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# *In-vitro* Evaluation of Bio Agents and Botanical Extracts against *Alternaria alternata* Causing Alternaria Blight of Dahlia

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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 effectiveness of various bio-agents (*Trichoderma viride* and *Trichoderma harzianum*) and plant-based botanical extracts (neem, marigold, eucalyptus, ashwagandha, tulsi) in managing *Alternaria alternata*, a fungal pathogen responsible for Alternaria blight in Dahlia (*Dahlia variabilis*). The research was conducted using the poisoned food technique to assess the antifungal properties of these treatments at different concentrations (5%, 10%, 15%). In the case of botanicals Neem leaf extract showed the minimum growth a maximum inhibition (19.75 mm, 75.99%) and least were Tulsi leaf extract with maximum growth and minimum inhibition (61.60 mm, 25.13%) against *A. alternata* on the other hand *Trichoderma viride* showed the minimum growth a maximum inhibition (19.68 mm, 72.73%) and least were *Trichoderma harzianum* with maximum growth and minimum inhibition (24.51 mm, 66.03%) against *A. alternata*. Results indicate that both bioagents,

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particularly *Trichoderma viride*, and botanical extracts, especially neem and eucalyptus, significantly inhibited the mycelial growth of the pathogen. Mancozeb, a chemical fungicide used as a treated check, exhibited the highest inhibitory effect. These findings suggest that integrating bio-agents and botanicals could be an effective strategy for sustainable management of Alternaria blight in Dahlia cultivation.

*Keywords: Dahlia; bio-agents; fungal pathogen; neem leaf; Tulsi leaf.*

## 1. INTRODUCTION

Dahlia (*Dahlia variabilis*) is a widely cherished flowering plant cultivated globally for its stunning, ornamental blooms that come in an array of vibrant colours, making it ideal for garden decoration and as a source for cut flowers. This tuberous-rooted, half-hardy perennial belongs to the family Asteraceae. Dahlia is an important flowering plant cultivated globally for garden decoration and cut flowers. *Alternaria alternata* is an important disease that affects all plant parts [1]. Chromosome count of 2n = 64, these plants can reach heights of up to 1.2 m and are known for their triangular leaves and vibrant blooms [2]. The 2023-24 report from the Agricultural and Processed Food Products Export Development Authority (APEDA) highlights significant growth in India's floriculture exports, encompassing products such as cut flowers, tubers, and rooted plants. This sector has generated substantial revenue, reaching an estimated 717.83 crore INR from April 2023 onward. The report also underscores the expanding demand for Indian floricultural products in global markets, with primary importers including the United States, the Netherlands, the UAE, and the UK [3].

Dahlias are susceptible to various diseases, particularly *Alternaria alternata*, which can severely impact ornamental value and marketability, especially in humid conditions [4,5]. Symptoms include dark leaf spots, yellowing, and premature defoliation, leading to potential yield losses of 30-40% [5]. Control measures like the broad-spectrum fungicide Mancozeb have been standard; however, natural plant extracts (*e.g.*, neem, marigold) and biological controls using *Trichoderma* species have emerged as promising alternatives [6,7]. This study evaluates the efficacy of these bioagents and selected botanicals against *Alternaria alternata*, aiming to enhance sustainable management practices in dahlia cultivation.

## 2. MATERIALS AND METHODS

A comprehensive study was conducted in Prayagraj, Uttar Pradesh, from 2023 to evaluate bio agents and botanical extracts against *Alternaria alternata* causing Alternaria blight of Dahlia. This study targeted to find alternatives way to managed Alternaria blight of dhalia by avoiding chemical fungicide and using botanicals and bio-agents. In this study, firstly isolated *Alternaria alternata* from the affected dahlia plants in horticulture field of SHUATS, Prayagraj and also two species of *Trichoderma*, *Trichoderma viride* and *Trichoderma harzianum*, were evaluated for their efficacy against the previously identified *Alternaria alternata* using the dual culture technique as described by Dennis and Webster [8].

**Table 1. Details of bioagents tested against *Alternaria alternata***

| Treatments | Bio control agent |
|---|---|
| $T_0$ | Control |
| $T_1$ | *Trichoderma viride* |
| $T_2$ | *Trichoderma harzianum* |

The botanical extracts tested included Neem [9], Marigold [10], Eucalyptus [11], Ashwagandha and Tulsi [12] applied at concentrations of 5%, 10%, and 15%.

