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Comparative *in vitro* evaluation of fungicides, botanicals and bioagents for suppressing *Alternaria alternata* causing Alternaria leaf spot in ber

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ABSTRACT

The present experiment was conducted during 2024-25 in the laboratory of the Department of Fruit Science, College of Horticulture and Forestry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, to evaluate the efficacy of eleven chemical fungicides at four concentrations (750 ppm, 1000 ppm, 1500 ppm, and 2000 ppm), along with ten botanical extracts and two bio-agents tested at three concentrations (10%, 15%, and 20%) against *Alternaria alternata*. The study was carried out using poisoned food technique for chemical fungicides, botanical extracts and bio-agent with Completely Randomized Design (CRD), Among the fungicides Hexaconazole and Tebuconazole proved to be the most effective chemical fungicide recording complete growth inhibition (100%) at all the four tested concentrations which was followed by Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC (100%) at 1500ppm and 2000ppm, Kasugamycin 5% + Copper oxychloride 45% WP (7.90mm, 91.22%) followed by Thiophanate methyl 38% + Kasugamycin 2.21% SC (8.13mm, 90.97%) at 2000ppm whereas, at 750ppm Copper oxychloride (38.40mm) was least effective in reducing fungal growth (57.33%). Among the botanical extracts and bioagents, recording average highest growth inhibition observed in Garlic bulb extract (80.59%) followed by Eucalyptus (76.11%), *Trichoderma viride* (72.95%) and minimum inhibition percent (31.30%) was observed in Ashok. The study indicated better performance of some chemical fungicides even at lower concentrations i.e. 750ppm and 1000ppm also. So, such effective fungicides could be used to minimize hazardous effect. Significant effect of some botanical extracts and bio-agent against pathogen growth suggests their application as potential control agent alternative to chemicals.

INTRODUCTION

The Ber (*Zizyphus jujube* Lamk.) is mainly grown as a fruit tree in wide range of tropical and sub-tropical and Mediterranean region, is native of India, ber covers 1.49% of total area and 1.39% total fruit production. The total area is 1 lakh hectares with annual production of 32.13 Lakhs tonnes (APEDA, 2023-24). Uttar Pradesh has the largest production 4.85 lakhs tonnes every year. The total productivity of ber in India is 10 metric tonnes per ha. "Zizaif" is the Arabic name of the fruit which has been derived from the genus *Zizyphus*. It is commonly called as Poor man's fruit. In India and it is also popularly called the king of arid zone fruit (Kumar and Singh, 2020). It is commercially cultivated in Haryana, Punjab, Maharashtra, Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, Andhra Pradesh and Tamil Nadu. In U.P., ber orchards are found in Varanasi, Faizabad, Agra, Raebareli districts (National Horticulture Board 2023). The fruit are widely acknowledged as a rich and cheap source of minerals and other nutrients such as iron, calcium and phosphorus, ascorbic acid, carbohydrates and essential minerals (Abbas *et al.*, 1988; Pareek *et al.*, 2002; Alam *et al.*, 2017). The importance of ber is due to the fact that, it is hardy fruit which can be grown in alkaline soils with pH as high as 9.2. However, deep sandy loam to loamy soils with neutral or slightly alkaline pH are considered optimum for growth. A principal limiting factor in profitable cultivation of ber tree is the attack of several diseases mainly fungi which cause heavy crop loss at all the stages, right after initiation of leaves to fruiting and harvest stage. Some important fungal diseases are Alternaria leaf spot (*Alternaria alternata* (Fr.) Keissler), Powdery mildew (*Oidium erysiphoides* f. sp. *ziziph*), Black leaf spot (*Isariopsis indica* var. *ziziphi*), Cercospora leaf spot (*Cercospora ziziphi*), Cladiosporium leaf spot (*Cladosporium ziziphi*), Algal leaf spot and Rust (*Uredo ziziphi*). Among these diseases Alternaria leaf spot caused by *Alternaria alternata* leads to significant damaging disease and yield losses in ber cultivation (Kumar, *et al.*, 2020 and Chaudhary *et al.*, 2021a). To investigate innovative approaches on mitigate its impact in crop production. The disease leads to emergence of small, irregular brown spots on the upper surface of the leaves of ber. Dark brown to black dots appears on the lower surface (Gupta and Madaan, 1977; Kumar *et al.*, 2020; Kumar *et al.*, 2022; Kumar *et al.*, 2023). Alternaria leaf spot was a minor disease, but due to climatic changes, it emerged moderately to severe form now a days. Therefore, keeping in view the importance of the orchard and seriousness of the disease the present study was conducted during 2024-2025 to evaluate *in vitro* efficacy of some fungicides, plant extracts and bio-agent against *Alternaria alternata* causing infection on ber.

