



***In vitro* Screening of Botanicals, Bio-Agents and Fungicides against *Alternaria alternata* (Fr.) Keissler, Causing Brinjal Blight Disease**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Blight disease of brinjal caused by *Alternaria alternata* is one of the most destructive disease of brinjal which causes both qualitative and quantitative crop losses. In the present investigation, all plant extracts evaluated at 10% concentration exhibited antifungal activity against *A. alternata*.

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Soapnut rind extract was found the most effective which recorded least colony growth of 18.66 mm and the highest inhibition of 79.26%. Wild brinjal leaf extract was next best treatment with 31.33 mm growth and 65.18% inhibition. Among the eight bio-agents tested, *Trichoderma koningii* and *T. longibrachiatum* showed complete inhibition of mycelial growth of *A. alternata*. *T. harzianum* showed significant effectiveness, with the lowest colony growth of 20.33 mm and 77.41% inhibition. Among the fungicides, Tebuconazole 25.9% EC, Hexaconazole 5% EC, Propiconazole 25% EC, Hexaconazole 4% + Zineb 68% (72% WP), Captan 70% + Hexaconazole 5% (75% WP) and Carboxin 37.5% + Thiram 37.5% (75% WS) each @ 0.1% concentration were found most significantly effective in inhibiting the mycelial growth of *A. alternata* with 100 per cent inhibition over control.

Keywords: *Alternaria alternata*; botanicals; bio-agents; fungicides; blight; brinjal.

1. INTRODUCTION

Brinjal, also known as eggplant or aubergine, is a member of the nightshade family (Solanaceae). Unripe brinjal fruits are highly regarded for their inclusion in numerous dishes, especially curries. They are packed with vital vitamins and minerals, including phosphorus, calcium and iron. Brinjal contains a significant amount of total water-soluble sugars, free reducing sugars and amide proteins. Studies have shown that oblong-fruited eggplant varieties typically have higher overall soluble sugar content, whereas long-fruited varieties are generally richer in free reducing sugars, anthocyanins, phenolic compounds, glycoalkaloids, dry matter and amide proteins (Bajaj et al., 1979). In India, brinjal is predominantly cultivated by marginal, small and medium-scale farmers for both subsistence and income generation. However, its production and productivity are influenced by numerous factors contributing to low crop yields, with biotic factors being the most significant. The crop is susceptible to various diseases, including those caused by fungi, bacteria, viruses and phytoplasmas, which can adversely affect its overall yield. Among the various fungal diseases affecting brinjal, leaf blight caused by *Alternaria alternata* (Fr.) Keissler is the most prevalent and destructive, impacting a wide range of hosts and resulting in both quantitative and qualitative losses. The first report of leaf spot disease on brinjal in India was documented at the Indian Agricultural Research Institute (IARI) in New Delhi (Kapoor and Hingorani, 1958). Pandey and Vishwakarma (1998) noted that *Alternaria* species responsible for leaf spot and fruit rot in brinjal contribute to significant yield reductions. Additionally, Balai and Ahir (2013) recorded yield losses of up to 25% in the Jaipur district of Rajasthan attributed to leaf spot disease caused by *Alternaria alternata* (Fr.) Keissler. Current public perceptions and

environmental concerns necessitate the exploration of alternative eco-friendly disease management strategies. An effective solution may lie in the integration of diverse cultural, biological and chemical methods to address these challenges (Barnwal et al., 2011; Jagan et al., 2013). Recently, there has been growing interest in employing biological control methods, including bio-agents and plant extracts, in both conventional and organic farming systems. Numerous plant extracts have shown significant antifungal activity, highlighting their potential as bio-fungicides within a comprehensive framework for environmental protection. Therefore, it is essential to implement an integrated disease management strategy that incorporates readily available plant extracts, bio-agents and fungicides. This comprehensive approach aims to reduce the reliance on fungicides to the minimum necessary level for effective plant disease control. By promoting sustainable and eco-friendly disease management practices, farmers can mitigate the impact of leaf blight disease, thereby minimizing yield losses and enhancing brinjal production.

2. MATERIALS AND METHODS

The present study was conducted at the Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli during the years 2022-23 and 2023-24.