Prepared plant extracts by the using the 1:1 weight/volume ratio. Plant extracts were mixed into potato dextrose agar (PDA) media, and a 90 mm disc of *A*. *alternata* was inoculated to assess their effectiveness through the poisoned food technique. The plates were incubated at 26° C, and radial growth was measured on the 3rd, 5th, and 7th days following inoculation. Observations from the dual culture method were recorded at 24-hour intervals until the control plates were completely covered. Mycelial inhibition for both methods was calculated using the formula provided by Arora and Upadhyay [13].

$$I = (C - T / C) \times 100$$

Where (I) represents the percentage of mycelium inhibition, (C) is the mycelium growth (mm) in the control, and (T) is the mycelium growth (mm) in the treatment. The gathered data were analyzed using ANOVA, and significant differences among treatments were assessed at a 5% probability level.

**Table 2. Treatment details (botanicals)**

| Sl no | Botanical name | Local name | Family | Medicinal use | Plant part used | References |
|---|---|---|---|---|---|---|
| $T_0$ | Control | untreated | | | | |
| $T_1$ | *Azadirachta indica* | Neem | Meliaceae | Antifungal | leaves | Choudhary et al. [9] |
| $T_2$ | *Tagetes erecta* | Marigold | Asteraceae | Antifungal | leaves | Mugao et al. [10] |
| $T_3$ | *Eucalyptus globulus* | Eucalyptus | Myrtaceae | Antifungal | leaves | Choudhary and Singh [11] |
| $T_4$ | *Withania somnifera* | Ashwagandha | Solanaceae | Antifungal | leaves | Nene et al. [12] |
| $T_5$ | *Ocimum sanctum* | Tulsi | Lamiaceae | Antifungal | leaves | Nene et al. [12] |
| $T_6$ | Mancozeb@0.2% | Check treated | | | | Gholve et al. (2014) |

## 3. RESULTS

### 3.1 Efficacy of Botanical Extracts

Throughout the study, all treatments exhibited significant differences from the untreated control (T0) at 3rd, 5th and 7th days after inoculation (DAI). Mancozeb (0.2%) (T6) consistently demonstrated the highest efficacy against *Alternaria alternata*, achieving maximum mycelial inhibition of 85.29% by the 7th DAI, with radial growth limited to 12.29 mm. Neem (T1) and Eucalyptus (T3) also proved effective, with inhibition percentages of 56.18% and 52.04%, respectively. Other treatments, including Ashwagandha (T4), Marigold (T2), and Tulsi (T5), showed varying levels of control, generally declining in effectiveness over time. Overall, while Mancozeb was the most effective treatment, neem and eucalyptus emerged as promising natural alternatives for managing this fungal pathogen.

At the 3rd, 5th and 7th days after inoculation (DAI), all treatments showed significant differences from the untreated control ($T_0$), which consistently recorded the highest radial growth with no inhibition. Mancozeb (0.2%) ($T_6$) was the most effective treatment at all intervals, achieving the highest mycelial inhibition, restricting radial growth to 9.45 mm at the 3rd DAI (71.77% inhibition), 11.55 mm at the 5th DAI (81.17% inhibition), and 12.29 mm at the 7th DAI (85.29% inhibition). Neem ($T_1$) and Eucalyptus ($T_3$) were the next most effective, with neem showing 37.28%, 54.19%, and 65.49% inhibition across the three intervals, and eucalyptus achieving 30.96%, 47.92%, and 61.81% inhibition. Ashwagandha ($T_4$) also demonstrated moderate control, while Marigold ($T_2$) and Tulsi ($T_5$) were the least effective, with inhibition percentages decreasing over time. Overall, Mancozeb provided the greatest control, while neem and eucalyptus emerged as promising natural alternatives.

At the 3rd, 5th, and 7th days after inoculation (DAI), Mancozeb (0.2%) ($T_6$) consistently showed the highest inhibition against *Alternaria alternata*, with the lowest radial growth across all intervals— 9.45 mm (71.77% inhibition) at the 3rd DAI, 11.55 mm (81.17% inhibition) at the 5th DAI, and 12.29 mm (85.29% inhibition) at the 7th DAI. Neem ($T_1$) and Eucalyptus ($T_3$) also demonstrated significant control, with inhibition percentages of 55.58% and 47.99% at the 3rd DAI, and improving to 75.99% and 71.24% at the 7th DAI, respectively. Ashwagandha ($T_4$) showed moderate effectiveness, while Marigold ($T_2$) and Tulsi ($T_5$) were the least effective, with inhibition percentages steadily declining over time. Overall, Mancozeb provided the highest level of control, with Neem and Eucalyptus emerging as strong natural alternatives.

### 3.2 *In vitro* Efficacy of Bioagents

The biocontrol potential of *Trichoderma viride* ($T_1$) and *Trichoderma harzianum* ($T_2$) against *Alternaria alternata* was evaluated over a period of 144 hours, with significant differences observed between treatments and the control. At 48 hours, *T. viride* reduced mycelium growth by 50.16%, while *T. harzianum* achieved 46.69% inhibition, both significantly outperforming the control. As the experiment progressed, *T. viride* continued to show superior effectiveness, achieving 72.73% inhibition at 144 hours, with mycelium growth limited to 19.68 mm. *T. harzianum*, while effective, showed slightly lower inhibition at 66.03% with mycelium growth of 24.51 mm. The untreated control exhibited the highest mycelium growth, reaching 72.17 mm with no inhibition observed. These results indicate that *T. viride* consistently demonstrated greater biocontrol potential than *T. harzianum* across all time points, making it a more effective option for managing *A. alternata*.