Materials and Methods

The experiment was carried out during 2024-2025 at the laboratory of the Department of Fruit Science, College of Horticulture and Forestry, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) India, in Completely Randomized Design using poisoned food technique. Eleven chemical fungicides *i.e.* Flusilazole 12.5% + Carbendazim 25% SE, Kasugamycin 5% + Copper oxychloride 45% WP, Thiophanate methyl 38% + Kasugamycin 2.21% SC, Tebuconazole 25% + Trifloxystrobin 25% WG, Propiconazole 25% EC, Hexaconazole 25% EC,, Copper Oxychloride 50% WG, Carbendazim 12% + Mancozeb63% WP, Azoxystrobin 23% SC, Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC, Zineb 68% + Hexaconazole 4% WP were evaluated at four different concentrations such as 750, 1000, 1500 and 2000 ppm and ten botanical extracts (Garlic, Ashok, Madar, Lantana, Turmeric, Neem, Eucalyptus, Onion, Tulsi, Karanj) and two bio-agents *i.e. Pseudomonas fluorescens*, *Trichoderma viride* were evaluated at three different concentrations *viz.* 10%, 15% and 20%, respectively. Each of the treatment was replicated in four times.

Pathogenic *Alternaria alternata* was isolated from infected leaf of ber collected from MES Horticulture Farm, ANDUAT, Ayodhya, Uttar Pradesh. Spores were teased from infected portion for microscopic examination to check the presence of pathogenic fungus. After confirming the presence of *Alternaria alternata*, Koch postulate was proved. Infected leaves were cut into small pieces (1-1.5mm) with sterile blade. These pieces were disinfected with 0.5% sodium hypochlorite (NaOCl) solution for two minutes followed by three washings with distilled water and excessive moisture was removed using sterile blotting paper. The sterilized leaf pieces were placed on PDA medium using sterilized forceps and incubated at 25 ± 1ºC for 7 days. Then the culture was purified by transferring small piece of agar containing spore to another Petri-plate containing media and incubated at 25 ± 1ºC for 7 days. The pathogen was sub cultured three times obtain pure culture and pure culture thus obtained was preserved in PDA slant at 4ºC.

***In-vitro* evaluation of newely molecules fungicides, botanical and bioagents**

Botanical extract was prepared as per methods used by Ul-Haq *et al*. (2014) and Thaware *et al*. (2010). Fresh and healthy leaves, bulbs and rhizomes were collected, thoroughly washed in tap water followed by sterilized distilled water, then air dried and grounded with mortar and pestle with the addition of distilled water at the ratio of 1:1 w/v. Then the extract obtained was filtered through double layered muslin cloth and centrifuged at 4000 rpm for 5 minutes. The supernatant was filtered through Whatman's filter paper No. 1 and boiled at 80ºC for 10 minutes in a hot water bath. Thus, obtained filtrate was taken as 100% basic stock solution. After autoclaving PDA media and cooling it to 50ºC required amount of this standard solution was mixed into PDA to get final concentration (%) of 10, 15 and 20 for poisoned food technique. Similarly for the evaluation of chemical fungicides, calculated amount of stock solution was mixed in sterilized PDA to make final concentration in ppm of 750, 1000, 1500 and 2000. Twenty ml of amended PDA was poured in each 90 mm sterilized Petri-plate and allowed to solidify. Control treatment was maintained without adding plant extracts or fungicides on PDA. A circular disc of 7mm diameter from 9 days old culture of *Alternaria alternata* was cut with sterilized cork borer and inoculated in the centre of solidified amended as well as in control treatment. Each treatment was replicated in four Petri-plates were incubated at 25 ± 1 ºC for nine days.