2.1 *In vitro* Efficacy of Botanicals

Aqueous extract of locally available nine botanicals each at 10% concentration were evaluated *In vitro* against the *A. alternata* by applying standard poisoned food technique (Nene and Thapliyal, 1993) and by using PDA as basal medium. Extracts from nine different plant species viz., garlic, neem, lantana, wild brinjal,

glyricidia, tulsi, soapnut, rui and congress grass were prepared as per the methodology given by Bhatti (1998). For obtaining 10% plant extract, 10 ml of standard plant extract was mixed with 90 ml of PDA in 250 ml conical flask. Later, 20 ml of the PDA medium containing plant extracts was poured into sterilized Petri plates under aseptic conditions. Mycelial discs of 5mm size from seven days old actively grown pure culture were used to inoculate the poured Petri plates. Control was maintained on PDA without adding any plant extract in a medium. Three replications were taken for each treatment. These plates were incubated at $27 \pm 2^\circ\text{C}$ for seven days and radial colony growth was measured.

2.2 In vitro Evaluation of Bio-agents

A total of nine bio-agents viz., *Trichoderma harzianum*, *T. koningii*, *T. longibrachiatum*, *T. viride*, *Aspergillus niger*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Saccharomyces cerevisiae* var. *ellipsoideus* were evaluated against *A. alternata* by applying Dual culture technique (Dennis and Webster, 1971) and using PDA as a basal medium. Seven days old culture of test fungus and test bio-agents were used for the study. Disc of PDA along with culture growth of test fungus and test bio-agents were cut out with cork borer and placed on Petri plates containing PDA at equidistance and exactly opposite to each other and the plates were incubated at $27 \pm 2^\circ\text{C}$. PDA plates inoculated with only culture disc of test fungus were maintained as untreated control. Each treatment was replicated thrice in Completely Randomized Design.

2.3 In vitro Evaluation of Fungicides

Various standard commercial formulation of fungicides were evaluated *in-vitro* against test pathogen by applying standard poisoned food technique (Nene and Thapliyal, 1993) and using potato dextrose agar as basal culture medium. Based on active ingredient, the required quantity of fungicide was calculated and mixed thoroughly with sterilized potato dextrose agar (PDA) medium in conical flasks to obtained desired concentration of fungicides. PDA medium without fungicides was served as untreated control. Fungicides amended PDA poured in Petri plates and allow to solidify at room temperature. After solidification of the medium, all the plated were inoculated with 5mm culture disc of the test fungus obtained from a week old growing pure culture of *A.alternata*. The disc was placed on

PDA in inverted position in the centre of the Petri plate and plates were incubated at $27 \pm 2^\circ\text{C}$ temperature. Each treatment was replicated thrice.

Observation on radial mycelial growth were recorded when untreated control plate was fully covered with mycelial growth of test fungus. Percent mycelial growth inhibition of the test fungus over untreated control was calculated by formula given by Vincent, (1947).

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

Where,

I = Per cent Inhibition

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates.

3. RESULTS AND DISCUSSION

3.1 In vitro Evaluation of Botanicals

The results (Table 1, Plate 1 and Fig. 1) revealed that all the plant extracts tested each @ 10% concentration showed antifungal activity against *A. alternata*. The mycelial growth of test fungus was ranged from 18.66 to 46.83 mm. Whereas, inhibition of mycelial growth of test fungus was ranged from 47.96 to 79.26 per cent. Among the nine plant extracts evaluated soapnut rind extract @ 10% concentration was found significantly superior to all treatments with least colony growth of 18.66 mm and highest inhibition of mycelial growth (79.26%) of *A. alternata*. The next best treatment was wild brinjal leaf extract (10%) which showed 31.33 mm mycelial growth & 65.18 per cent mycelial growth inhibition of *A. alternata*. Lantana leaf extract at 10 per cent concentration was also found effective with minimum mycelial growth of 34.83 mm and highest inhibition of mycelial growth with 61.30 per cent. It was followed by neem leaf extract (39.83 mm & 55.74%), congress grass extract (40.00 mm & 55.55%), garlic clove extract (44.66 mm & 50.37%), glyricidia leaf extract (44.66 mm & 50.37%) and rui leaf extract (44.83 mm & 50.18%) at 10% concentration. Least mycelial inhibition of *A. alternata* was recorded in tulsi leaf extract by 47.96% over control.