## 4. DISCUSSION

The study aimed to evaluate the efficacy of various treatments, including chemical and natural biocontrol agents, against *Alternaria alternata*. Mancozeb (0.2%) ($T_6$) consistently emerged as the most effective treatment across all time intervals (3rd, 5th, and 7th DAI), achieving the highest levels of mycelial inhibition. By the 7th DAI, Mancozeb reduced radial growth to 12.29 mm, with an inhibition percentage of 85.29%. This strong performance highlights the potency of Mancozeb as a chemical fungicide, offering rapid and sustained control of *A. alternata*. Among the natural treatments, Neem ($T_1$) and Eucalyptus ($T_3$) demonstrated considerable biocontrol potential, with inhibition percentages improving over time. By the 7th DAI, Neem achieved a 65.49% inhibition, while Eucalyptus followed closely with 61.81%. These findings suggest that both plant-based treatments are promising natural alternatives to chemical control. Neem's consistent efficacy throughout the study aligns with previous reports highlighting its broad-spectrum antifungal properties,

**Table 3. Effect of selected plant leaf extracts on the radial growth (mm) and inhibition (%) at 5 % 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 | 34.23 | 00.00 | 58.38 | 0.00 | 82.28 | 0.00 |
| $T_1$ | Neem | 26.32 | 22.26 | 32.47 | 44.38 | 36.05 | 56.18 |
| $T_2$ | Marigold | 32.40 | 5.34 | 48.51 | 16.90 | 63.44 | 22.89 |
| $T_3$ | Eucalyptus | 29.42 | 14.05 | 36.72 | 37.10 | 39.46 | 52.04 |
| $T_4$ | ashwagandha | 32.59 | 4.79 | 40.49 | 30.64 | 45.47 | 44.73 |
| $T_5$ | Tulsi | 33.63 | 1.75 | 52.53 | 10.02 | 71.53 | 13.06 |
| $T_6$ | Mancozeb (0.2%) | 9.45 | 71.77 | 11.55 | 81.17 | 12.29 | 85.29 |

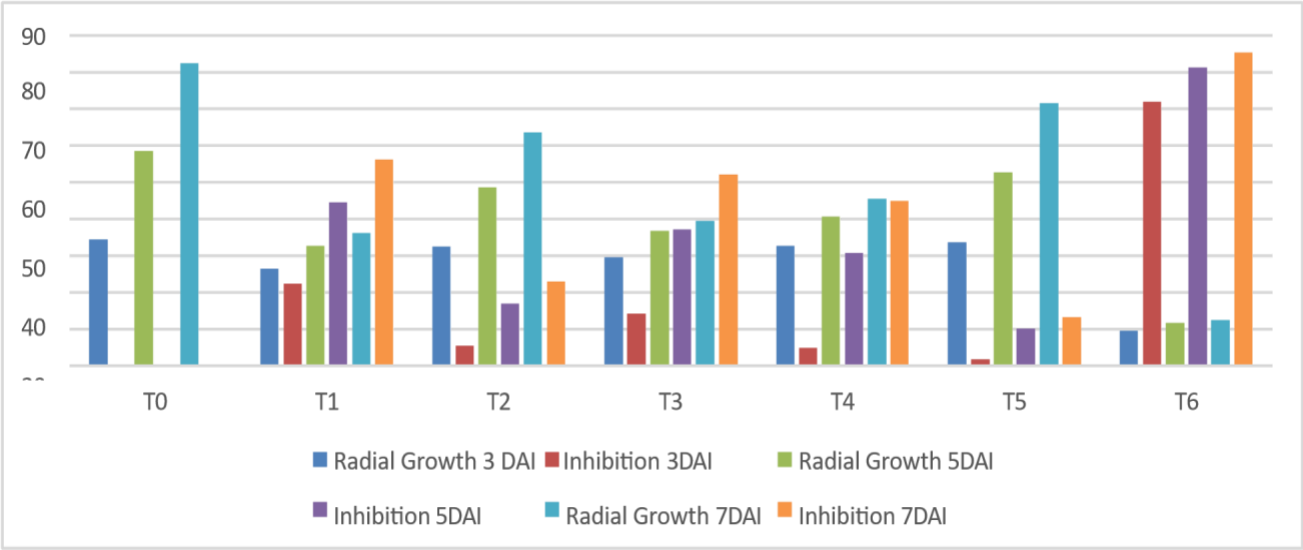


**Fig. 1. Effect of selected botanical extracts on radial growth (mm) and radial inhibition (%) of *Alternaria alternata* at 5% concentration**

