

***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 of bioagents and botanicals in eco-friendly management strategies for Alternaria blight in sunflower cultivation.

**Keywords:** *Trichoderma* species, botanical extracts, *Alternaria helianthi* , Alternaria blight, sunflower

**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.

**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 <sub>0</sub> )	20.35 <sup>a</sup>	00.00	33.35 <sup>a</sup>	00.00	51.40 <sup>a</sup>	00.00	63.28 <sup>a</sup>	00.00	74.48 <sup>a</sup>	00.00	00.00
<i>T.viride</i> (T <sub>1</sub> )	10.92 <sup>b</sup>	46.33	14.60 <sup>b</sup>	56.22	16.34 <sup>b</sup>	68.21	20.17 <sup>b</sup>	68.12	21.87 <sup>b</sup>	70.63	61.90
<i>T.harzianum</i> (T <sub>2</sub> )	9.62 <sup>c</sup>	52.72	11.39 <sup>c</sup>	65.84	13.48 <sup>c</sup>	73.77	16.16 <sup>c</sup>	74.25	17.63 <sup>c</sup>	76.32	68.58
C.D. (5%)	0.47		0.49		0.49		0.77		0.64		

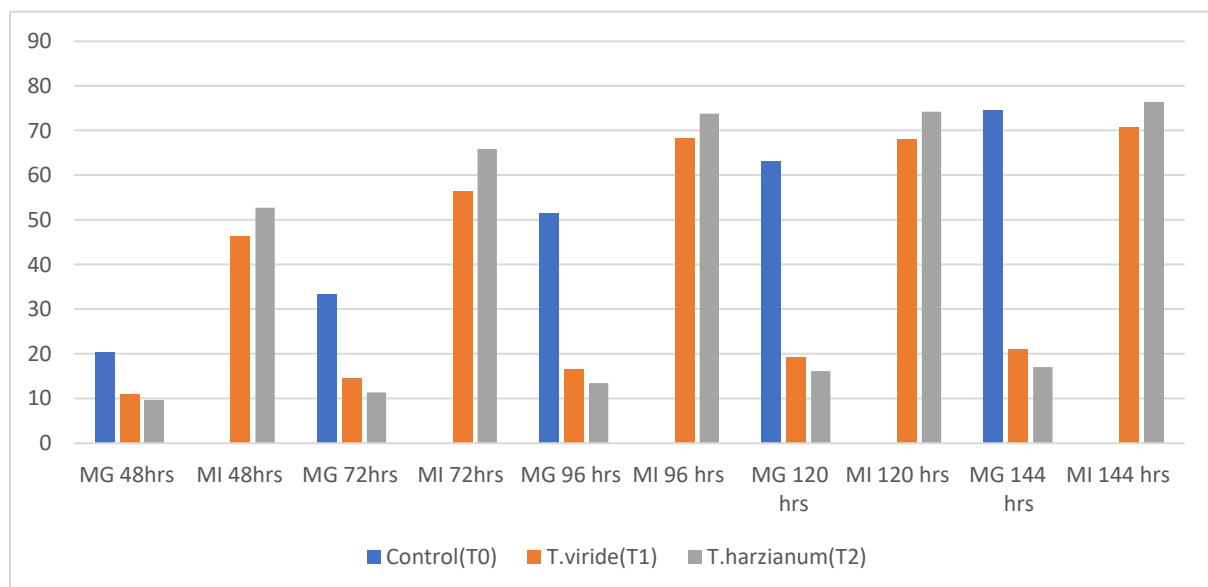


Fig 1. Comparison of mycelium growth (mm) and inhibition (%) among the treatment

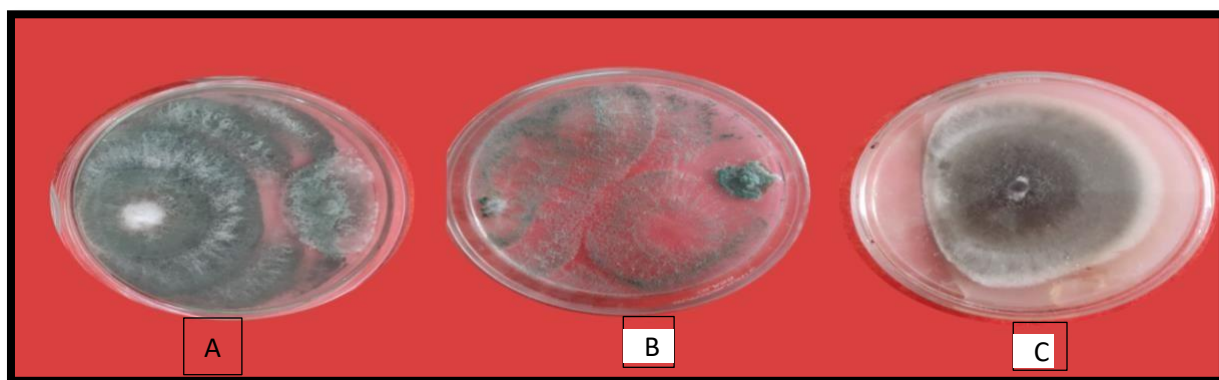


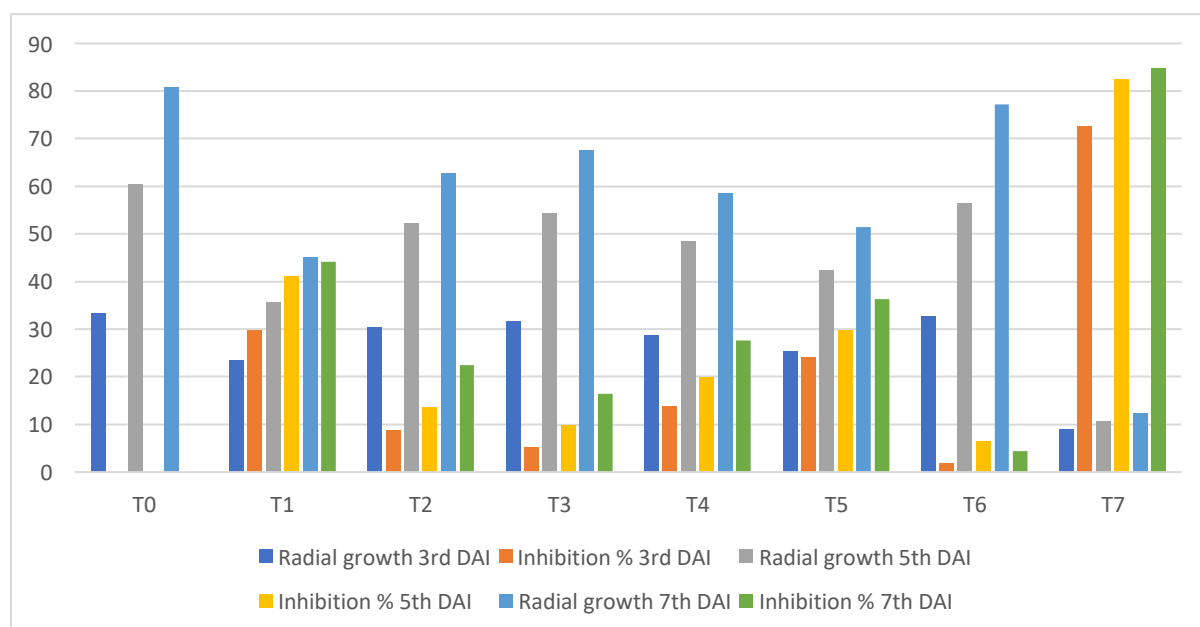
Plate 1. A) *T. viride* B) *T. harzianum* C) control at 144 hrs.