These results are in conformity with the earlier findings of those researchers who reported

botanicals viz., garlic clove, neem leaf, tulsi leaf and lantana leaf extract at various concentrations had significantly inhibited mycelial growth of *A. alternata*, inciting leaf spot / blight disease of brinjal (Bochalya, 2010; Rajput, 2010; Sidhdhapara, 2014; Jakatimath et al., 2017; Kumar, 2017; Rajput and Chaudhari, 2018; Nagaraju et al., 2020; Rajkar et al., 2021; Chauhan et al., 2023, Ghule et al. 2023, Shekhada et al. 2023, Sudani, 2023 and Yadav et al. 2024).

3.2 In vitro Evaluation of Bio-agents

The results depicted in Table 2, Plate 2 and Fig. 2 indicated that, all the bio-agents evaluated were significantly effective in inhibiting the mycelial growth of *A. alternata*. Among the eight bio-agents tested, complete mycelial growth inhibition of *A. alternata* was recorded due to *Trichoderma koningii* and *T. longibrachiatum*. However, *T. harzianum* was also found effective

with least colony growth of test pathogen (20.33 mm) with 77.41 per cent inhibition of mycelial growth followed by *T. viride* (30.83 mm & 65.74 %), *Aspergillus niger* (31.16 mm & 65.37 %) and *Saccharomyces cerevisiae* var. *ellipsoideus* (41.50 mm & 53.88 %). Maximum colony growth and least inhibition was observed in case of *Bacillus subtilis* (56.50 mm & 37.22 %) and *Pseudomonas fluorescens* (59.00 mm & 34.44 %), respectively.

The results of present investigation are in consonance with the earlier findings of those researchers who reported bio-agents viz., *T. viride*, *T. harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Aspergillus niger* had significantly inhibited mycelial growth of *A. alternata*, causing leaf spot / blight disease of brinjal (Rajput, 2010; Jakatimath et al., 2017; Dhere, 2021; Khursheed et al., 2021, Rajkar et al., 2021, Ghule et al. 2023 and Shekhada et al. 2023).

Table 1. In vitro efficacy of botanicals against *A. alternata*

Tr. No.	Common Name	Botanical Name	Conc.(%) used	Mean colony diameter (mm)	Per cent inhibition
T ₁	Garlic	<i>Allium sativum</i>	10	44.66	50.37
T ₂	Neem	<i>Azadirachta indica</i>	10	39.83	55.74
T ₃	Lantana	<i>Lantana camara</i>	10	34.83	61.30
T ₄	Wild brinjal	<i>Solanum torvum</i>	10	31.33	65.18
T ₅	Glyricidia	<i>Glyricidia sepium</i>	10	44.66	50.37
T ₆	Tulsi	<i>Ocimum tenuiflorum</i>	10	46.83	47.96
T ₇	Soapnut	<i>Sapindus mukorossi</i>	10	18.66	79.26
T ₈	Rui	<i>Calotropis gigantea</i>	10	44.83	50.18
T ₉	Congressgrass	<i>Parthenium hysterophorus</i>	10	40.00	55.55
T ₁₀	Control	-	-	90.00	-
S.E.m ±				0.53	
C.D. at 1%				1.59	

Table 2. In vitro efficacy of bio-agents against *A. alternata*

Tr. No.	Bio agents evaluated	Mean colony diameter (mm)	Per cent inhibition
T ₁	<i>Trichoderma harzianum</i>	20.33	77.41
T ₂	<i>T. koningi</i>	0.00	100.00
T ₃	<i>T. longibrachiatum</i>	0.00	100.00
T ₄	<i>T. viride</i>	30.83	65.74
T ₅	<i>Aspergillus niger</i>	31.16	65.37
T ₆	<i>Pseudomonas fluorescens</i>	59.00	34.44
T ₇	<i>Bacillus subtilis</i>	56.50	37.22
T ₈	<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i>	41.50	53.88
T ₉	Control	90.00	-
S.E. m ±		0.28	
C.D. at 1%		0.86	