**Table 4. Effect of selected plant leaf extracts on the radial growth (mm) and inhibition (%) 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 | 34.23 | 00.00 | 58.38 | 0.00 | 82.28 | 0.00 |
| $T_1$ | Neem | 21.82 | 37.28 | 26.74 | 54.19 | 28.39 | 65.49 |
| $T_2$ | Marigold | 28.49 | 16.76 | 42.44 | 27.30 | 57.56 | 30.04 |
| $T_3$ | Eucalyptus | 23.63 | 30.96 | 30.40 | 47.92 | 41.42 | 61.81 |
| $T_4$ | ashwagandha | 26.73 | 21.91 | 34.58 | 40.76 | 37.51 | 54.41 |
| $T_5$ | Tulsi | 30.50 | 12.06 | 48.51 | 16.90 | 67.32 | 18.18 |
| $T_6$ | Mancozeb (0.2%) | 9.45 | 71.77 | 11.55 | 81.17 | 12.29 | 85.29 |

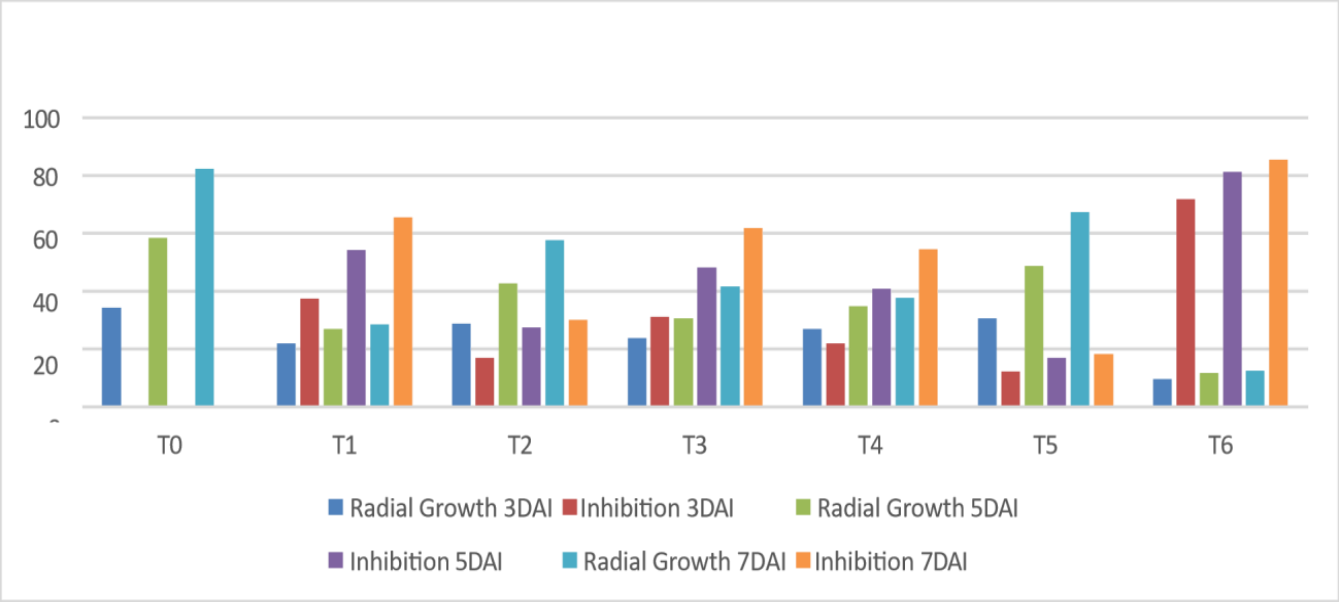


**Fig. 2. Effect of selected botanical extracts on radial growth(mm) and radial inhibition (%) of *Alternaria alternata* at 10% concentration**

**Table 5. Effect of selected plant leaf extracts on the radial growth (mm) and inhibition (%) at 15% 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 | 34.23 | 00.00 | 58.38 | 0.00 | 82.28 | 0.00 |
| $T_1$ | Neem | 15.38 | 55.58 | 18.49 | 68.32 | 19.75 | 75.99 |
| $T_2$ | Marigold | 24.42 | 28.65 | 36.66 | 37.20 | 54.79 | 34.41 |
| $T_3$ | Eucalyptus | 17.80 | 47.99 | 21.64 | 62.29 | 23.66 | 71.24 |
| $T_4$ | ashwagandha | 19.51 | 43.00 | 22.50 | 61.45 | 25.68 | 68.78 |
| $T_5$ | Tulsi | 28.81 | 15.83 | 46.81 | 19.81 | 61.60 | 25.13 |
| $T_6$ | Mancozeb (0.2%) | 9.45 | 71.77 | 11.55 | 81.17 | 12.29 | 85.29 |

