In vitro screening of botanicals, bio-agents and fungicides against *Alternaria alternata* (Fr.) Keissler, causing brinjal blight disease.

Abstract:

Blight disease of brinjal caused by *Alternariaalternata*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*. Soapnut rind extract was found the most effective which recorded leastcolony 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, *Trichodermakoningii* and *T. longibrachiatum*showed complete inhibition of mycelial growthof *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

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 *etal.*, 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 *Alternariaalternata* (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 *Alternariaalternata* (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.

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.

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 ± 2^0C for seven days and radial colony growth was measured.

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 ± 2°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..

3. ***In vitro* evaluation of fungicides**

Various standard commercial formulation of fungicides were evaluated *in-vitro* against test pathogenby 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 ± 2^0C 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.

Results and Discussion

1. *Invitro* evaluation of botanicals

The results (Table 1, Plate I 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 *etal*., 2017; Kumar, 2017; Rajput and Chaudhari, 2018; Nagaraju *etal*., 2020; Rajkar *etal*., 2021; Chauhan *etal*., 2023, Ghule *et al.* 2023, Shekhada *et al.* 2023, Sudani, 2023 and Yadav *etal.* 2024).

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	*Allium sativum*	10	44.66	50.37
T_2	Neem	*Azadirachta indica*	10	39.83	55.74
T_3	Lantana	*Lantana camara*	10	34.83	61.30
T_4	Wild brinjal	*Solanum torvum*	10	31.33	65.18
T_5	Glyricidia	*Glyricidia sepium*	10	44.66	50.37
T_6	Tulsi	*Ocimum tenuiflorum*	10	46.83	47.96
T_7	Soapnut	*Sapindus mukorossi*	10	18.66	79.26
T_8	Rui	*Calotropis gigantea*	10	44.83	50.18
T_9	Congressgrass	*Parthenium hysterophorus*	10	40.00	55.55
T_{10}	Control	-	-	90.00	-
	S.E.m ±			**0.53**	
	C.D. at 1%			**1.59**	

2. *Invitro* evaluation of bio-agents

The results depicted in Table 2, Plate II 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 koningiiand 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 cerevisae*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, Pseudomans 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 *etal.*, 2021, Ghule *et al.* 2023 and Shekhada *et al.* 2023).

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	*Trichoderma harzianum*	20.33	77.41
T_2	*T. koningi*	0.00	100.00
T_3	*T. longibrachiatum*	0.00	100.00
T_4	*T. viride*	30.83	65.74
T_5	*Aspergillus niger*	31.16	65.37
T_6	*Pseudomonas fluorescens*	59.00	34.44
T_7	*Bacillus subtilis*	56.50	37.22
T_8	*Saccharomyces cerevisae* var.*ellipsoideus*	41.50	53.88
T_9	Control	90.00	-
	S.E.m ±	**0.28**	
	C.D. at 1%	**0.86**	

3. *Invitro* evaluation of fungicides

The results furnished in Table 3, Plate III 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 concnentartions had significantly inhibited mycelial growth of *A. alternata*, inciting leaf spot / blight disease of brinjal (Rajput, 2010; Jakatimath *etal*., 2017; by Kumar, 2017; Rajput and Chaudhari, 2018; Kumar, 2019, Ghule *et al*. 2023, Shekhada *et al*. 2023, Maurya *et al*., 2023 and Yadav *etal*. ,2024).

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**	

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 koningiiand 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 effectivewhich completely inhibits mycelial growth of *A. alternatainvitro*.

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2.

3.

