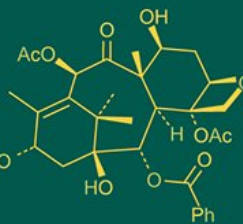
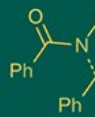
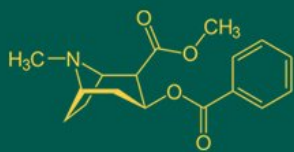


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Evaluation of bioagents, plant extracts and fungicides for the management of Alternaria leaf spot of okra caused by *Alternaria alternata*: An *in vitro* study

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Abstract

Alternaria leaf spot caused by *Alternaria alternata* (Fr.) Keissler is one of the major foliar diseases limiting okra (*Abelmoschus esculentus* L. Moench) production by reducing both yield and quality. The present investigation was undertaken to evaluate the efficacy of different biocontrol agents, plant extracts and fungicides against *A. alternata* under *in vitro* conditions. Among the five biocontrol agents tested by the dual culture technique, *Trichoderma harzianum* recorded the highest mycelial growth inhibition (65.28%), followed by *T. asperellum* (59.31%), while *Pseudomonas fluorescens* was the least effective (36.25%). Among the nine plant extracts evaluated by the poisoned food technique, Datura leaf extract at 10 per cent concentration exhibited the highest mycelial growth inhibition (63.15%), whereas at 20 per cent concentration, Neem leaf extract (75.37%) and Eucalyptus leaf extract (75.00%) proved most effective and were statistically at par. Among the systemic fungicides, Propiconazole 25% EC recorded the maximum inhibition of mycelial growth (77.22, 84.44 and 84.25%) at 250, 500 and 1000 ppm, respectively. Copper oxychloride 50% WP was the most effective non-systemic fungicide, recording mycelial growth inhibition of 71.67, 78.70 and 84.44 per cent at 1000, 1500 and 2000 ppm, respectively. Among the ready-mix fungicides, Azoxystrobin 18.2% + Difenconazole 11.4% SC and Tebuconazole 50% + Trifloxystrobin 25% WG completely inhibited the mycelial growth of *A. alternata* (100%) at all the concentrations tested. The study demonstrated that *T. harzianum*, Neem and Eucalyptus leaf extracts and the ready-mix fungicides Azoxystrobin + Difenconazole and Tebuconazole + Trifloxystrobin were highly effective in suppressing the growth of *A. alternata* under laboratory conditions and may be considered as promising components for the integrated management of Alternaria leaf spot of okra.

Keywords: *Alternaria alternata*, Alternaria leaf spot, okra, bioagents, plant extracts, fungicides, *in vitro* evaluation.

Introduction

Okra (*Abelmoschus esculentus* L. Moench), belonging to the family Malvaceae, is one of the most important vegetable crops cultivated in tropical and subtropical regions of the world (Naveed *et al.*, 2009; Oyelade *et al.*, 2003)^[12, 13]. It is valued for its tender green pods, which are rich in vitamins, minerals, dietary fibre and antioxidants and contribute significantly to nutritional security and farmers' income. India is the largest producer of okra in the world, accounting for more than 70 per cent of the global production (Anonymous, 2025). Among the major okra-growing states, Gujarat occupies a leading position, with Surat, Tapi and Navsari districts being the principal okra-producing regions of South Gujarat (Anonymous, 2024-25).

Despite its economic importance, okra production is severely constrained by several biotic and abiotic stresses. Among the fungal diseases, Alternaria leaf spot caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive foliar diseases of okra. The disease initially appears as small light brown lesions on leaves, which later enlarge into dark brown concentric necrotic spots, leading to premature defoliation, reduction in photosynthetic area and poor fruit yield. Under favourable environmental conditions, the disease may cause yield losses ranging from 30 to 50 per cent or even higher (Hafiz, 1986; Jha and Dubey, 2000; Thippeswamy *et al.*, 2007; Pansambal *et al.*, 2015)^[5, 7, 14, 19]. *Alternaria alternata* is a

Ubiquitous fungal pathogen capable of infecting a wide range of cultivated crops and surviving on infected plant debris and seeds. The pathogen proliferates rapidly under warm and humid conditions and produces abundant airborne conidia, which facilitate rapid disease spread. Because of its broad host range, efficient dissemination and adaptability to diverse environmental conditions, management of *Alternaria* leaf spot remains a major challenge in okra cultivation (Maheshwari *et al.*, 2000; Dube, 2013; Singh, 2015) [11]. Recent studies have also emphasized that *A. alternata* remains one of the major foliar pathogens of okra and highlighted the need for continuous evaluation of effective management strategies due to its persistent occurrence and economic impact (Choudhary and Panchouli, 2024) [3]. Management of *Alternaria* leaf spot largely depends on fungicidal application. However, indiscriminate and repeated use of chemical fungicides may lead to the development of fungicide resistance, environmental pollution and residual toxicity. Therefore, the use of biological control agents and plant-derived extracts, either alone or in combination with effective fungicides, has emerged as an environmentally safe and sustainable approach for disease management. Evaluation of these management options under *in vitro* conditions is an essential prerequisite for identifying effective treatments before their validation under field conditions. Recent investigations have demonstrated that integrating fungicides with biological agents and botanicals provides effective suppression of *A. alternata* while promoting sustainable disease management in okra (Mahanty and Zacharia, 2024) [10].

Material and methods

Isolation and pathogenicity

The culture of *A. alternata* used in the present investigation was isolated from infected okra leaves collected from the RHRS (Regional Horticultural Research Station) field at Navsari Agricultural University, Navsari. For isolation, infected leaf samples were cut along with a small portion of healthy tissue and surface sterilized with 0.1 per cent mercuric chloride or 1 per cent sodium hypochlorite solution for one minute, followed by three washings with sterile distilled water. The sterilized bits were transferred aseptically to Potato Dextrose Agar (PDA) medium under laminar airflow. The inoculated plates were incubated at 25 ± 2 °C for 7-10 days. The isolated fungus was identified as *A. alternata* based on cultural and morphological characteristics using standard literature. The fungal growth developing around the tissue bits was transferred to PDA slants for sub-culturing. The isolate was purified by the hyphal tip technique, maintained on PDA slants by periodic sub-culturing and stored at 5 °C until further use (Tuite, 1969) [20]. For pathogenicity, a spore suspension of *A. alternata* (2×10^6 spores ml^{-1}) was prepared from the pure culture and sprayed on 30 day old healthy okra plants grown in earthen pots under glasshouse conditions. Control plants were sprayed with sterile distilled water. The inoculated and uninoculated plants were covered with polyethene bags for 48 h to maintain high humidity. The pathogen was re-isolated from the inoculated plants for confirmation of Koch's postulates.