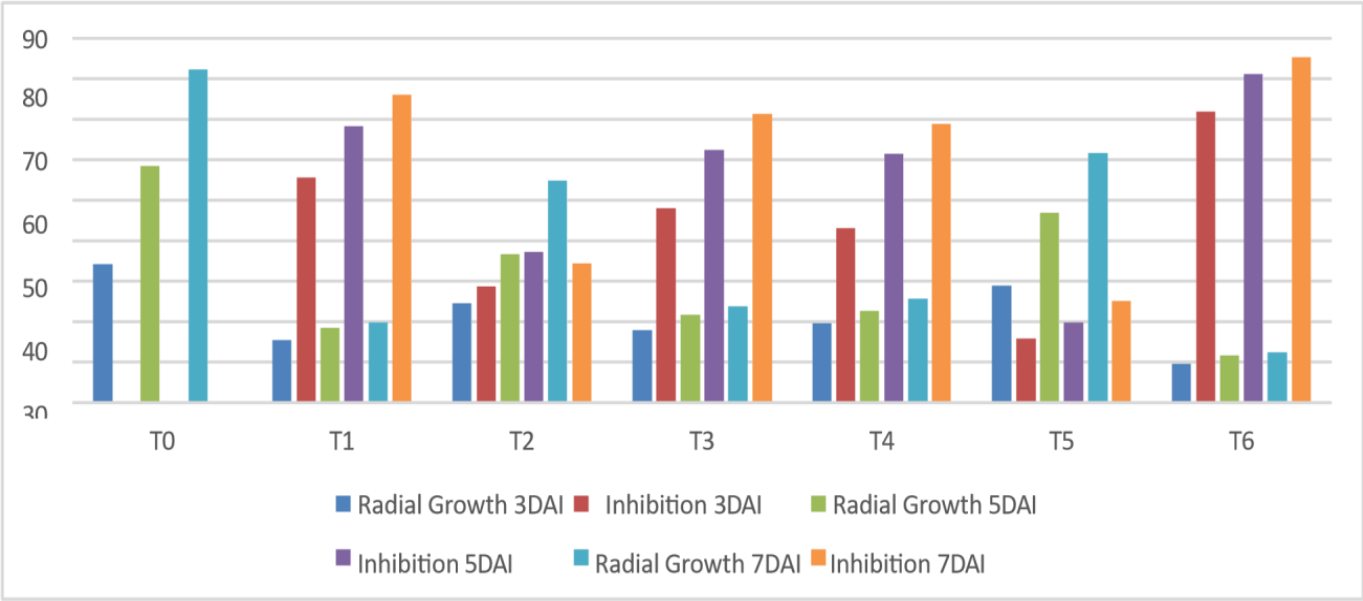


**Fig. 3. Effect of selected botanical extracts on radial growth(mm) and radial inhibition (%) of *Alternaria alternata* at 15% concentration**

