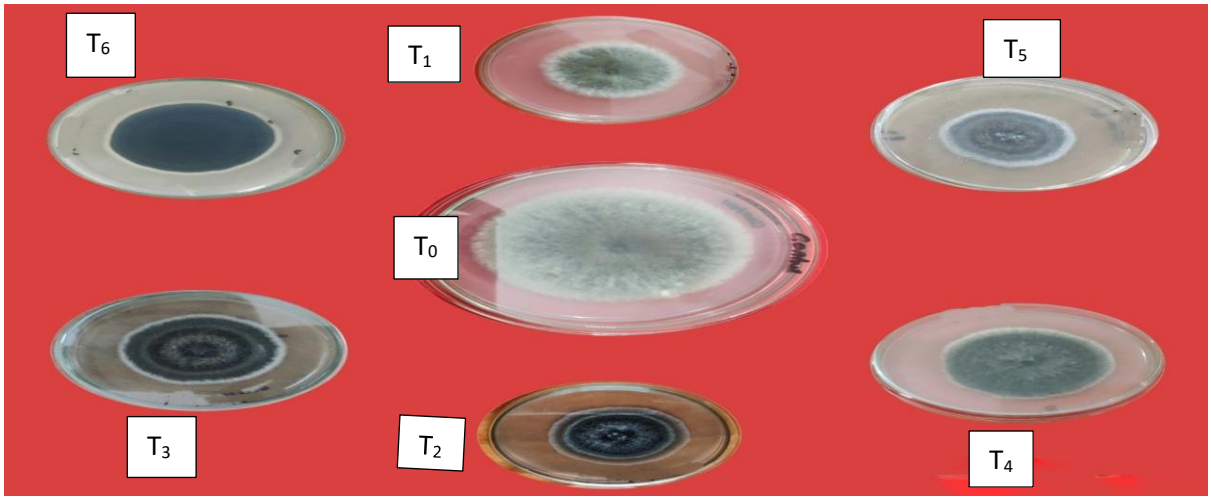

Plate 3. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 10% concentration on 7Th DAI.

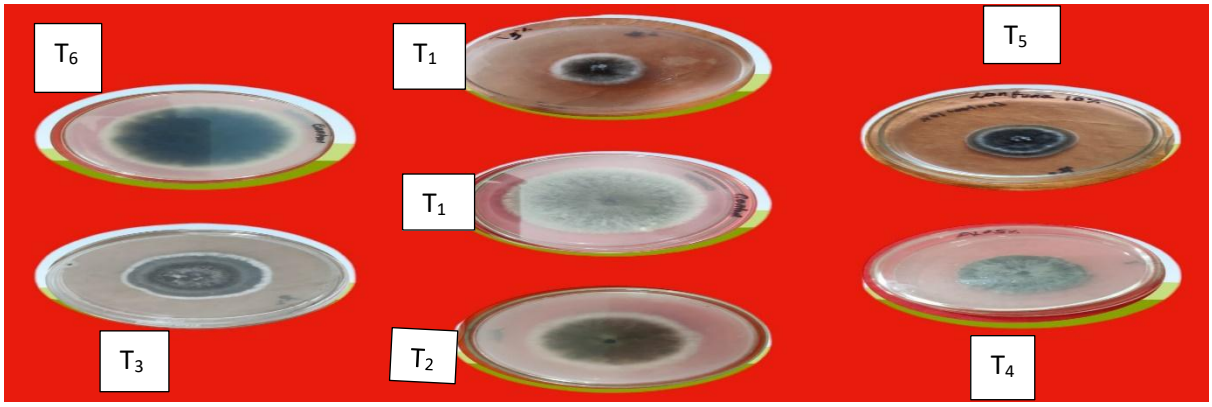

Plate 4. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 15% concentration on 7Th DAI.

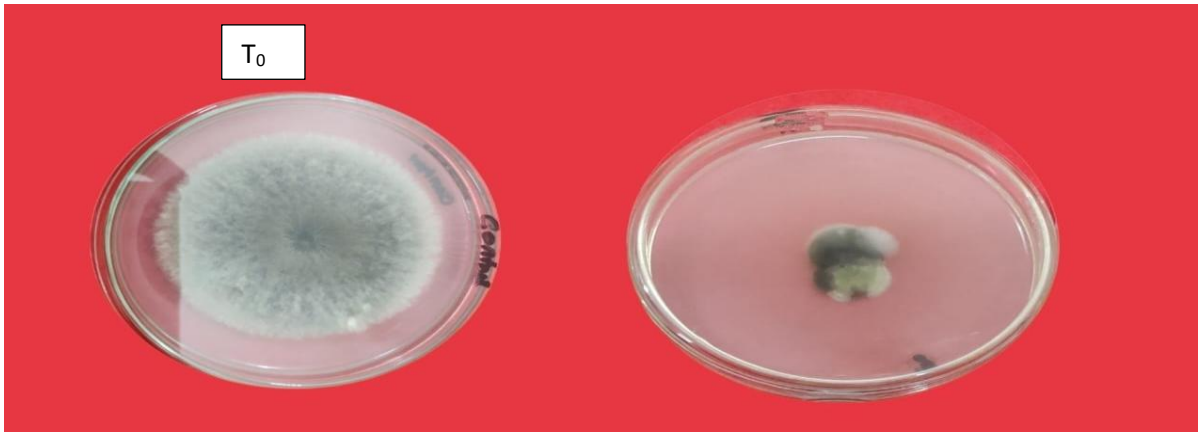

Plate 5. Mycelial growth of *Alternaria helianthi* on PDA media with Hexaconazole 0.2% (Treated check) on 7th DAI.

Discussion:

This study highlights the effectiveness of bioagents and botanical extracts in inhibiting the mycelium growth of *Alternaria helianthi*, a significant fungal pathogen. Both *Trichoderma viride* and *Trichoderma harzianum* demonstrated considerable antifungal activity, with *T. harzianum* exhibiting superior inhibition, achieving an average of 68.58% compared to 61.90% for *T. viride*. This suggests that *T. harzianum* may possess more potent antagonistic mechanisms, possibly due to its ability to produce mycotoxins and enzymes that disrupt fungal cell walls. The observed time-dependent increase in mycelium inhibition indicates that these bioagents can effectively suppress fungal growth over time, emphasizing the importance of application timing in integrated pest management strategies.

In evaluating the botanical extracts, neem and ashwagandha stood out as the most effective treatments, particularly at higher concentrations. Neem consistently showed high inhibition rates, likely due to its active compounds, such as azadirachtin, which are known to interfere with fungal growth and reproduction. Ashwagandha also demonstrated significant antifungal properties, supporting its potential use in disease management. Other botanicals, like turmeric and aloe vera, exhibited moderate inhibition, suggesting that while they may have some beneficial effects, they are less effective than neem and ashwagandha.

The study underscores the critical role of concentration in the efficacy of both bioagents and botanical extracts, with higher doses leading to increased inhibition rates. This finding is essential for practical applications, as proper dosage can enhance disease control. Furthermore, while hexaconazole provided a strong benchmark for antifungal activity, the promising results from neem and ashwagandha suggest that they could serve as sustainable alternatives to

conventional chemical fungicides, which may have detrimental environmental impacts and contribute to pathogen resistance.

Overall, the findings of this research support the potential of integrating biological and botanical treatments into crop protection strategies, paving the way for more sustainable agricultural practices. Future studies should explore field trials to validate these results in real-world conditions and further investigate the mechanisms underlying the antifungal activities of these agents.

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 7^{th}DAI. 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.

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