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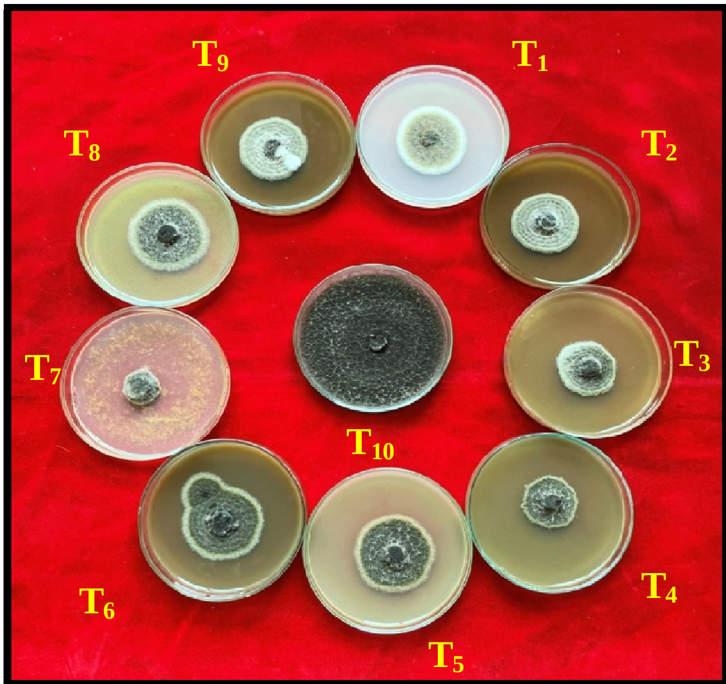