Growth inhibition test

The observation on mycelial growth was recorded after 9 days of incubation in each treatment using vernier caliper scale. The per cent growth inhibition of mycelial growth over control was calculated by using the formula given by Vincent, 1947 and Kantwa *et al*., 2014).

$$\text{Growth Inhibition (\%)} = \frac{\text{Radial growth in control} - \text{Radial growth of treated value}}{\text{Radial growth in control}} \text{ x } 100$$

Statistical analysis

All the data were entered in MS Excel (2010) and analysis of variance was done using Opstat software. Mean comparison was done using Fisher-LSD test at 0.05 level of significance.

Results and Discussion

***In-vitro* evaluation of newely molecules of fungicides**

The efficacy of different chemical fungicides against test fungus was evaluated *in vitro* using poisoned food technique. The data on mycelial growth in table 1 and fig. 1 and inhibition per cent presented in table 2 and fig. 2. The data was reveals that the chemicals tested Hexaconazole and Tebuconazole 25% + Trifloxystrobin 25% proved to be the most effective chemical fungicide recording 100% growth inhibition at all four tested concentrations which was followed by Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC (100%) at 1500ppm and 2000ppm, respectively. At 2000ppm minimum mycelial growth and maximun per cent inhibition was recorded with Kasugamycin 5% + Copper oxychloride 45% WP (7.90mm, 91.22%) followed by Thiophanate methyl 38% + Kasugamycin 2.21% SC (8.13mm, 90.97%), Zineb 68% + Hexaconazole 4% WP (8.57mm, 90.48%), Propiconazole 25% EC (9.97mm, 88.92%), Flusilazole 12.5% + Carbendazim 25% SE (10.47mm, 88.37%) and Carbendazim 12% + Mancozeb 63% WP (15.23mm, 83.08%), respectively. Similarly, Chaudhary *et al*. (2021) evaluation of fungal growth was performed using chemical fungicides and tested nine fungicides at concentrations of 100, 250, 500, 1000, 1500, 2000 and 2500 ppm, including Hexaconazole (97.95%) showed maximum inhibition of mycelial growth followed by Tebuconazole (96.57%) against Alternaria leaf spot of ber. Kumar *et al*. (2022) was conducted to study the efficacy of fungicides viz., Cymoxnil + Mancozeb, Hexaconazole, Tebuconazole, Metalaxyl + Mancozeb, Propiconazole, Carbendazim, Kresoxim methyl and Mancozeb against the mycelial growth of *A. alternata*. Among fungicides, Cymoxnil + Mancozeb proved to be the most effective fungicides showing complete mycelial growth inhibition at all concentration (250, 500, 1000, 1500 and 2000 ppm) followed by Hexaconazole (99.35%) followed by Tebuconazole (98.35%).

Table-1: Efficacy of newely molecules fungicides on mycelial growth of *A. alternata*

S. No.	Treatments	Mycelia growth (mm) at ppm				Average mycelial growth(mm)
		750	1000	1500	2000	
1	Flusilazole 12.5% + Carbendazim 25% SE	24.07 (29.36)	19.67 (26.30)	18.8 (25.68)	10.47 (17.93)	18.25
2	Kasugamycin 5% + Copper oxychloride 45% WP	22.07 (28.01)	17.53 (24.89)	13.60 (21.62)	7.90 (16.31)	13.01
3	Thiophanate methyl 38% + Kasugamycin 2.21% SC	22.53 (28.32)	17.73 (24.96)	15.00 (22.77)	8.13 (16.55)	13.62
4	Tebuconazole 25% + Trifloxystrobin 25% WG	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00
5	Propiconazole 25% EC	23.20 (28.78)	18.87 (25.72)	17.73 (24.96)	9.97 (17.38)	17.44