In vitro evaluation of bio-agents by dual culture method

The efficacy of different bio-agents against *A. alternata* was evaluated by the dual culture technique described by Foldes

et al. (2000). The pathogen and test bio-agents were grown separately on PDA medium. A 5 mm diameter mycelial disc from the periphery of a 10 day old culture of the bio-agent was placed at one end of a sterilized Petri plate containing PDA medium and a similar disc of the pathogen was placed on the opposite side of the plate, approximately 70 mm away. Plates inoculated with the pathogen alone served as the control. The inoculated plates were incubated at 25 ± 2 °C until the control plates were completely covered with the mycelial growth of the pathogen. Each treatment was replicated four times. Radial mycelial growth of the pathogen was recorded and per cent growth inhibition over the control was calculated by using the formula suggested by Vincent (1947).

$$\text{PGI} = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Average diameter (mm) of mycelial colony in control plate

T = Average diameter (mm) of mycelial colony in treated plate

In vitro evaluation of phytoextracts by poisoned food technique

Aqueous leaf extracts of the test phytoextracts were prepared by grinding healthy fresh leaves (50 g) with an equal volume (50 ml) of sterile distilled water using a grinder. The homogenate was filtered through double-layered muslin cloth and centrifuged at 2500 rpm for 10 min to obtain the crude (100%) extract. The efficacy of different phytoextracts against *A. alternata* was evaluated by the poisoned food technique. Required quantities of the crude extract were incorporated into sterilized PDA medium to obtain 10 and 20 per cent concentrations, mixed thoroughly and poured into sterilized Petri plates (20 ml per plate). PDA plates without phytoextract served as the control. After solidification of the medium, the plates were inoculated aseptically with a 5 mm diameter mycelial disc taken from the periphery of a seven day old culture of *A. alternata* and incubated at 25 ± 2 °C. Each treatment was replicated three times. Radial mycelial growth was recorded after seven days of incubation and per cent growth inhibition over the control was calculated by using the formula suggested by Vincent (1947) mentioned above.

In vitro evaluation of fungicides by poisoned food technique

Poisoned food technique (Nene and Thapliyal, 1979) was used to evaluate the efficacy of systemic, non-systemic and ready-mix fungicides against *A. alternata*. PDA medium was prepared and dispensed at the rate of 100 ml in 250 ml conical flasks and sterilized in an autoclave. Before solidification of the medium, the required quantity of fungicides at their respective concentrations was incorporated aseptically into the sterilized PDA and mixed thoroughly. About 20 ml of poisoned medium was poured into each sterilized Petri plate. PDA plates without fungicide served as the control. After solidification, the plates were inoculated aseptically with a 5 mm diameter mycelial disc cut from the periphery of a seven day old culture of *A. alternata* and incubated at 25 ± 2 °C. Each treatment was replicated three times. Observations on radial mycelial growth were recorded after seven days of incubation and per

cent growth inhibition over the control was calculated by using above formula.

Results and Discussion

In vitro evaluation of bio-agents against *A. alternata*

Five bio-agents were evaluated against the mycelial growth of *A. alternata* along with an untreated control by the dual culture technique. The observations on mycelial growth (mm) and per cent growth inhibition (PGI) were recorded seven days after inoculation and are presented in Table 1, Fig. 1 and Photo 1. All the bio-agents tested significantly inhibited the mycelial growth of *A. alternata* over the untreated control. The untreated control recorded maximum mycelial growth (90.00 mm). Among the bio-agents, *Trichoderma harzianum* proved to be the most effective by recording the minimum mycelial growth of 31.25 mm with the highest per cent growth inhibition of 65.28 per cent. The next best antagonist was *T. asperellum*, which recorded 36.63 mm mycelial growth with 59.31 per cent inhibition,

followed by *T. koningii*, which recorded 39.00 mm mycelial growth with 56.67 per cent inhibition. Among the bacterial antagonists, *Bacillus subtilis* and *Pseudomonas fluorescens* recorded 41.53 and 36.25 per cent growth inhibition, respectively and were comparatively less effective than the fungal antagonists.

The present findings are in accordance with Jakatimath *et al.* (2017) [6], who reported *Trichoderma* spp. to be more effective than bacterial antagonists against *A. alternata*. Similar observations were reported by Shingne *et al.* (2020) [18], who found *Trichoderma* spp. to be superior over *Pseudomonas fluorescens* and *Bacillus subtilis* in suppressing the mycelial growth of *A. alternata*. Kharbadkar *et al.* (2022) [9] also reported that *T. harzianum* exhibited maximum inhibition of the pathogen among the bio-agents tested. Likewise, Sahu *et al.* (2025) [15] confirmed the superior antagonistic potential of *Trichoderma* spp. against *A. alternata*, thereby supporting the results obtained in the present study.

Table 1: Bio-efficacy of biocontrol agents against *A. alternata* under *in vitro* conditions

Tr. No.	Biocontrol agents	Mycelial growth 7 DAI (mm)	PGI (%)
T ₁	<i>Trichoderma asperellum</i>	37.23 ^{b*} (36.62)	47.96 ^b (59.31)
T ₂	<i>Trichoderma harzianum</i>	33.97 ^a (31.25)	52.52 ^a (65.28)
T ₃	<i>Trichoderma koningii</i>	38.63 ^c (39.00)	45.99 ^c (56.67)
T ₄	<i>Pseudomonas fluorescens</i>	49.22 ^e (57.37)	31.19 ^e (36.25)
T ₅	<i>Bacillus subtilis</i>	46.49 ^d (52.62)	35.01 ^d (41.53)
T ₆	Control	71.54 ^f (90.00)	0.00 ^f (0.00)
	S.E.m. ±	0.260 (0.446)	
	CD at 5 %	0.779 (1.335)	
	CV %	1.127 (1.743)	

*Figures outside the parentheses are arc transformation values, whereas figures in parentheses indicate original values

Means followed by a common letter are not significantly different at the 5% level by DNMRT