### Efficacy of botanical extracts

The data presented in Table 2 revealed the mean radial growth and inhibition percentages of *Alternaria helianthi* at 3<sup>rd</sup> days after inoculation (DAI), 5<sup>th</sup> DAI and 7<sup>th</sup> DAI across various treatments at 5% concentration. The untreated control (T<sub>0</sub>) exhibited a radial growth of 33.28 mm at 3<sup>rd</sup> DAI, increasing to 60.32 mm at 5<sup>th</sup> DAI, and 80.76 mm at 7<sup>th</sup> DAI. Among the treatments, Neem extract (T<sub>1</sub>) showed the highest inhibition, with radial growth of 23.4 mm (29.68% inhibition) at 3<sup>rd</sup> DAI, 35.58 mm (41.01% inhibition) at 5<sup>th</sup> DAI and 45.10 mm (44.15% inhibition) at 7<sup>th</sup> DAI. Lantana (T<sub>2</sub>) had moderate effects, with growth at 30.38 mm (8.71% inhibition) at 3<sup>rd</sup> DAI, 52.22 mm (13.42% inhibition) at 5<sup>th</sup> DAI and 62.58 mm (22.51% inhibition) at 7<sup>th</sup> DAI. Turmeric (T<sub>3</sub>) and Aloe vera (T<sub>4</sub>) also demonstrated some inhibition, with growth measurements of 31.58 mm (5.10% inhibition) and 28.72 mm (13.70% inhibition) at 3<sup>rd</sup> DAI, respectively. Ashwagandha (T<sub>5</sub>) had a radial growth of 25.28 mm (24.03% inhibition) at 3<sup>rd</sup> DAI, with increasing inhibition percentages over time. Pudina (T<sub>6</sub>) exhibited minimal inhibition, with growth at 32.70 mm (1.74% inhibition) at 3<sup>rd</sup> DAI, while Hexaconazole at 0.2% (T<sub>7</sub> treated check) was the most effective treatment, showing only 8.85 mm growth (72.58% inhibition) at 3<sup>rd</sup> DAI, 10.65 mm (82.34% inhibition) at 5<sup>th</sup> DAI and 12.39 mm (84.69% inhibition) at 7<sup>th</sup> 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**

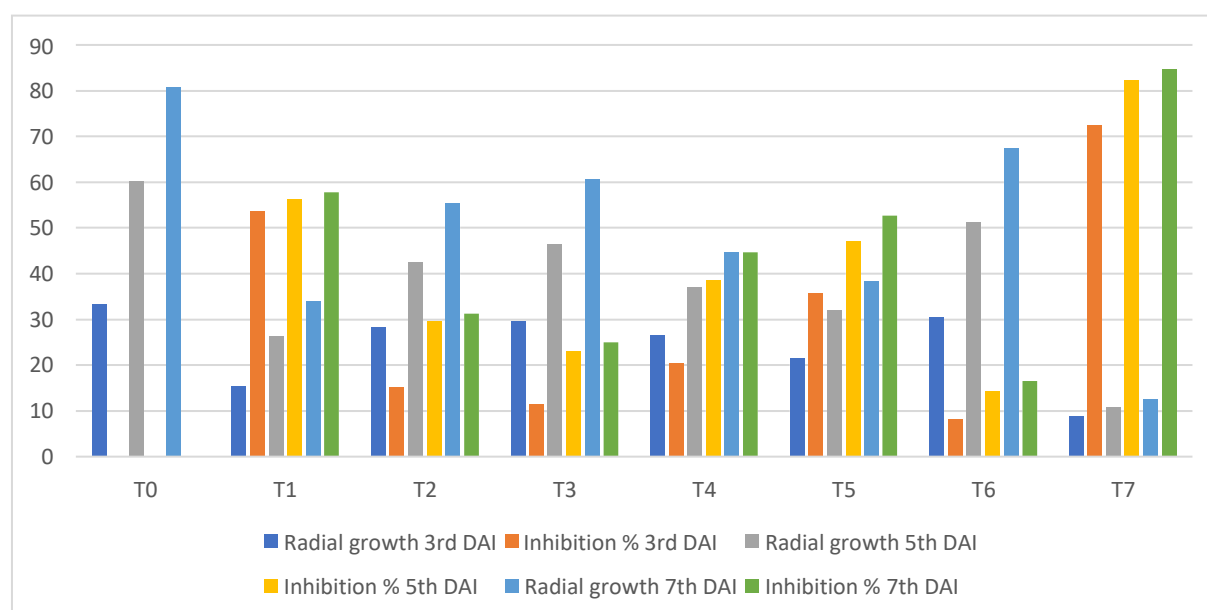
Treatm ent no	Treatments	Radial growth @3 <sup>rd</sup> DAI	Inhibition% @ 3 <sup>rd</sup> DAI	Radialgr owth@ 5 <sup>th</sup> DAI	Inhibitio n% @ 5 <sup>th</sup> DAI	Radial growth @7 <sup>th</sup> DAI	Inhibition % @ 7 <sup>th</sup> DAI
T <sub>0</sub>	Untreated check	33.28	0.00	60.32	0.00	80.76	0.00
T <sub>1</sub>	Neem	23.4	29.68	35.58	41.01	45.10	44.15
T <sub>2</sub>	Lantana	30.38	8.71	52.22	13.42	62.58	22.51
T <sub>3</sub>	Turmeric	31.58	5.10	54.34	9.91	67.46	16.46
T <sub>4</sub>	Aloe vera	28.72	13.70	48.42	19.72	58.40	27.68
T <sub>5</sub>	Ashwagandha	25.28	24.03	42.36	29.77	51.44	36.30
T <sub>6</sub>	Pudina	32.70	1.74	56.50	6.33	77.18	4.43
T <sub>7</sub>	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**