6	Hexaconazole 25% EC	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00
7	Copper oxychloride 50% WG	38.40 (38.27)	36.17 (36.95)	27.56 (31.65)	20.93 (27.20)	30.76
8	Carbendazim 12% + Mancozeb 63% WP	29.00 (32.56)	27.17 (31.39)	21.73 (27.77)	15.23 (22.95)	23.28
9	Azoxystrobin 23% SC	36.80 (37.33)	33.83 (35.54)	27.2 (31.41)	18.93 (25.77)	29.19
10	Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC	6.43 (14.68)	2.03 (8.18)	0.00 (0.0)	0.00 (0.0)	2.11
11	Zineb 68% + Hexaconazole 4% WP	22.50 (22.01)	17.98 (25.80)	16.86 (24.66)	8.57 (16.98)	16.47
12	Control (water spray)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00
	SE(m)	**1.894**	**0.586**	**0.4**	**0.45**	
	CD	**5.562**	**1.722**	**1.17**	**1.31**	
	CV	**12.768**	**4.307**	**3.49**	**4.92**	

Table within parentheses represent arcsine or transformed values, as indicated by the bracketed entries.

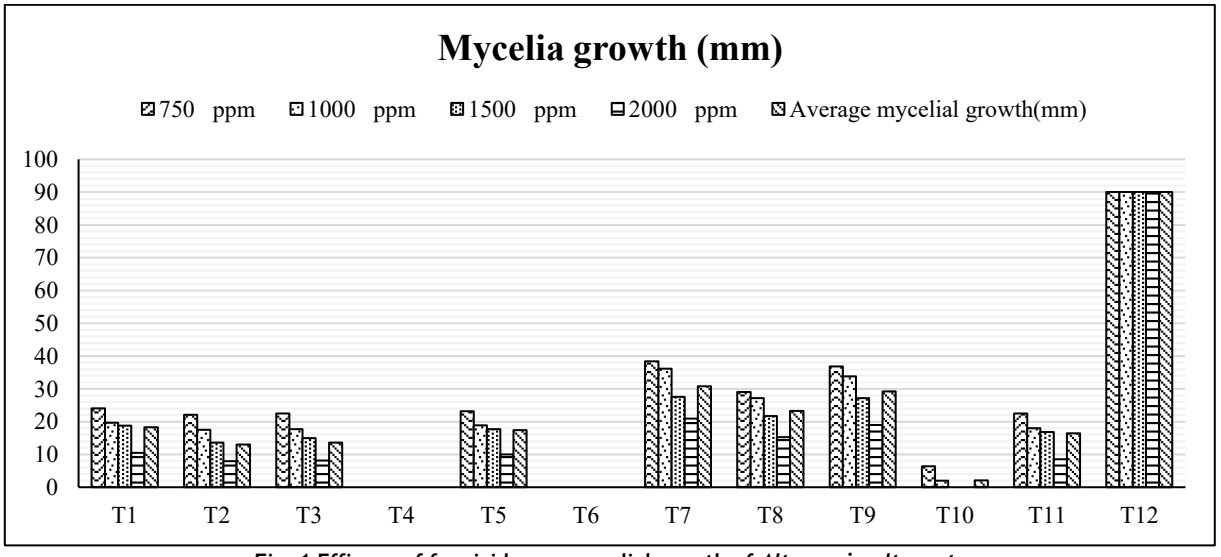

Fig. 1 Efficacy of fungicides on mycelial growth of *Alternaria alternata*

Table-2: Efficacy of newely molecules fungicides on *A. alternata* growth inhibition (%)