DAI: Day after inoculation, PGI: Per cent growth inhibition

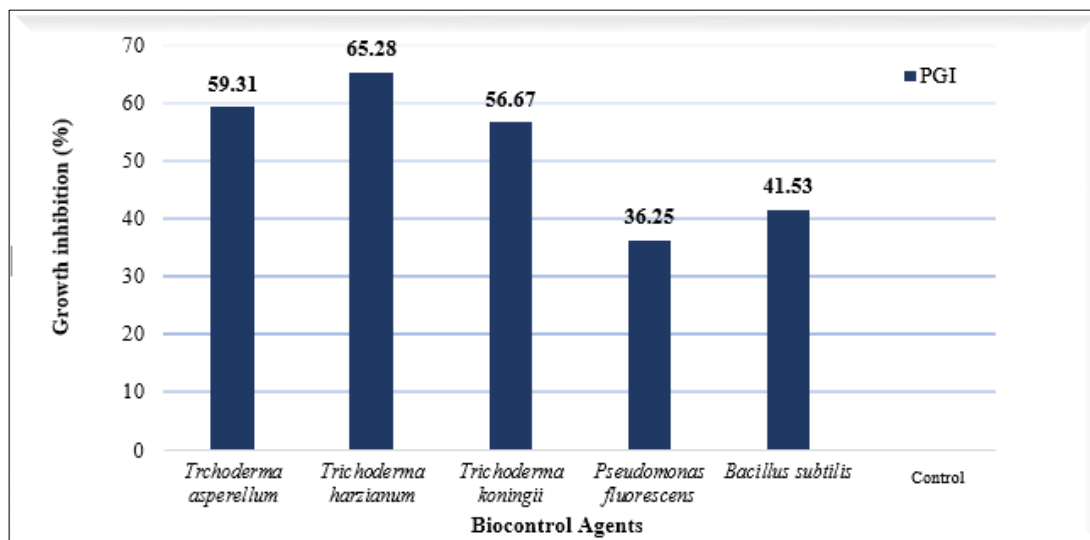


Fig. 1: Bio-efficacy of biocontrol agents on per cent growth inhibition (PGI) of *A. alternata* in vitro

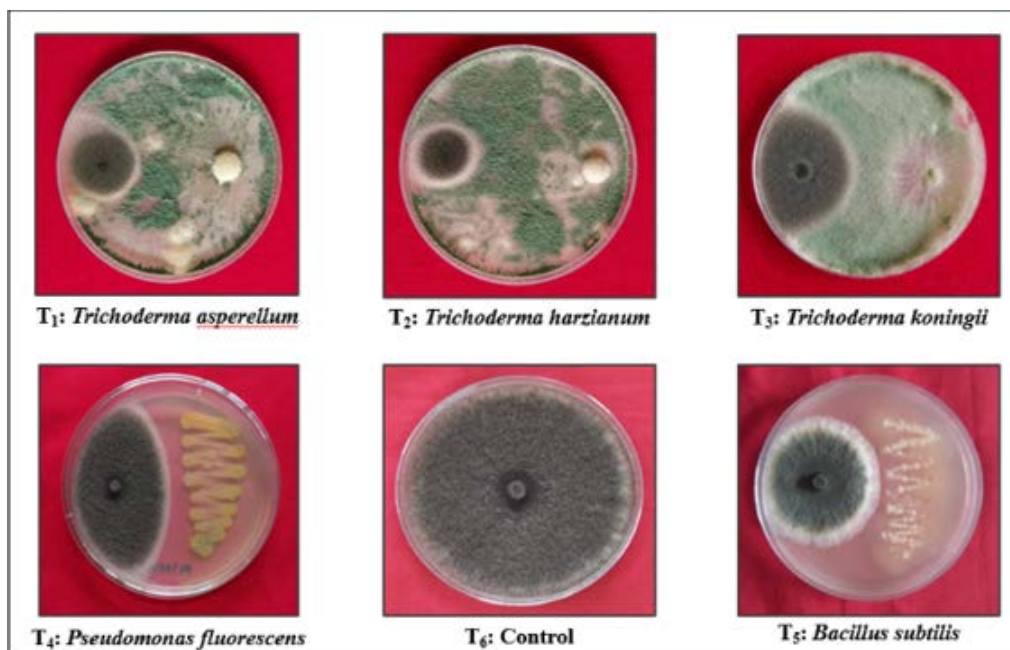


Photo 1: Bio-efficacy of biocontrol agents against *A. alternata* under *in vitro* conditions

In vitro* evaluation of phytoextracts against *A. alternata

Nine plant extracts were evaluated against the mycelial growth of *A. alternata* along with an untreated control at two concentrations (10 and 20%) by the poisoned food technique. The observations on mycelial growth (mm) and per cent growth inhibition (PGI) were recorded seven days after inoculation and are presented in Table 2, Fig. 2 and Photo 2. All the plant extracts tested significantly inhibited the mycelial growth of *A. alternata* over the untreated control. The maximum mycelial growth (90.00 mm) was recorded in the untreated control. Among the plant extracts, Neem leaf extract proved to be the most effective and recorded the minimum mycelial growth of 35.50 and 22.17 mm with the highest per cent growth inhibition of 60.56 and 75.37 per cent at 10 and 20 per cent concentrations, respectively. The next best treatment was Eucalyptus leaf extract, which recorded mycelial growth of 34.50 and 22.50 mm with 61.67 and 75.00 per cent growth inhibition, respectively, followed by Datura leaf extract with mycelial growth of 33.17 and 29.50 mm and corresponding inhibition

of 63.15 and 67.22 per cent at 10 and 20 per cent concentrations. Aak leaf extract also showed appreciable inhibition, whereas Bougainvillea, Tulsi, Karanj, Marigold and Barmasi leaf extracts were comparatively less effective in suppressing the mycelial growth of the pathogen.

The present findings are in close agreement with Sharma *et al.* (2021b) [17], who reported that garlic extract was the most effective, followed by neem leaf extract, against *A. alternata*. Similar observations were made by Kharbadkar *et al.* (2022) [9], who found neem leaf extract among the most effective botanicals in inhibiting the mycelial growth of *A. alternata*. Ghule *et al.* (2023) [4] also reported significant inhibition of *A. alternata* by different botanicals, while Kansotia *et al.* (2024) observed that neem leaf extract recorded maximum inhibition of mycelial growth among the phytoextracts tested. These findings support the effectiveness of botanicals, particularly neem and eucalyptus leaf extracts, observed in the present investigation.