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

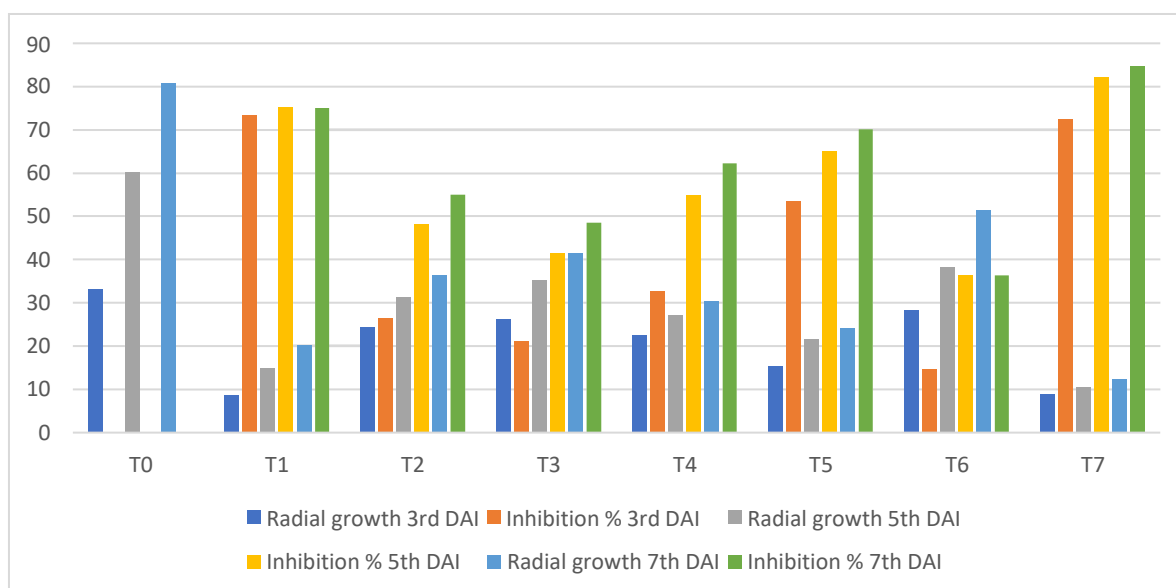


**Fig 3. Effect of selected plant extracts on radial growth (mm) of *Alternaria helianthi* and percent inhibition (%) at 10% concentration**