S. No.	Treatments	Per cent growth inhibition (%) at ppm				Average inhibition (%)
		750	1000	1500	2000	
1	Flusilazole 12.5% + Carbendazim 25% SE	73.26 (60.64)	78.15 (63.70)	79.11 (64.32)	88.37 (72.05)	79.72
2	Kasugamycin 5% +Copper oxychloride 45% WP	75.47 (61.99)	80.52 (65.11)	84.89 (68.38)	91.22 (73.69)	83.03
3	Thiophanate methyl 38% + Kasugamycin 2.21% SC	74.96 (61.68)	80.30 (65.04)	83.33 (67.23)	90.97 (73.45)	82.39
4	Tebuconazole 25% + Trifloxystrobin 25% WG	100 (90)	100 (90)	100 (90)	100 (90)	100
5	Propiconazole 25% EC	74.22 (61.22)	79.04 (64.28)	80.30 (65.04)	88.92 (72.62)	80.62
6	Hexaconazole 25% EC	100 (90)	100 (90)	100 (90)	100 (90)	100
7	Copper oxychloride 50% WG	57.33 (51.73)	59.8 (53.05)	69.38 (58.35)	76.74 (62.80)	65.82
8	Carbendazim 12% + Mancozeb 63% WP	67.77 (55.44)	69.82 (58.61)	75.85 (62.23)	83.07 (67.05)	74.12
9	Azoxystrobin 23% SC	59.11 (52.67)	62.41 (54.46)	69.78 (58.59)	78.96 (64.23)	67.57
10	Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC	92.85 (75.32)	97.74 (81.82)	100 (90)	100 (90)	97.64
11	Zineb 68% + Hexaconazole 4% WP	75.00	80.02	81.27	90.48	81.69

		(67.99)	(64.20)	(65.34)	(73.02)	
12	Control (water spray)	0	0	0	0	0
	SE(m)	**1.06**	**1.027**	**0.786**	**0.607**	
	CD	**3.145**	**3.031**	**2.32**	**1.793**	
	CV	**2.379**	**2.201**	**1.604**	**1.17**	

Table within parentheses represent arcsine or transformed values, as indicated by the bracketed entries.

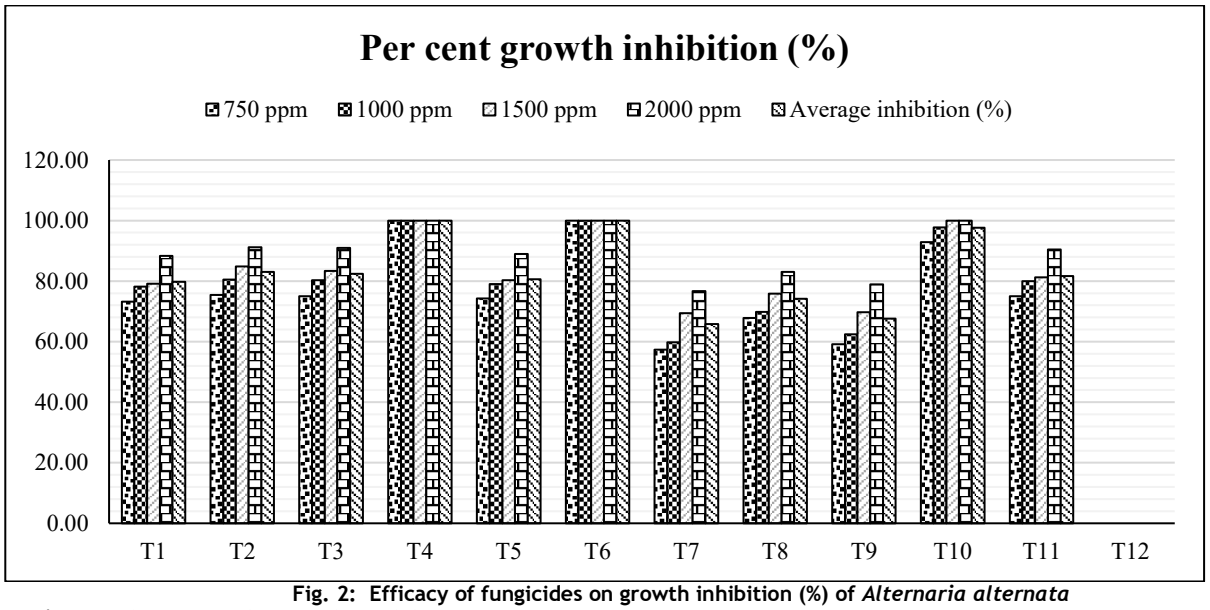

Fig. 2: Efficacy of fungicides on growth inhibition (%) of *Alternaria alternata*

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

Ten different botanical extracts and two bio-agents were evaluated at 10, 15, and 20% concentrations for their efficacy against *A. alternata*. The result revealed that, all the tested botanicals and bioagents inhibited the growth of pathogen over untreated control in table 3 and fig. 3 and table 4 and fig. 4.