Table 2: Bio-efficacy of plant extracts against *A. alternata* under *in vitro*

Tr. No.	Treatments		Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)
	Common name	Botanical name	10%		20%	
T ₁ , T ₂	Bougainvillea leaf extract	Bougainvillea glabra	48.14 ^{fe*} (55.50)	32.71 ^{fe} (38.33)	47.56 ^f (54.50)	33.52 ^f (39.44)
T ₃ , T ₄	Tulsi leaf extract	Ocimum basilicum L.	47.85 ^e (55.00)	33.11 ^e (38.89)	46.80 ^f (53.16)	34.58 ^f (40.93)
T ₅ , T ₆	Neem leaf extract	Azadiracta indica A. Juss.	36.56 ^b (35.50)	48.90 ^b (60.56)	28.07 ^a (22.17)	60.75 ^a (75.37)
T ₇ , T ₈	Karanj leaf extract	Pongamia pinnata L. Pierre	48.91 ^{gf} (56.83)	31.63 ^{gf} (36.85)	45.65 ^e (51.17)	36.18 ^e (43.15)
T ₉ , T ₁₀	Marigold leaf extract	Tagetes erecta L.	49.87 ^g (58.50)	30.29 ^g (35.00)	46.89 ^f (53.33)	34.45 ^f (40.74)
T ₁₁ , T ₁₂	Aak leaf extract	Calotropis gigantea L.	39.89 ^c (41.17)	44.23 ^c (54.26)	38.82 ^d (39.33)	45.72 ^d (56.30)
T ₁₃ , T ₁₄	Datura leaf extract	Datura stramonium L.	35.14 ^a (33.17)	50.87 ^a (63.15)	32.88 ^b (29.50)	54.03 ^b (67.22)
T ₁₅ , T ₁₆	Barmasi leaf extract	Catharanthus roseus L.	44.50 ^d (49.17)	37.79 ^d (45.37)	35.75 ^c (34.16)	50.02 ^c (62.04)
T ₁₇ , T ₁₈	Eucalyptus leaf extract	Eucalyptus spp.	35.96 ^{ba} (34.50)	49.74 ^{ba} (61.67)	28.30 ^a (22.50)	60.43 ^a (75.00)
T ₁₉	Control	-	71.54 ^h (90.00)	0.00 ^h (0.00)	71.54 ^g (90.00)	0.00 ^g (0.00)
	S.Em. ±		0.324(0.477)		0.216(0.354)	
	CD at 5 %		0.969(1.418)		0.641(1.050)	
	CV %		1.637(1.623)		0.884(1.361)	

*Figures outside the parentheses are arc transformation values, whereas figures in parentheses indicate original values

Means followed by a common letter are not significantly different at the 5% level by DNMRT

DAI: Day after inoculation, PGI: Per cent growth inhibition

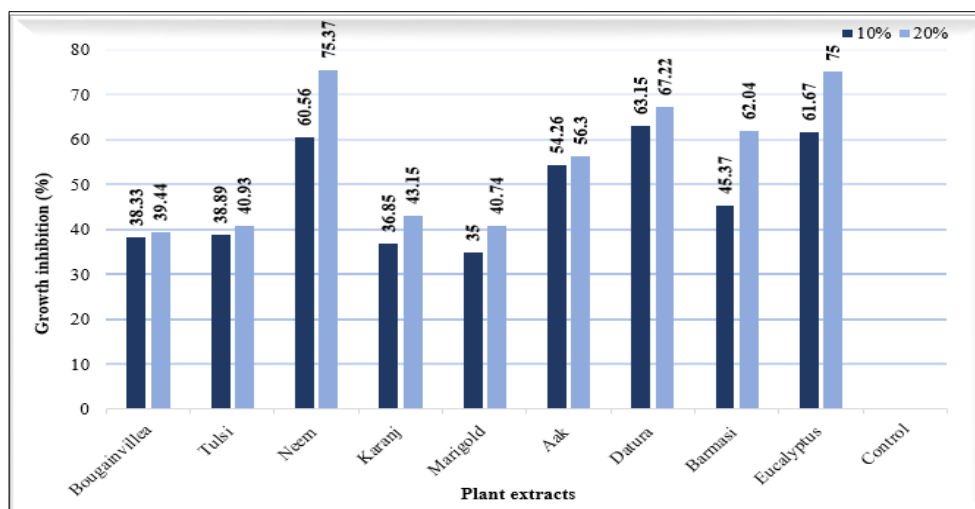


Fig. 2: Bio-efficacy of plant extracts on per cent growth inhibition (PGI) of *A. alternata* *in vitro*

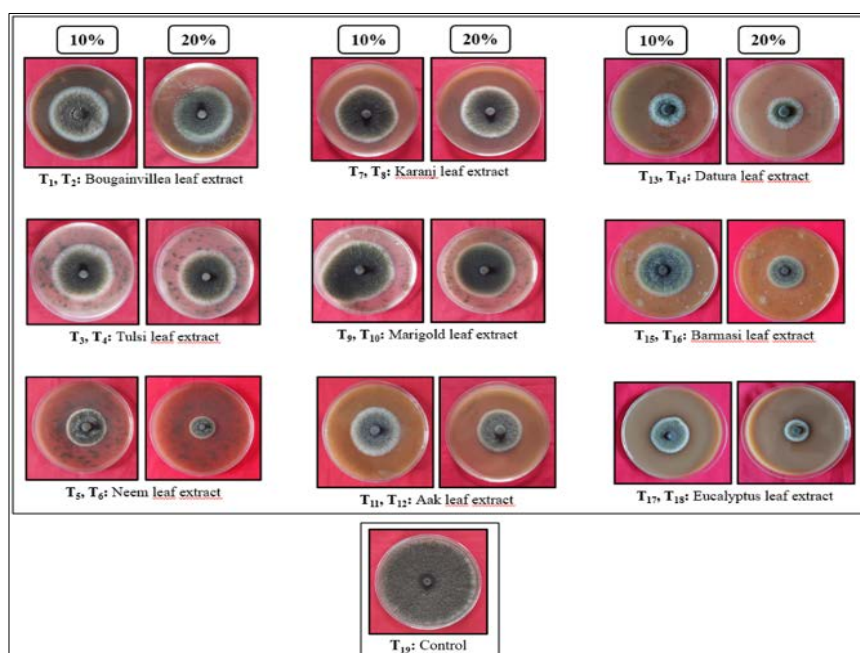


Photo 2: Bio-efficacy of plant extracts against *A. alternata* under *in vitro* conditions

In vitro* evaluation of fungicides against *A. alternata

The experiment was conducted to evaluate the efficacy of different fungicides against *Alternaria alternata* by the poisoned food technique as described under materials and methods. The fungicides were grouped into systemic, non-systemic and ready-mix categories and tested at different concentrations based on active ingredient. Systemic fungicides were evaluated at 250, 500 and 1000 ppm, non-systemic fungicides at 1000, 1500 and 2000 ppm, while ready-mix fungicides were tested at 500, 1000 and 1500 ppm.