Plate I:*Invitro* efficacy of botanicals against *A. alternata*

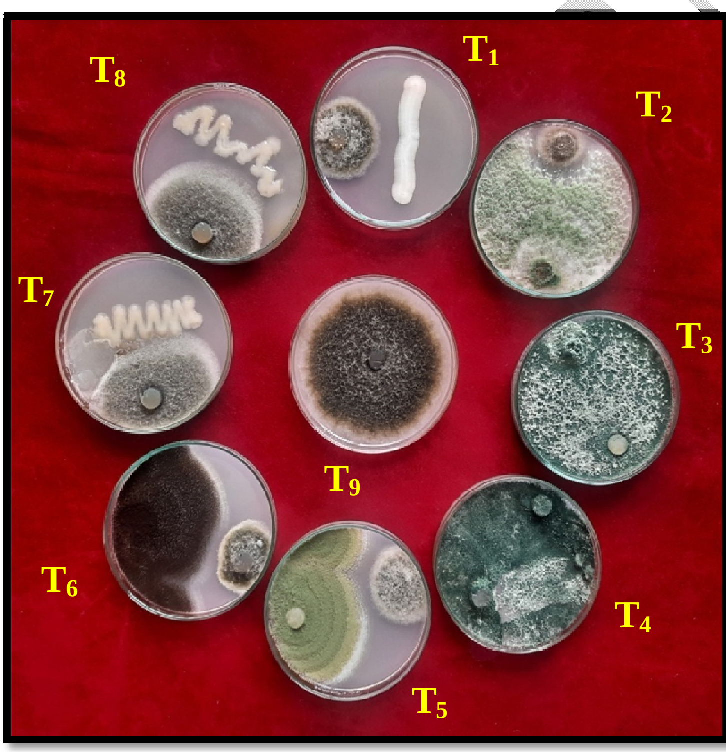


Plate II:*Invitro* efficacy of bio-agents against *A. alternata*

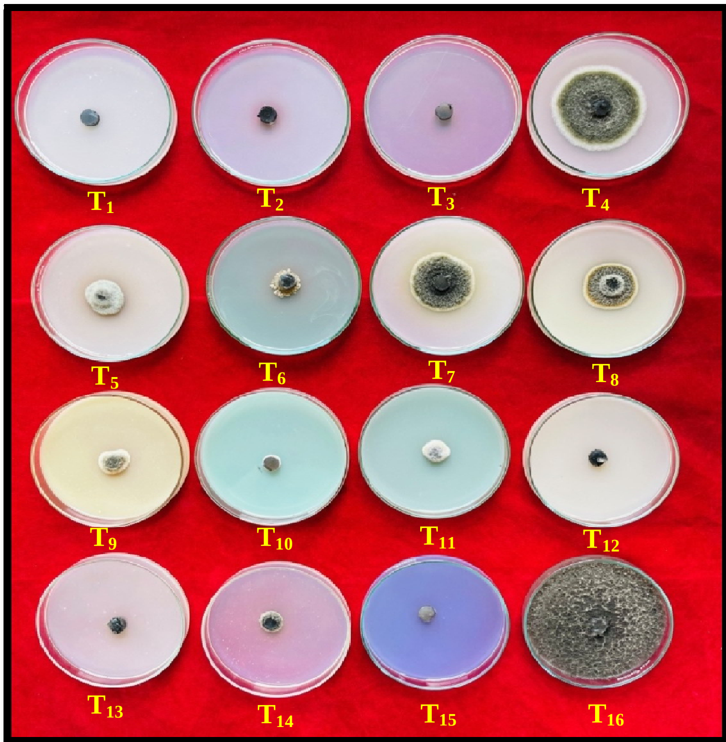


Plate III:*Invitro* efficacy of fungicides against *A. alternata*

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