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<sub>0</sub>) had a radial growth of 33.28 mm at 3<sup>rd</sup> DAI, increasing to 80.76 mm by 7<sup>th</sup> DAI. Neem (T<sub>1</sub>) was effective, with growth reduced to 15.4 mm (53.72% inhibition) at 3<sup>rd</sup> DAI and 34.08 mm (57.80% inhibition) at 7<sup>th</sup> DAI. Lantana (T<sub>2</sub>) showed moderate inhibition, with growth at 28.28 mm (15.02% inhibition) at 3<sup>rd</sup> DAI and 55.50 mm (31.27% inhibition) at 7<sup>th</sup> DAI. Turmeric (T<sub>3</sub>) had growth of 29.46 mm (11.47% inhibition) at 3<sup>rd</sup> DAI and 60.58 mm (24.98% inhibition) at 7<sup>th</sup> DAI. Aloe vera (T<sub>4</sub>) showed 26.48 mm (20.43% inhibition) at 3<sup>rd</sup> DAI, while Ashwagandha (T<sub>5</sub>) had 21.42 mm (35.63% inhibition) at 3<sup>rd</sup> DAI and 38.20 mm (52.69% inhibition) at 7<sup>th</sup> 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							
Treatm ent no	Treatments	Radial growth @ 3 <sup>rd</sup> DAI	Inhibitio n% @ 3 <sup>rd</sup> DAI	Radial growth @ 5 <sup>th</sup> DAI	Inhibition % @ 5 <sup>th</sup> DAI	Radial growth@ 7 <sup>th</sup> DAI	Inhibition % @ 7 <sup>th</sup> DAI
T <sub>0</sub>	Untreated check	33.28	0.00	60.32	0.00	80.76	0.00
T <sub>1</sub>	Neem	8.82	73.49	15.00	75.13	20.16	75.03
T <sub>2</sub>	Lantana	24.46	26.50	31.26	48.17	36.32	55.02
T <sub>3</sub>	Turmeric	26.28	21.03	35.28	41.51	41.54	48.56
T <sub>4</sub>	Aloe vera	22.42	32.63	27.22	54.87	30.52	62.20
T <sub>5</sub>	Ashwagandha	15.42	53.66	21.68	65.05	24.18	70.05
T <sub>6</sub>	Pudina	28.38	14.72	38.30	36.50	51.40	36.35
T <sub>7</sub>	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<sub>0</sub>) showed radial growth of 33.28 mm at 3<sup>rd</sup> DAI, increasing to 80.76 mm by 7<sup>th</sup> DAI. Neem (T<sub>1</sub>) was the most effective treatment, with growth reduced to 8.82 mm (73.49% inhibition) at 3<sup>rd</sup> DAI, 15.00 mm (75.13% inhibition) at 5<sup>th</sup> DAI, and 20.16 mm (75.03% inhibition) at 7<sup>th</sup> DAI. Lantana (T<sub>2</sub>) demonstrated moderate inhibition, with growth at 24.46 mm (26.50% inhibition) at 3<sup>rd</sup> DAI and 36.32 mm (55.02% inhibition) at 7<sup>th</sup> DAI. Turmeric (T<sub>3</sub>) had radial growth of 26.28 mm (21.03% inhibition) at 3<sup>rd</sup> DAI and 41.54 mm (48.56% inhibition) at 7<sup>th</sup> DAI. Aloe vera (T<sub>4</sub>) showed 22.42 mm (32.63% inhibition) at 3<sup>rd</sup> DAI, while Ashwagandha (T<sub>5</sub>) had 15.42 mm (53.66% inhibition) at 3<sup>rd</sup> DAI and 24.18 mm (70.05% inhibition) at 7<sup>th</sup> DAI. Pudina (T<sub>6</sub>) exhibited lower inhibition, with growth of 28.38 mm (14.72% inhibition) at 3<sup>rd</sup> 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.