Evaluation of systemic fungicides

All the systemic fungicides tested significantly inhibited the mycelial growth of *A. alternata* over the untreated control. The maximum mycelial growth (90.00 mm) was recorded in the control. Among the fungicides, Propiconazole 25% EC proved to be the most effective by recording the minimum mycelial growth of 20.50, 14.00 and 13.67 mm with the highest per cent growth inhibition of 77.22, 84.44 and 84.25 per cent at 250, 500 and 1000 ppm, respectively. The next

effective fungicide was Azoxystrobin 25% SC, which recorded mycelial growth of 30.33, 26.50 and 24.50 mm with 66.30, 70.56 and 86.85 per cent inhibition, respectively, followed by Hexaconazole 5% EC, which recorded 34.00, 29.83 and 24.00 mm mycelial growth with 62.22, 66.85 and 73.33 per cent inhibition at the corresponding concentrations (Table 3, Fig. 3 and Photo 3). The present findings are in conformity with those of Pansambal *et al.* (2015) [14], who reported complete inhibition of *Alternaria* spp. by Propiconazole and Hexaconazole. Similar results were reported by Kadam *et al.* (2018) [8], where Propiconazole exhibited maximum inhibition followed by Hexaconazole against *A. alternata*. Likewise, Kharbadkar *et al.* (2022) [9] observed Propiconazole to be the most effective systemic fungicide against *A. alternata*, while Uniyal *et al.* (2023) [21] and Choudhary and Panchouli (2024) [3] also reported superior efficacy of Propiconazole under *in vitro* conditions. More recently, Sahu *et al.* (2025) [15] confirmed complete inhibition of *A. alternata* by Propiconazole even at lower concentrations, thereby supporting the present investigation.

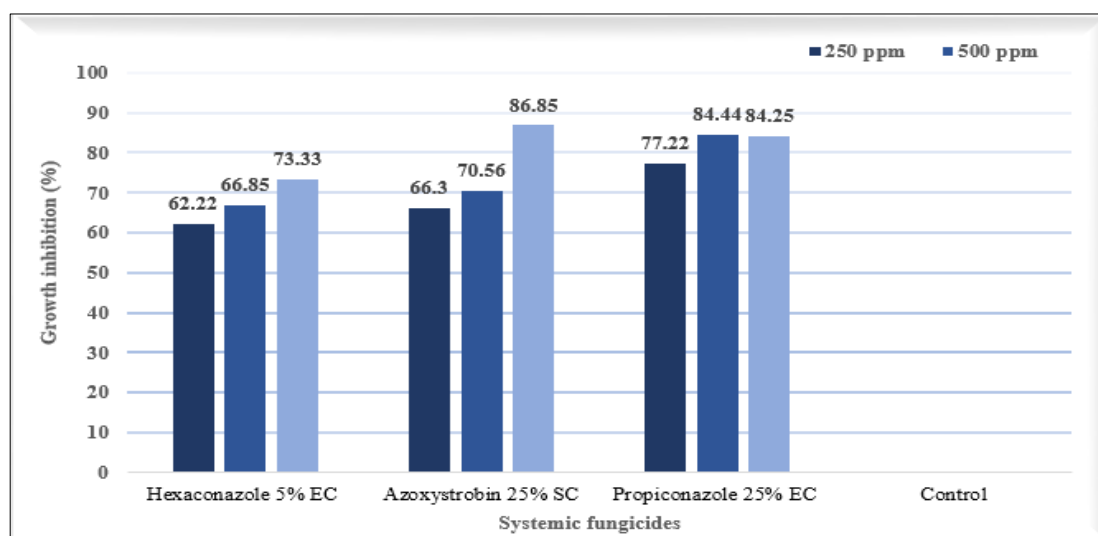
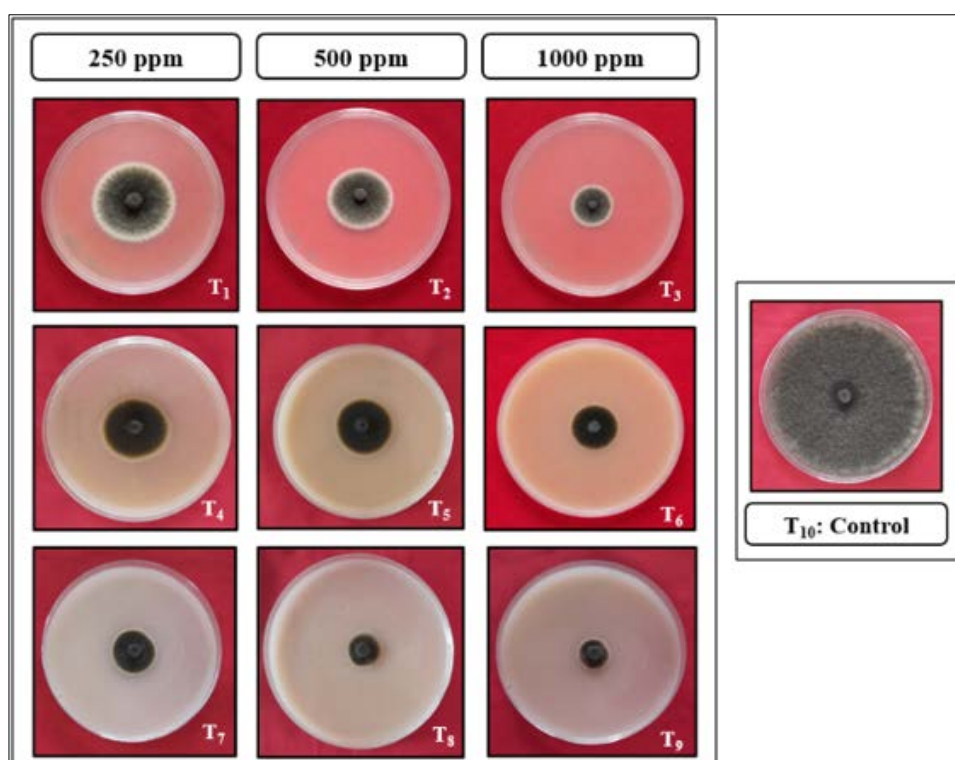
Table 3: Efficacy of systemic fungicides against *A. alternata* under *in vitro*