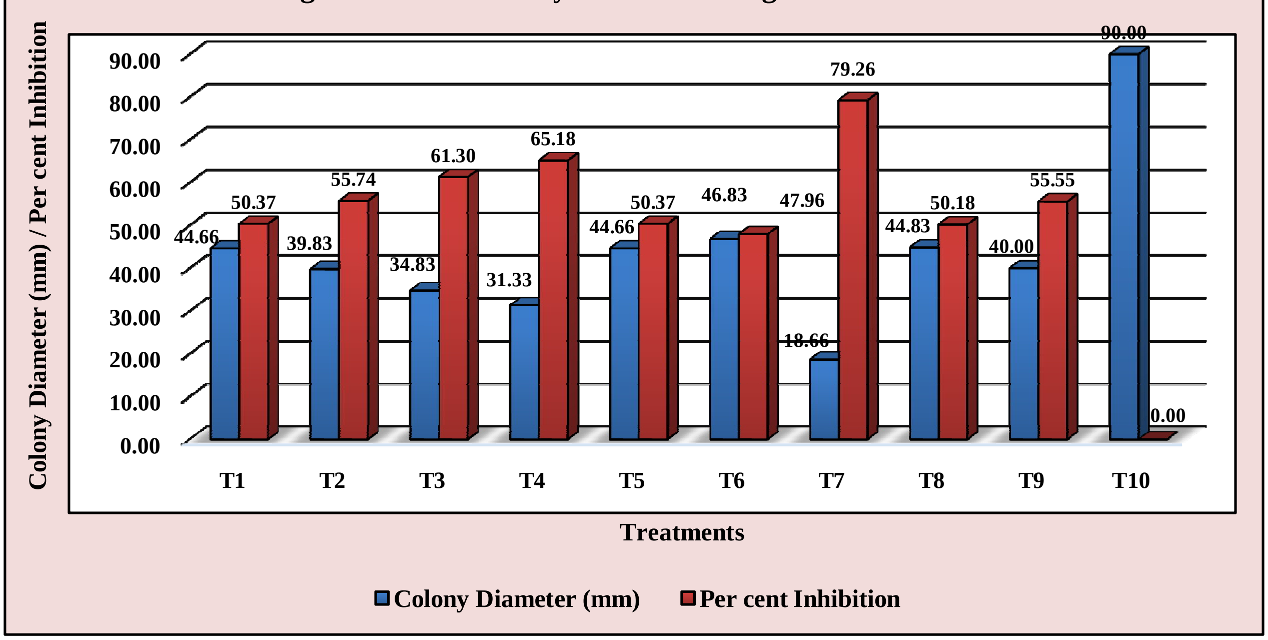


Fig. 2 : *In vitro* efficacy of bio-agents against *A. alternata*

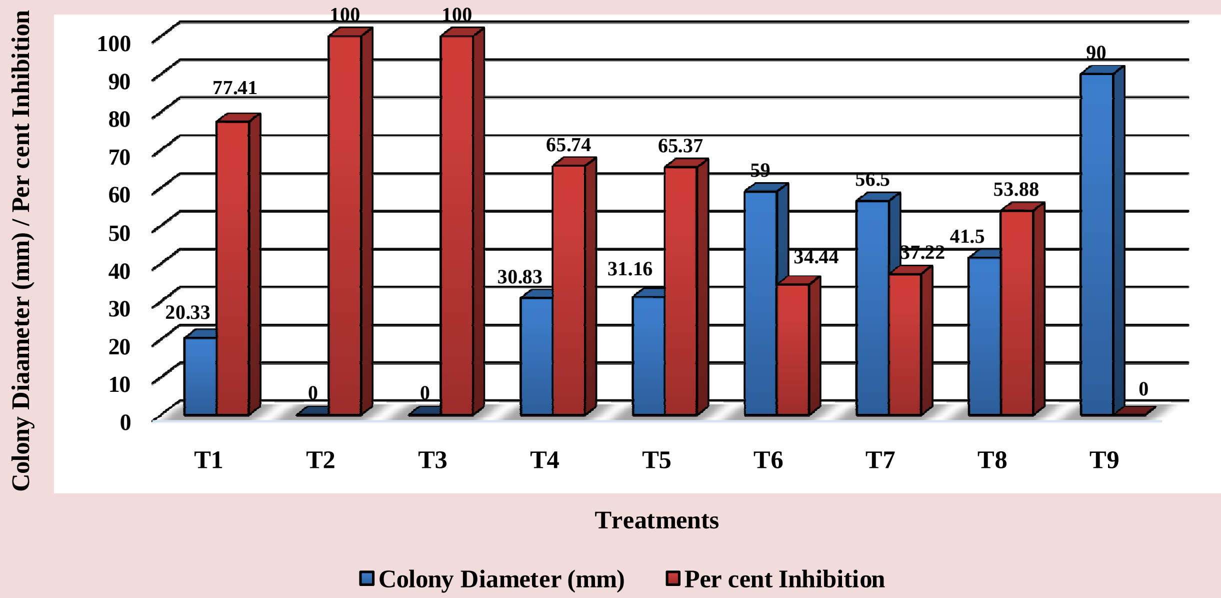
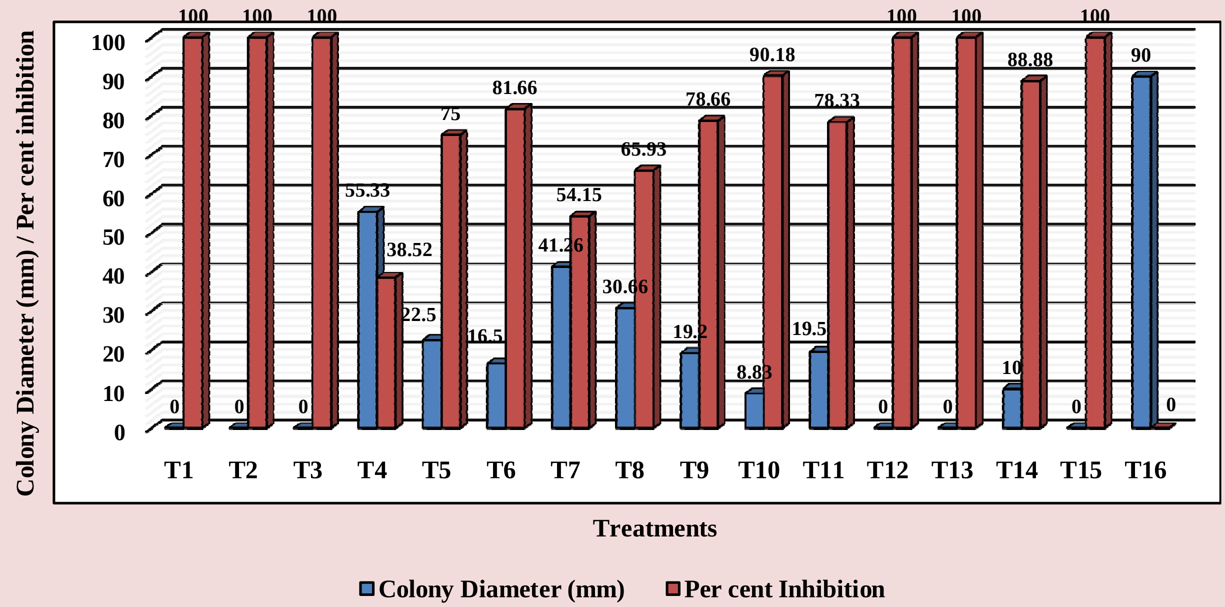


Fig. 3 : *In vitro* efficacy of fungicides against *A. alternata*



Pure Culture of *Alternaria alternata*

Microscopic view of *Alternaria alternata*