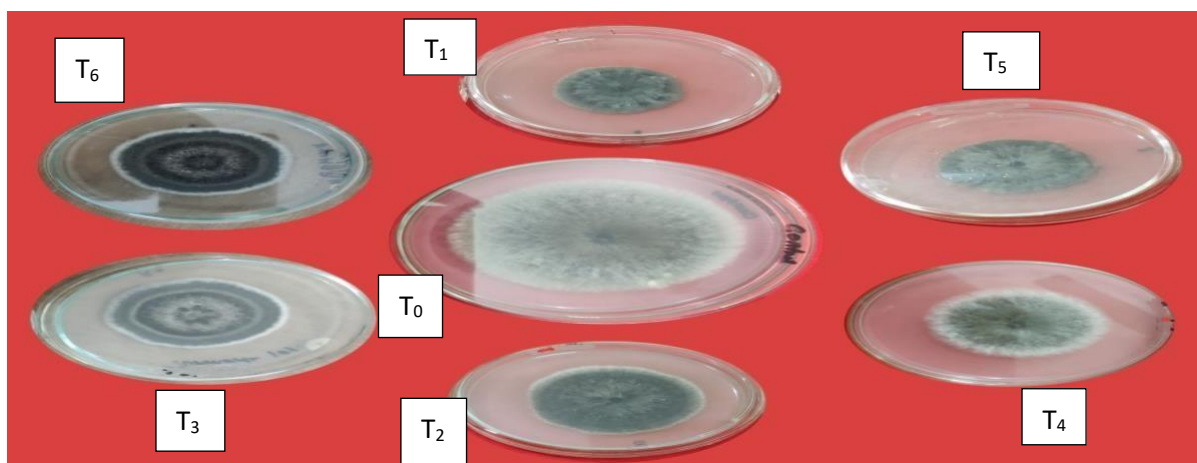


Plate 2. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 5% concentration on 7<sup>th</sup> DAI.

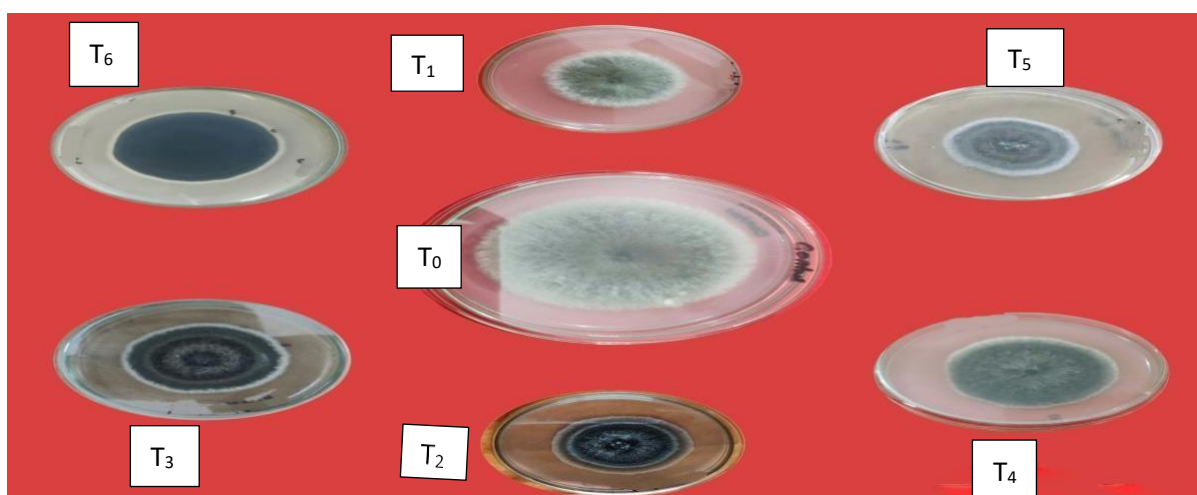


Plate 3. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 10% concentration on 7<sup>th</sup> DAI.