Tr. No.	Systemic fungicides	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)
		250 ppm		500 ppm		1000 ppm	
T ₁ , T ₂ , T ₃	Hexaconazole 5% EC	35.65 ^{c*} (34.00)	50.16 ^c (62.22)	33.10 ^c (29.83)	53.73 ^c (66.85)	29.32 ^b (24.00)	59.01 ^b (73.33)
T ₄ , T ₅ , T ₆	Azoxystrobin 25% SC	33.40 ^b (30.33)	53.31 ^b (66.30)	30.97 ^b (26.50)	56.70 ^b (70.56)	29.65 ^b (24.50)	58.54 ^b (86.85)
T ₇ , T ₈ , T ₉	Propiconazole 25% EC	26.91 ^a (20.50)	62.38 ^a (77.22)	21.96 ^a (14.00)	69.31 ^a (84.44)	21.68 ^a (13.67)	69.69 ^a (84.25)
T ₁₀	Control	71.54 ^d (90.00)	0.00 ^d (0.00)	71.54 ^d (90.00)	0.00 ^d (0.00)	71.54 ^c (90.00)	0.00 ^c (0.00)
	S.Em. ±	0.227 (0.363)		0.261 (0.333)		0.257 (0.363)	
	CD at 5 %	0.753 (1.203)		0.866 (1.104)		1.852 (1.203)	
	CV %	0.940 (1.439)		1.149 (1.440)		1.172 (1.654)	

*Figures outside the parentheses are arc transformation values, whereas figures in parentheses indicate original values

Means followed by a common letter are not significantly different at the 5% level by DNMR

DAI: Day after inoculation, PGI: Per cent growth inhibition

**Fig. 3:** Efficacy of systemic fungicides on per cent growth inhibition of *A. alternata* *in vitro***Photo 3:** Efficacy of systemic fungicides against *A. alternata* under *in vitro* conditions

Evaluation of non-systemic fungicides

All the non-systemic fungicides significantly reduced the mycelial growth of

A. alternata compared with the untreated control. The untreated control recorded maximum mycelial growth (90.00 mm). Among the fungicides tested, Copper oxychloride 50% WP was the most effective and recorded the minimum mycelial growth of 25.50, 19.17 and 14.00 mm with corresponding inhibition of 71.67, 78.70 and 84.44 per cent at 1000, 1500 and 2000 ppm, respectively. Chlorothalonil 75% WP was the next effective fungicide, recording mycelial growth of 38.50, 33.00 and 31.83 mm with inhibition of 57.22, 63.33 and 64.63 per cent. Mancozeb 75% WP ranked next by recording 35.50, 34.83 and 33.83 mm mycelial growth with 60.56, 61.30 and 62.41

per cent inhibition at the respective concentrations (Table 4, Fig. 4 and Photo 4).

These findings corroborate the reports of Kantwa *et al.* (2014), who observed high inhibition of *A. alternata* with Copper oxychloride. Similar observations were recorded by Shingne *et al.* (2020) [18], where Copper oxychloride proved highly effective against *A. alternata*. Kharbadkar *et al.* (2022) [9] also reported that Copper oxychloride was superior to Chlorothalonil in suppressing mycelial growth of the pathogen, which closely agrees with the present results. Furthermore, Sahu *et al.* (2025) [15] demonstrated that Copper oxychloride remained one of the effective non-systemic fungicides against *A. alternata* under laboratory conditions.

Table 4: Efficacy of non-systemic fungicides against *A. alternata* under *in vitro*

Tr. No.	Non-systemic fungicides	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)
		1000 ppm		1500 ppm		2000 ppm	
T ₁ , T ₂ , T ₃	Mancozeb 75% WP	36.56 ^{b*} (35.50)	48.90 ^b (60.56)	36.16 ^c (34.83)	49.45 ^c (61.30)	35.55 ^c (33.83)	50.30 ^c (62.41)
T ₄ , T ₅ , T ₆	Chlorothalonil 75% WP	38.34 ^c (38.50)	46.41 ^c (57.22)	35.05 ^b (33.00)	51.00 ^b (63.33)	34.33 ^b (31.83)	52.01 ^b (64.63)
T ₇ , T ₈ , T ₉	Copper Oxychloride 50% WP	30.32 ^a (25.50)	57.62 ^a (71.67)	25.95 ^a (19.17)	63.73 ^a (78.70)	21.96 ^a (14.00)	69.30 ^a (84.44)
T ₁₀	Control	71.54 ^d (90.00)	0.00 ^d (0.00)	71.54 ^d (90.00)	0.00 ^d (0.00)	71.54 ^d (90.00)	0.00 ^d (0.00)
	S.Em. ±	0.154 (0.250)		0.272 (0.425)		0.278 (0.373)	
	CD at 5 %	0.510 (0.828)		0.902 (1.407)		0.921 (1.234)	
	CV %	0.603 (0.914)		1.118 (1.663)		1.179 (1.522)	

*Figures outside the parentheses are arc transformation values, whereas figures in parentheses indicate original values

Means followed by a common letter are not significantly different at the 5% level by DNMR