Garlic bulb extract at 10% concentration showed that the minimum radial mycelial growth (24.23mm) and highest inhibition rate (73.07%) was found most effective subsequently followed by Eucalyptus (29.83mm, 66.85%), *Trichoderma viride* (34.50mm, 61.66%), Tulsi (37.50mm, 58.88%), *Pseudomonas fluorescens* (37.00mm, 58.33%), Neem (44.83mm, 50.18%), Onion bulb extract (47.33mm and 47.71%) and so no.

The investigation also concluded that, when compared to the control, all the plant extracts at 15% doses were noticeably better in inhibiting the growth of *A. alternata*. Garlic bulb extract exhibited the lowest radial mycelial growth (19.66mm) and the highest inhibition rate (82.77%), followed by Eucalyptus (21.66mm, 75.93%), *Trichoderma viride* (24.20mm, 73.14%), Tulsi (30.66mm, 65.93%), *Pseudomonas fluorescens* (33.83mm, 62.41%), Neem (35.20mm, 60.88%), Onion bulb extract (36.86mm and 59.04%) and so no.

At 20% concentrations, Garlic bulb extract exhibited the radial mycelial growth and the highest inhibition rate (13.00mm, 85.55%), followed by Eucalyptus (14.43mm, 83.96%), *Trichoderma viride* (17.46mm, 84.07%), Tulsi (19.66mm, 80.60%), *Pseudomonas fluorescens* (27.70mm, 69.44%), Neem (32.86mm, 63.48%) and Onion bulb extract (33.93mm, 62.30%). This showed that these botanical extracts have significant potential in suppressing *A. alternata* growth under laboratory conditions.

Among three concentrations used, maximum average mycelial growth and inhibition observed in Garlic bulb extract (18.96mm, 80.46%) followed by Eucalyptus (21.97mm, 75.58%) and minimum inhibition percent (61.82mm, 31.30%) was observed in Ashok.

According to Chaudhary and Singh (2021) were tested at 10%, 15% and 20% concentration, out of which garlic (74.88%) showed maximum mycelium growth inhibition followed by ginger (73.51%) and *neem* (69.25%), while minimum inhibition was found by Parthenium (34.64%) followed by Dhatura (53.37). It was concluded that the botanicals are also effective in controlling the alternaria leaf spot of ber. Zade *et al*. (2018), reported that the extracts of garlic clove @ 10% resulted in maximum inhibition (87.50%) of mycelial growth followed by Neem leaf extract and Onion bulb extract recorded 47.34 and 43.47 percent mycelial inhibition of *A. alternata*. Singh *et al*. (2013) in Alternaria blight of rapeseed-mustard.

Table-3: Efficacy of botanicals and bioagents on mycelial growth of *Alternaria alternata* on PDA

S. No.	Treatment	Mycelia growth (mm)			Average mycelial growth (mm)
		10% Cons.	15% Cons.	20% Cons.	
1	Eucalyptus	29.83 (33.09)	21.66 (27.72)	14.43 (22.31)	21.97
2	Ashok	73.66 (59.10)	62.16 (52.02)	49.66 (44.79)	61.82
3	Madar	63.16 (52.61)	52.26 (46.28)	44.16 (41.63)	53.19
4	Lantana	55.93 (49.12)	49.20 (44.52)	40.26 (39.37)	48.46
5	Turmeric	49.33 (44.60)	41.83 (40.28)	36.50 (37.15)	42.55
6	Neem	44.83 (42.01)	35.20 (36.37)	32.86 (34.48)	37.63
7	Onion	47.33 (43.45)	36.86 (37.37)	33.93 (35.61)	39.37
8	Garlic	24.23 (29.47)	19.66 (26.31)	13.0 (21.11)	18.96

9	Tulsi	37.00 (37.45)	30.66 (33.61)	19.66 (26.31)	29.10
10	Karanj	60.26 (50.90)	50.83 (45.45)	43.03 (40.97)	51.37
11	*Pseudomonas fluorescens*	37.50 (37.74)	33.83 (35.55)	27.70 (31.74)	33.01
12	*Trichoderma viride*	34.50 (35.95)	24.20 (29.45)	17.46 (24.68)	25.38
13	Control (water spray)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00
	SE(m)	**0.357**	**0.454**	**0.379**	
	CD	**1.044**	**1.327**	**1.109**	
	CV	**1.237**	**1.864**	**1.109**	

Table within parentheses represent arcsine or transformed values, as indicated by the bracketed entries.