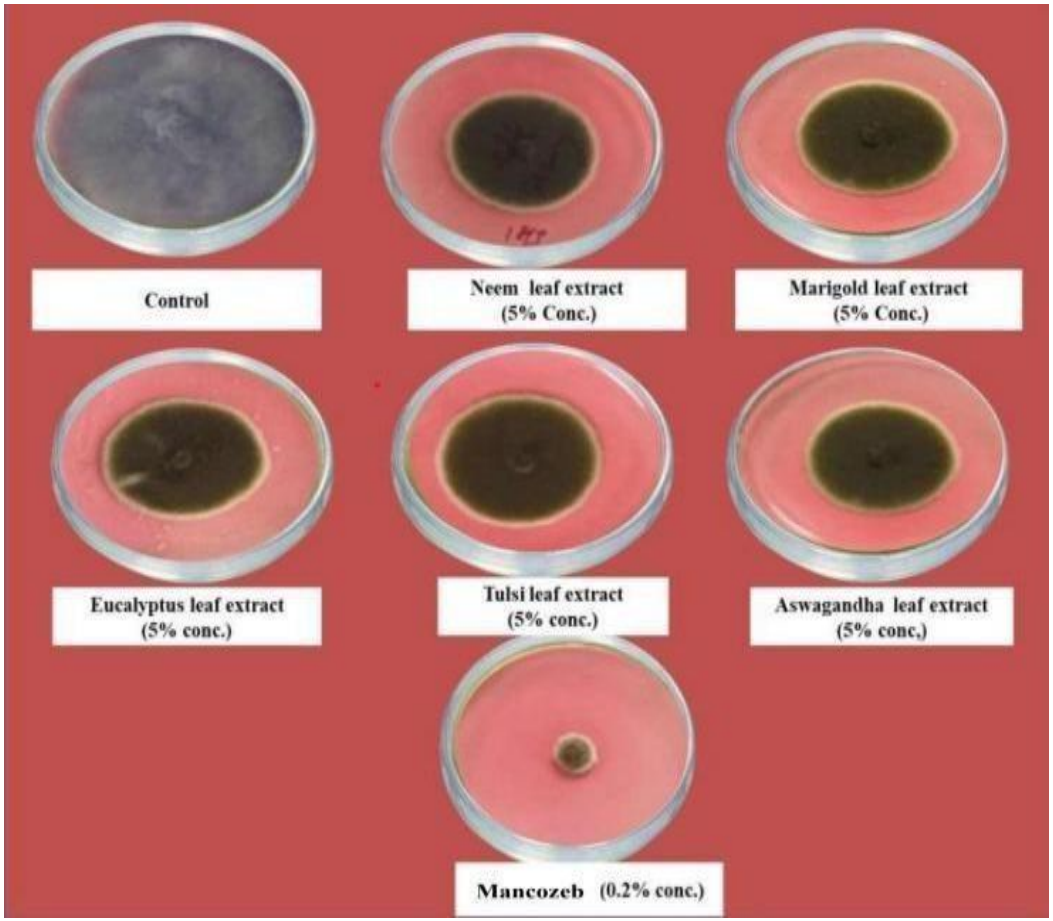


**Plate 1. Effect of botanicals on *Alternaria alternata* at 5% concentration with respect to mancozeb @0.2% concentration**

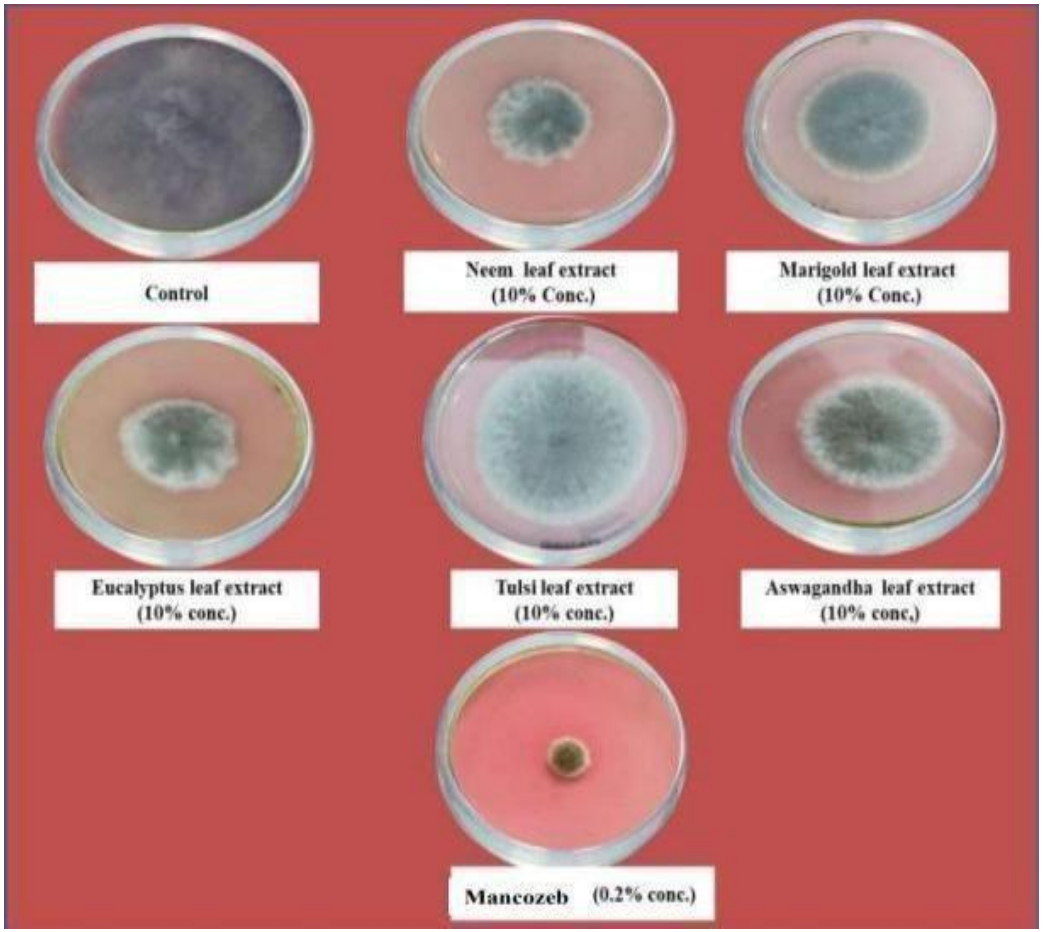


**Plate 2. Effect of botanicals on *Alternaria alternata* at 10% concentration with respect to mancozeb @0.2% concentration**