DAI: Day after inoculation, PGI: Per cent growth inhibition

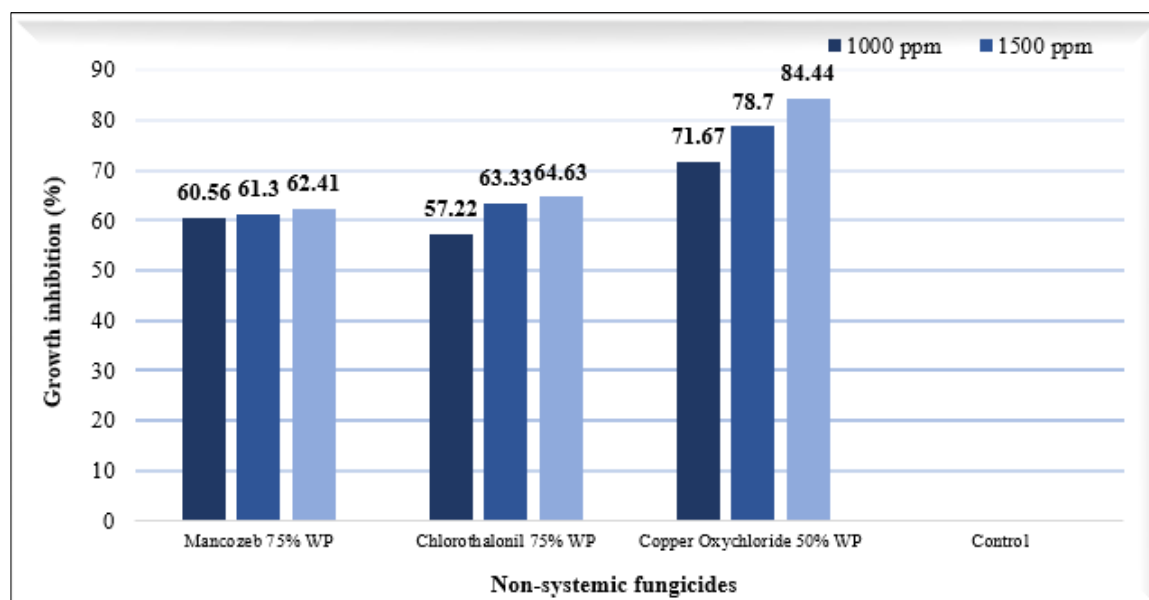


Fig. 4: Efficacy of non-systemic fungicides on per cent growth inhibition of *A. alternata* *in vitro*

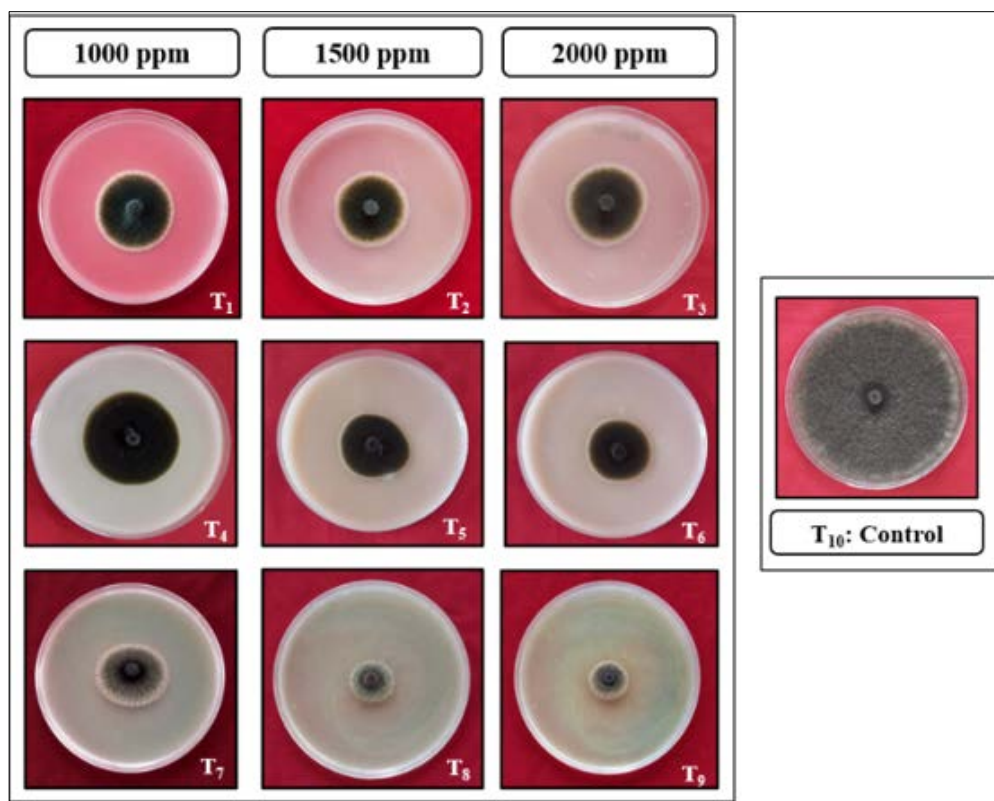


Photo 4: Efficacy of non-systemic fungicides against *A. alternata* under *in vitro* conditions

Evaluation of ready-mix fungicides

All the ready-mix fungicides significantly inhibited the mycelial growth of *A. alternata* over the untreated control. The untreated control recorded maximum mycelial growth (90.00 mm). Among the fungicides, Azoxystrobin 18.2% + Difenconazole 11.4% SC and Tebuconazole 50% + Trifloxystrobin 25% WG completely inhibited the mycelial growth of the pathogen and recorded cent per cent growth inhibition at all the tested concentrations (500, 1000 and 1500 ppm), proving significantly superior to all other treatments. Metiram 55% + Pyraclostrobin 5% WG ranked next by recording mycelial growth of 22.50, 21.00 and 9.00 mm with corresponding inhibition of 75.00, 76.67 and 90.00 per cent at 500, 1000 and 1500 ppm, respectively (Table 5, Fig. 5 and Photo 5).

The present findings are in close agreement with those of Yadav (2018), who reported Tebuconazole + Trifloxystrobin as the most effective ready-mix fungicide against *A. alternata*. Similar results were obtained by Ghule *et al.* (2023)^[4], who reported complete inhibition of *A. alternata* with Azoxystrobin + Difenconazole and complete inhibition by Tebuconazole + Trifloxystrobin at higher concentrations. Sharma *et al.* (2022)^[16] also observed cent per cent inhibition of *A. alternata* with Tebuconazole + Trifloxystrobin at 500 ppm. Likewise, Sahu *et al.* (2025)^[15] reported complete inhibition of the pathogen by Azoxystrobin + Difenconazole under *in vitro* conditions, thereby confirming the superiority of these ready-mix fungicides observed in the present study.