3.3 *In vitro* Evaluation of Fungicides

The results furnished in Table 3, Plate 3 and Fig. 3 revealed that all, the fungicides tested at different concentrations were found significantly effective in inhibiting the mycelial growth of *A. alternata*. Among the systemic fungicides, Tebuconazole 25.9% EC, Hexaconazole 5% EC and Propiconazole 25% EC (each @ 0.1% concentration) and among combi fungicides, Hexaconazole 4% + Zineb 68% (72% WP), Captan 70% + Hexaconazole 5% (75% WP) and Carboxin 37.5% + Thiram 37.5% (75% WS) (each @ 0.1% concentration) were found most significantly effective in inhibiting the mycelial growth of *A. alternata* with 100 per cent inhibition over control. Among rest of the fungicides, Copper Hydroxide 77% WP (0.1%) resulted with minimum mycelial growth of 8.83 mm and with inhibition of 90.18% followed by Tebuconazole 50% + Trifloxystrobin 25% WG (0.05%) (10.00 mm & 88.88%), Copper oxychloride 50% WP (0.25%) (16.50 mm & 81.66%), Mancozeb 75% WP (0.2%) (19.20 mm & 78.66%), Carbendazim + Mancozeb 63% (75% WP) (0.2%) (19.50 mm & 78.33%), Captan 50% WP (0.2 %) (22.50 mm &

75.00%) and Zineb 75% WP (0.2%) (30.66 mm & 65.93%), respectively with average mycelial growth and its corresponding average inhibition. Chlorothalonil 75% WP (0.2%) and Carbendazim 50% WP (0.1%) were found least effective in inhibiting the mycelial growth of test pathogen with maximum colony growth of 41.26 mm & 55.33 mm and least inhibition of mycelial growth by 54.15% & 38.52%, respectively.

These results are in conformity with the earlier findings of those researchers who reported fungicides viz., Propiconazole 25% EC, Difenconazole 25% EC, Hexaconazole 5% EC, Mancozeb 75% WP, Zineb 75% WP, Carbendazim 50% WP, Chlorothalonil 75% WP, Copper oxychloride 75% WP, Carbendazim 12% + Mancozeb 63% WP at different concentrations had significantly inhibited mycelial growth of *A. alternata*, inciting leaf spot / blight disease of brinjal (Rajput, 2010; Jakatimath *et al.*, 2017; by Kumar, 2017; Rajput and Chaudhari, 2018; Kumar, 2019, Ghule *et al.* 2023, Shekhada *et al.* 2023, Maurya *et al.*, 2023 and Yadav *et al.* ,2024).