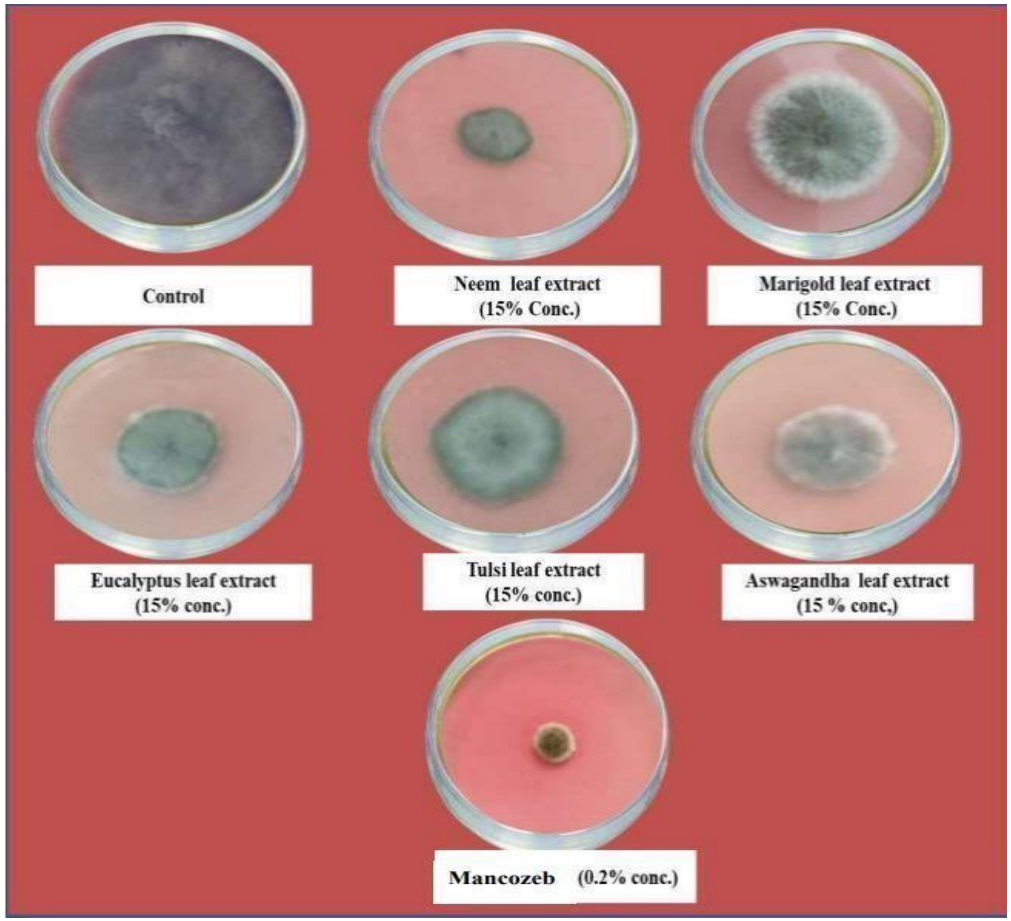


**Plate 3. Effect of botanicals on *Alternaria alternata* at 15% concentration with respect to mancozeb @0.2% concentration**

**Table 6. Efficacy of *Trichoderma Viride* and *T. harzianum* against mycelian growth and percent inhibition of *Alternaia alternata* at different time intervals**

| Bio agents | 48 hrs. | | 72 hrs. | | 96 hrs. | | 120 hrs. | | 144 hrs. | | Avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mycelium growth(mm) and inhibition (MI) in % | | | | | | | | | | |
| | MG | MI | MG | MI | MG | MI | MG | MI | MG | MI | MI |
| Control ($T_0$) | 21.63 | 00 | 32.68 | 00 | 50.66 | 00 | 59.61 | 00 | 72.17 | 00 | 00 |
| *T. viride* ($T_1$) | 10.78 | 50.16 | 12.40 | 62.05 | 14.54 | 71.29 | 16.50 | 72.32 | 19.68 | 72.73 | 65.71 |
| *T. harzianum* ($T_2$) | 11.53 | 46.69 | 13.68 | 58.13 | 16.68 | 67.07 | 19.52 | 67.25 | 24.51 | 66.03 | 61.03 |
| CD (5%) | 0.36 | | 0.42 | | 0.39 | | 0.48 | | 0.43 | | |