Table 5: Efficacy of ready-mix fungicides against *A. alternata* under *in vitro*

Tr. No.	Ready-mix fungicides	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)	Mycelial growth 7 DAI (mm)	PGI (%)
		500 ppm		1000 ppm		1500 ppm	
T ₁ , T ₂ , T ₃	Metiram 55% + Pyraclostrobin 5% WG	28.30 ^{b*} (22.50)	60.43 ^b (75.00)	27.26 ^b (21.00)	61.89 ^b (76.67)	17.43 ^b (9.00)	75.64 ^b (90.00)
T ₄ , T ₅ , T ₆	Azoxystrobin 18.2% + Difenconazole 11.4% SC	0.00 ^a (0.00)	100.00 ^a (100.00)	0.00 ^a (0.00)	100.00 ^a (100.00)	0.00 ^a (0.00)	100.00 ^a (100.00)
T ₇ , T ₈ , T ₉	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00 ^a (0.00)	100.00 ^a (100.00)	0.00 ^a (0.00)	100.00 ^a (100.00)	0.00 ^a (0.00)	100.00 ^a (100.00)
T ₁₀	Control	71.54 ^c (90.00)	0.00 ^c (0.00)	71.54 ^c (90.00)	0.00 ^c (0.00)	71.54 ^c (90.00)	0.00 ^c (0.00)
	S.E.m. ±	0.099 (0.144)		0.203 (0.289)		0.289 (0.289)	
	CD at 5 %	0.328 (0.478)		0.672 (0.956)		0.958 (0.956)	
	CV %	0.687 (0.889)		1.424 (1.802)		2.253 (2.020)	

*Figures outside the parentheses are arc transformation values, whereas figures in parentheses indicate original values

Means followed by a common letter are not significantly different at the 5% level by DNMRT

DAI: Day after inoculation, PGI: Per cent growth inhibition

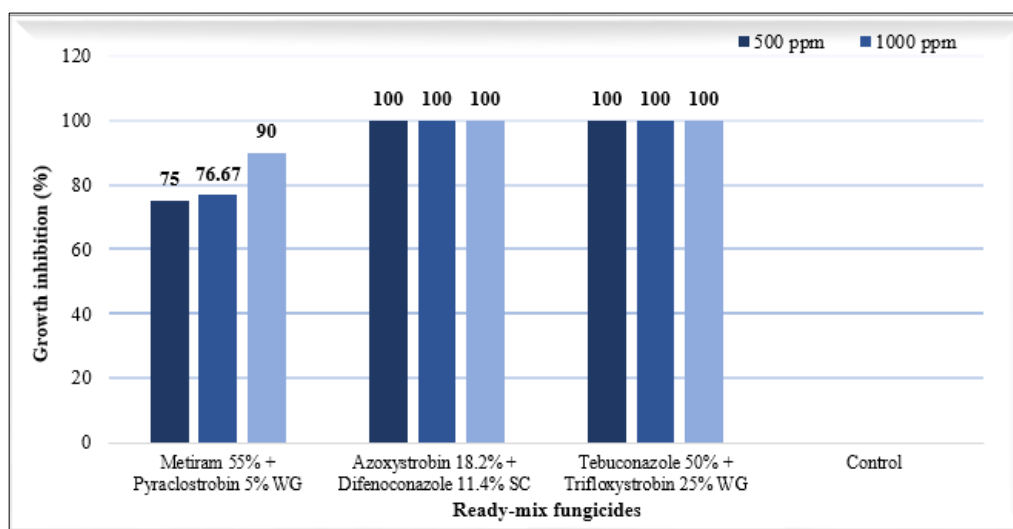


Fig. 5: Efficacy of ready-mix fungicides on per cent growth inhibition of *A. alternata* in vitro

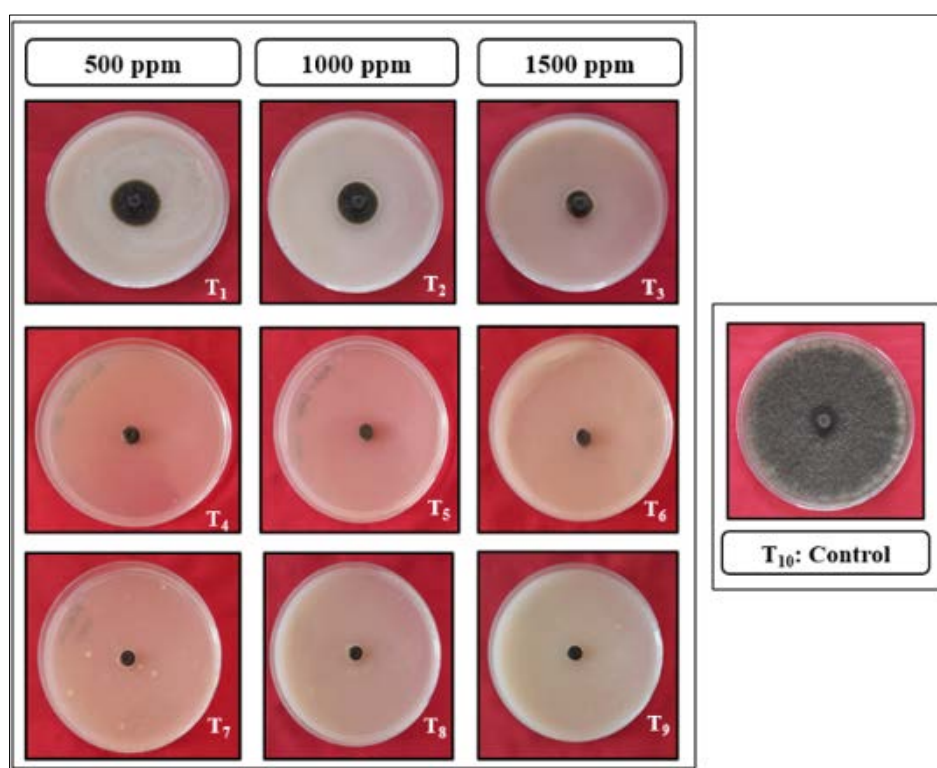


Photo 5: Efficacy of ready-mix fungicides against *A. alternata* under in vitro conditions

Conclusions

In vitro evaluations of biocontrol agents, plant extracts and fungicides revealed the two most effective biocontrol agents, viz., *T. harzianum* and *T. asperellum*, recorded minimum mycelial growth and highest per cent growth inhibition (31.25 mm and 65.28%) and (36.62 mm and 59.31%), respectively, against *A. alternata* under *in vitro* conditions. Among the plant extracts, Neem leaf extract and Eucalyptus leaf extract at 20 per cent concentration were found most effective with minimum mycelial growth and highest per cent growth inhibition (22.17 mm and 75.37%) and (22.50 mm and 75.00%), respectively. Among the ready-mix fungicides, Azoxystrobin 18.2% + Difenconazole 11.4% SC and Tebuconazole 50% + Trifloxystrobin 25% WG recorded cent per cent mycelial growth inhibition against *A. alternata* at 500, 1000 and 1500 ppm concentrations under *in vitro* conditions.

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