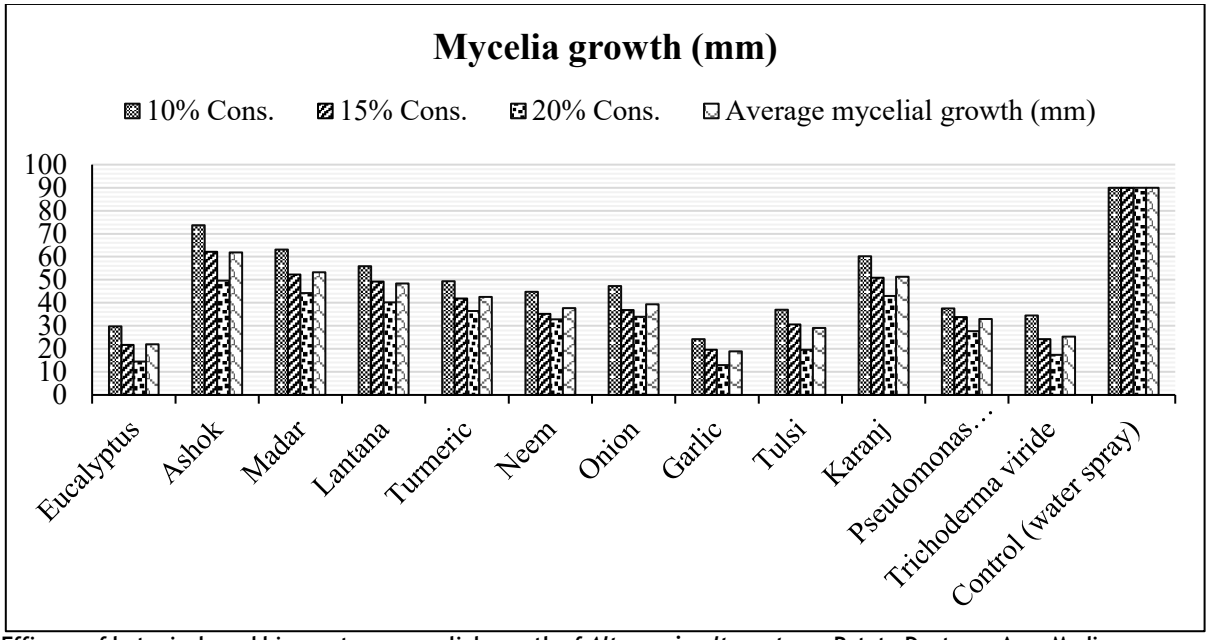

Fig. 3: Efficacy of botanicals and bioagents on mycelial growth of *Alternaria alternata* on Potato Dextrose Agar Media

Table-4. Efficacy of botanicals and bioagents on growth inhibition (%) of *Alternaria alternata* on PDA