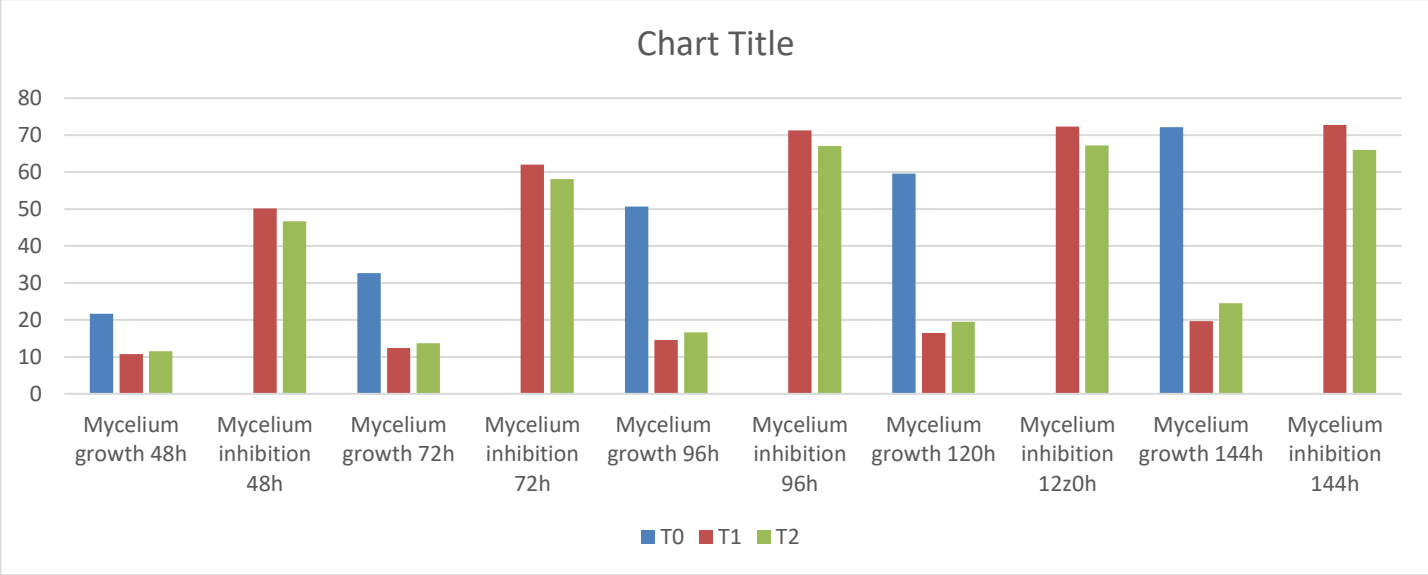


**Fig. 4. Comparison of mycelium growth (mm) and mycelium inhibition (%) among the treatments**

**Plate 4. Dual culture in 144 hrs. (*Alternaria alternata* vs *Trichoderma harzianum* and *Trichoderma viride*)**

while Eucalyptus's moderate but significant control demonstrates its potential for integrated disease management strategies.

Other treatments, such as Ashwagandha ($T_4$), Marigold ($T_2$), and Tulsi ($T_5$), displayed varying levels of efficacy. Although Ashwagandha provided moderate inhibition, Marigold and Tulsi were less effective, with their control diminishing over time. This suggests that while some plant extracts offer potential for disease suppression, their effectiveness may be limited compared to more potent treatments like Neem and Eucalyptus. Further optimization of concentrations or combinations with other biocontrol agents could enhance their performance.

In a separate experiment, *Trichoderma viride* ($T_1$) and *Trichoderma harzianum* ($T_2$) were tested for their biocontrol potential against *A. alternata*. Over a 144-hour period, both treatments significantly reduced mycelial growth compared to the untreated control. *T. viride* consistently outperformed *T. harzianum*, achieving an inhibition of 72.73% by the end of the study, while *T. harzianum* reached 66.03% inhibition. These results corroborate the well-documented biocontrol efficacy of *Trichoderma* species, particularly *T. viride*, which has been shown to inhibit various phytopathogens through mechanisms like competition, mycoparasitism, and the production of antifungal compounds.

Overall, the study underscores the potential of integrating chemical and biological control strategies for managing *Alternaria alternata*. While Mancozeb offers robust chemical control, Neem and Eucalyptus provide viable natural alternatives with fewer environmental impacts. Additionally, *Trichoderma viride* demonstrated excellent potential as a biocontrol agent, which could be further explored in combination with plant extracts for a more sustainable disease management approach.

## 5. CONCLUSION

This study concludes that Mancozeb (0.2%) is the most effective treatment for controlling *Alternaria alternata* in *Dahlia variabilis*, with *Trichoderma viride* emerging as the superior biocontrol agent among the bioagents tested. Neem and Eucalyptus also demonstrated strong antifungal potential, offering alternative botanical solutions. While Mancozeb provided the highest inhibition, the promising results from *T. viride* and select plant extracts suggest that integrating both synthetic and natural treatments can enhance disease management strategies for improved Dahlia productivity and health.

## 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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