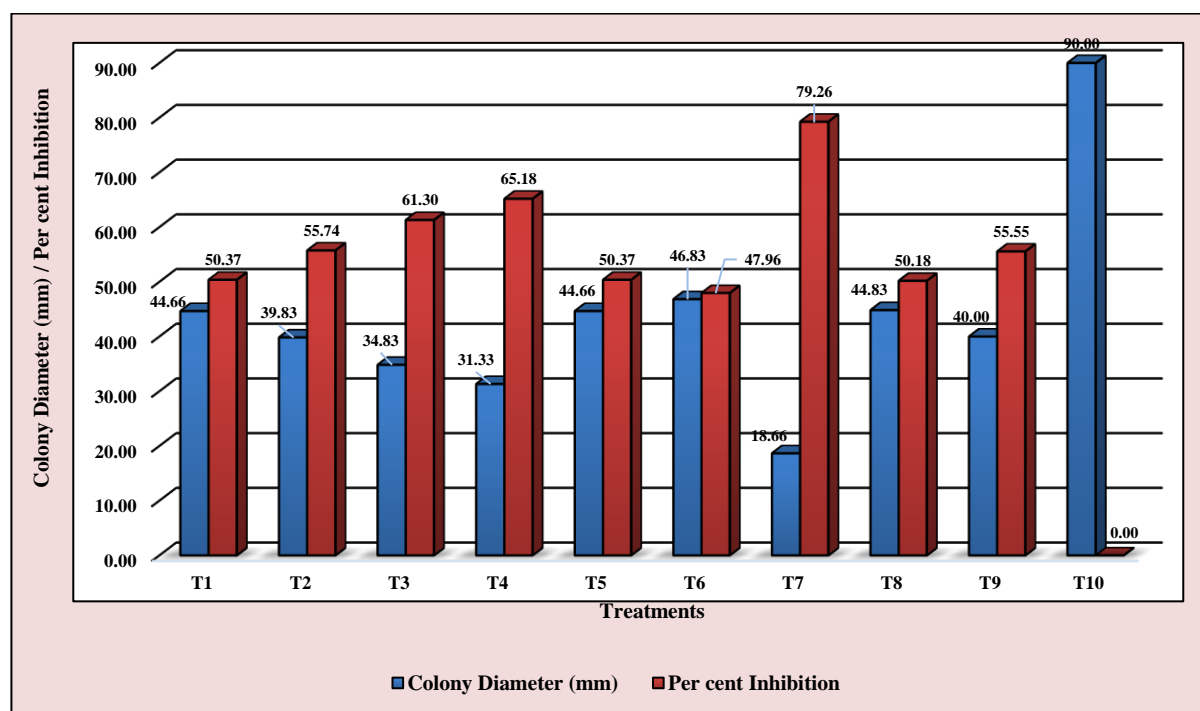


Fig. 1. *In vitro* efficacy of botanicals against *A. alternata*

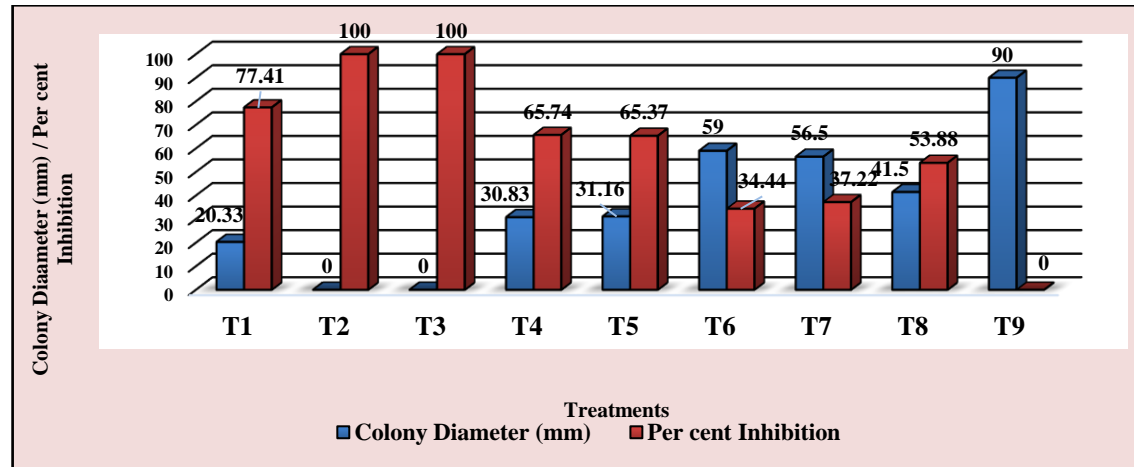


Fig. 2. *In vitro* efficacy of bio-agents against *A. alternata*

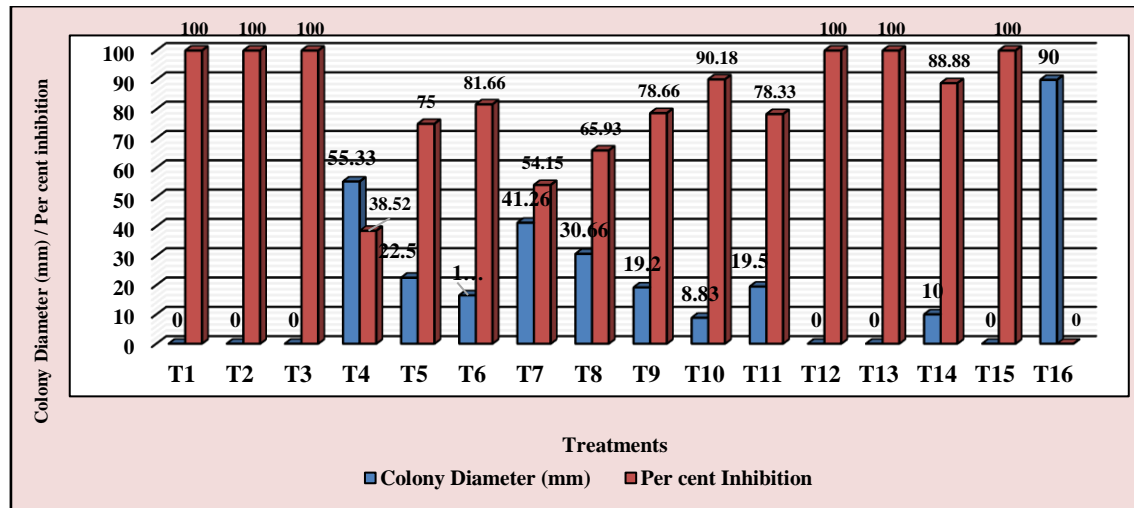


Fig. 3. *In vitro* efficacy of fungicides against *A. alternata*

Table 3. *In vitro* efficacy of fungicides against *A. alternata*

Tr. No.	Fungicides	Conc. (%) used	Mean colony diameter (mm)	Per cent inhibition
T ₁	Tebuconazole 25.9% EC	0.1	0.00	100
T ₂	Hexaconazole 5% EC	0.1	0.00	100
T ₃	Propiconazole 25% EC	0.1	0.00	100
T ₄	Carbendazim 50% WP	0.1	55.33	38.52
T ₅	Captan 50% WP	0.2	22.50	75.00
T ₆	Copper oxychloride 50% WP	0.25	16.50	81.66
T ₇	Chlorothalonil 75% WP	0.2	41.26	54.15
T ₈	Zineb 75% WP	0.2	30.66	65.93
T ₉	Mancozeb 75% WP	0.2	19.20	78.66
T ₁₀	Copper Hydroxide 77% WP	0.1	8.83	90.18
T ₁₁	Carbendazim + Mancozeb 63% (75% WP)	0.2	19.50	78.33
T ₁₂	Hexaconazole 4% + Zineb 68% (72% WP)	0.1	0.00	100
T ₁₃	Captan 70% + Hexaconazole 5% (75% WP)	0.1	0.00	100
T ₁₄	Tebuconazole 50% +Trifloxystrobin 25% WG	0.05	10.00	88.88
T ₁₅	Carboxin 37.5% + Thiram 37.5% (75% WS)	0.1	0.00	100