S. No.	Treatments	Per cent growth inhibition at %			Average inhibition (%)
		10% Cons.	15% Cons.	20% Cons.	
1	Eucalyptus	66.85 (54.83)	75.93 (60.60)	83.96 (65.97)	75.58
2	Ashok	18.15 (25.19)	30.93 (33.77)	44.82 (42.09)	31.30
3	Madar	29.82 (33.07)	41.93 (40.33)	50.92 (45.50)	40.89
4	Lantana	37.85 (38.96)	45.33 (42.30)	55.26 (48.00)	46.14
5	Turmeric	45.18 (42.21)	53.52 (47.00)	59.44 (50.42)	52.71
6	Neem	50.18 (45.08)	60.88 (51.26)	63.48 (53.48)	58.18
7	Onion	47.41 (43.49)	59.04 (50.18)	62.30 (52.10)	56.25
8	Garlic	73.07 (58.72)	82.77 (65.45)	85.55 (67.90)	80.46
9	Tulsi	58.88 (50.09)	65.93 (54.27)	80.60 (63.84)	68.28
10	Karanj	33.04 (35.07)	43.52 (41.25)	52.18 (46.23)	42.91
11	*Pseudomonas fluorescens*	58.33 (49.77)	62.41 (52.16)	69.44 (56.41)	63.39
12	*Trichoderma viride*	61.66 (51.72)	73.14 (58.76)	84.07 (66.37)	72.95
13	Control	0	0	0	0
	SE(m)	**0.788**	**0.731**	**0.71**	
	CD	**2.313**	**2.146**	**2.084**	
	CV	**2.838**	**2.185**	**1.815**	

Table within parentheses represent arcsine or transformed values, as indicated by the bracketed entries.

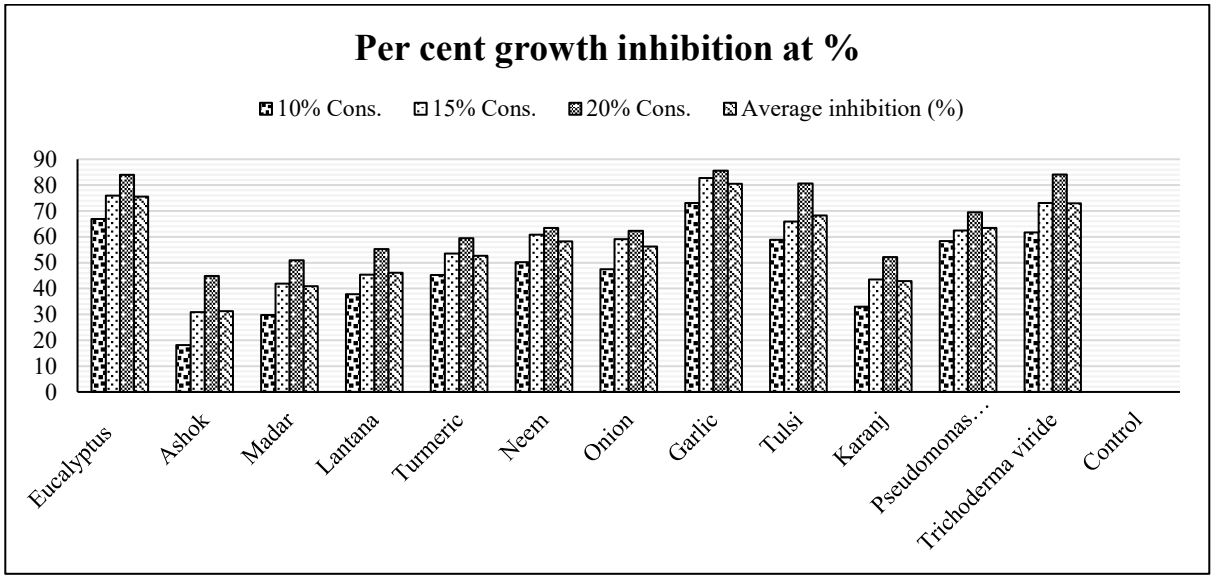

Fig 4: Efficacy of botanicals and bioagents on growth inhibition (%) of *Alternaria*

CONCLUSION

Alternaria leaf spot of ber is a worldwide disease of economic importance in ber crop. Different chemical fungicides are commercially available in market to control this disease. This study revealed significant inhibition effect of all the tested chemical fungicides and botanical extracts over control. In discriminate application of chemical fungicides have resulted several health hazards, negative impacts in environment so, the use of effective chemical at possible lower concentrations could be safer way to minimize health hazards and environmental pollutions Botanical extracts such as garlic and eucalyptus exhibited inhibition of *Alternaria alternata* at higher percentage. Therefore, this biological agent and plant extracts could be a potential to be used as novel fungicides alternative to harmful chemical fungicides. However, these *In vitro* research finding should be verified in the lab conditions before taking for field application.

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