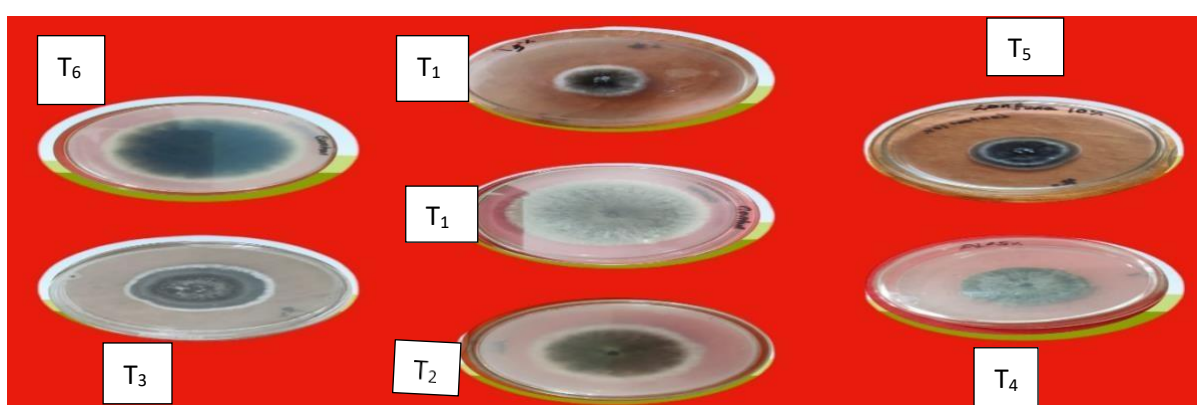


Plate 4. Effect of selected plant extracts on the mycelial growth of *Alternaria helianthi* at 15% concentration on 7<sup>th</sup> DAI.



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

### 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 et al., 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.

## 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<sup>th</sup>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.

Disclaimer (Artificial intelligence)

Option 1:

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 the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

## References:

- Arora, D. K. and Upadhyay, R. K. (1978).** Effect of fungal staling growth substances on colony interaction. *Plant and Soil*, pp: 685-690.
- Brand, S. I., Heldwein, A. B., Radons, S. Z., Maldaner, I. C., Hinnah, F. D., Guse, F. I. and da Silva, J. R. (2020).** Effect of Alternaria and Septoria spot on sunflower yield. *International Journal of Bio meteorology*. 64(12):2153-2160.
- Dennis, C. and Webster, J. (1971).** Antagonistic properties of species group of *Trichoderma* and hyphal interactions. *British Mycology Society*. 57:363-369.
- Gulya, T. J., Rashid, K. Y., and Masirevic, S. M. (1994).** Sunflower diseases: Important diseases worldwide and their occurrence in the USA. *USDA Agricultural Research Service Bulletin*. 49 (1): 10-15.
- Udayashankar, A. C., Nayaka, S. C., & Reddy, M. S. (2012).** Biocontrol of *Alternaria helianthi* causing Alternata blight of sunflower by rhizosphere bacteria. *Archives of Phytopathology and plant Protection*, 45(7): 837-847.
- Yegorov, B., Turpurova, S. E. and Bondar, Y. (2019).** Prospects of using by- products of sunflower oil production in compound feed, T. industry. *Journal of Food Science Technology Ukraine*.13: 106-113.

**Bharati, K., Gupta, A., & Sharma, K. (2010).** Efficacy of neem (*Azadirachta indica*) against various fungal pathogens. *Journal of Phytopathology*, 158(3), 205-211.

**Choi, W. K., Kim, H. K., & Lee, J. H. (2004).** Antifungal activities of plant extracts against *Alternaria* species. *Mycobiology*, 32(4), 217-220.

**Dhingra, O. D., & Sinclair, J. B. (1995).** *Basic Plant Pathology Methods*. CRC Press.

**Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Staples, R. C. (2004).** Trichoderma species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2(1), 43-56.

**Rajasekaran, T., Ravikumar, K., & Muthulakshmi, P. (2006).** Antifungal activity of turmeric (*Curcuma longa*) on *Fusarium* and *Alternaria* species. *Journal of Agricultural Technology*, 2(1), 145-152.

**Sharma, M., Sharma, S., & Kumar, V. (2018).** Efficacy of fungicides against various fungal diseases in plants. *Plant Disease Research*, 33(2), 157-163.

**Sinha, K. K., Mohan, K., & Ghosh, S. K. (2010).** Antifungal properties of *Withania somnifera*. *Phytotherapy Research*, 24(6), 856-861.

**Singh, R., & Kapoor, K. (2015).** Antifungal activities of *Ashwagandha* extracts. *International Journal of Scientific Research*, 4(5), 125-127.