T ₁₆	Control	-	90.00	-
S.E. m ±			0.31	
C.D. at 1%			0.91	



Plate 1. *In vitro* efficacy of botanicals against *A. alternata*



Plate 2. *In vitro* efficacy of bio-agents against *A. alternata*

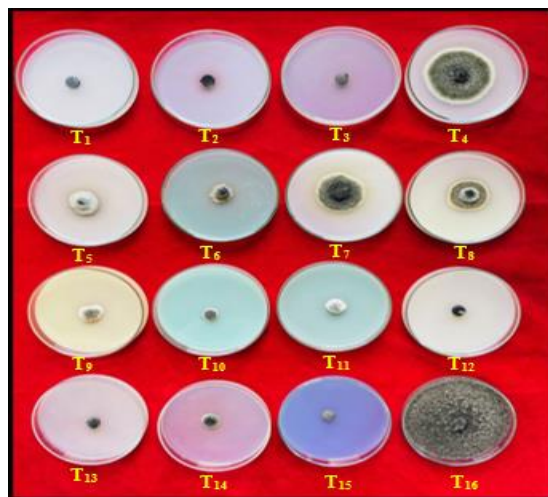


Plate. 3. *In vitro* efficacy of fungicides against *A. alternata*



Plate. 4. Pure Culture of *Alternaria alternata*



Plate. 5. Microscopic view of *Alternaria alternata*

4. CONCLUSION

From the results of present experiment, it is concluded that blight disease of brinjal incited by *A. alternata* can be effectively controlled by botanicals namely, soapnut rind extract and wild brinjal extract @ 10% concentration and bio-agents namely, *Trichoderma koningii* and *T. longibrachiatum*. Tebuconazole 25.9% EC, Hexaconazole 5% EC, Propiconazole 25% EC, Hexaconazole 4% + Zineb 68% (72% WP), Captan 70% + Hexaconazole 5% (75% WP) and Carboxin 37.5% + Thiram 37.5% (75% WS) (each @ 0.1%) are most effective which completely inhibits mycelial growth of *A. alternata* *in vitro*.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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