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Inhibitory Effect of Phytoextracts and Fungicides Against *Alternaria alternata* Causing Alternaria Leaf Spot of Ber

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ABSTRACT

Alternaria leaf spot caused by *Alternaria alternata* is one of the major disease in ber which affects the yield. Experiment was conducted to study the efficacy of phytoextracts viz., *Azadirachta indica, Alium sativum, Curcuma longa, Eucalyptus oblique, Saraca asoca, Argemone maxicana, Solanum torum, Milletia pinnata* and *Jasminum officinarum* and fungicides viz., Cymoxnil + Mancozeb, Hexaconazole, Tebuconazole, Metalaxyl + Mancozeb, Propiconazole, Carbendazim, Kresoxim methyl and Mancozeb against the mycelial growth of *A. alternata.* Among phytoextracts, clove extracts of *Allium sativum* was found highly effective @ 20 and 30% in inhibiting the complete mycelial growth of *A. alternata* respectively followed by *Azadirachta indica* leaves extracts. 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%), Tebuconazole (98.35%).

Keywords Ber, *Alternaria alternata*, *in vitro*, Phytoextracts, Fungicides.

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INTRODUCTION

Ber (*Ziziphus mauritiana*) is an important fruit cultivated in arid and semi-arid regions of the world and belongs to the family Rhamnaceae. It is indigenous to India and China but more associated with the Indian culture since ancient times. It is very nutritious and rich in Vitamin C, A and B complex nutrients such as iron, calcium and phosphorus, ascorbic acid, carbohydrates and essential minerals (Alam *et al.* 2017). It is a good source of income, providing food at low cost, therefore affordable to the poor. Hence, it is called the poor man's fruit. It is popularly known as King of arid zone fruits (Mishra *et al*. 2013). In India, it is commercially cultivated in Haryana, Punjab, Maharashtra, Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, Andhra Pradesh and Tamil Nadu. In Uttar Pradesh, ber orchards were found in Varanasi, Ayodhya, Agra and Raebareli (Kumar and Singh 2020). In India, it occupies an area of more than 50,000 ha of land with 5,13,000 MT production (Kaur *et al*. 2020).

A limiting factor in profitable cultivation of

Ber is the biotic factors mainly fungi which causes an economic yield loss at all the stages, right after commencement of leaves to fruiting and harvest stage. Ber are affected by over 30 diseases caused by fungi, bacteria and phytoplasma (Mirzaee 2013). Some important fungal diseases are powdery mildew *(Oidium erysiphoides* f. sp. *ziziphi),* rust *(Phakospora Ziziphus vulgaris),* leaf spot *(Alternaria alternata, Cercospora zizyphi, Cladosporium zizyphi, Phoma macrostoma)* and mouldy leaf spot *(Isariopsis indica* var. *zizyphi).* Ber black fruit spot disease caused by *Alternaria alternata* reduces the fruit quality and also yields of the ber (Manjot *et al*. 2019; Kaur *et al*. 2020). Alternaria leaf spot of ber caused by *Alternaria alternata* of ber has played a significant role in causing economical yield losses. Symptoms were observed from margin or tip of leaf which was irregular and light brown in color. In severe condition these spots or lesions gradually increased coalesced and covered the entire surface of leaf results earlier leaf drop (Mehmood *et al.* 2018; Chaudhary and Singh 2021). The disease did not have importance due to minor in nature but due to climate change the disease now has economic importance and occurs in moderate to severe form (Chaudhary *et al.* 2021). Therefore, keeping in view the importance of orchard and seriousness of the disease, present study was conducted to test the efficacy of fungicides and phytoextracts in various concentrations against *A. alternata in vitro.*

MATERIALS AND METHODS

The experiments were conducted in laboratory of Department of Fruit Science and Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya during 2020-21. It is situated at 26.27^{0}N latitude, 82.12^{0}E latitude and at an altitude of 113 meters from sea level in the north Indo gangetic plain. The region falls under sub tropical zone.

Isolation of pathogen

Infected leaf showing characteristic symptoms were collected from Main Experiment Station, Horticulture and brought to the laboratory and isolate them on PDA (Potato Dextrose Agar) medium. Small pieces of the infected leaves of ber were cut along with some healthy tissues and surface sterilized for 1 min. in 1.0% Sodium hypochlorite (NaOCl) solution followed by three washings with sterilized distilled water. Excess moisture was removed by placing these bits on sterilized blotter paper. These bits were transferred aseptically placed in Petri disc containing PDA medium which was supplemented with 100 ppm streptomycin to avoid bacterial contamination and incubated at 25±1°C for 7 days.

Preparation of plant extracts

The plant parts were collected from Main Experiment Station, Horticulture and campus of the University. The plant part was thoroughly washed with sterilized water and ground separately with pestle and mortar using equal amount of sterilized distilled water (1:1 w/v). The mixture was squeezed with double-layered sterilized cheese cloth. The extracts thus obtained were considered as of 100% concentration (i.e., stock solution).

Evaluation of fungicides and phytoextracts against *Alternaria alternata*

Inhibitory effects of eight fungicides (Table 1) and nine phytoextracts (Table 2) were tested at various concentrations against the mycelial growth of *A. alternata* through food poison technique. Required quantity of fungicides and botanical extracts were mixed in the 100 ml PDA at luke warm stage and mixed thoroughly by sacking prior amended PDA poured in the sterilized Petriplates. After pouring of PDA in Petriplates, the medium was allowed to solidify and these plates were centrally inoculated with the 6 mm diameter disc of *A. alternata* at the center of the Petriplate. The disc is cut by sterilized cork borer taken from the edge of robustly grown 7 days old culture. Control was used as such without treatment in the medium. Four replications of each treatment incubated at 25 ± 2^{0}C for growth of the pathogen. The efficacy of fungicides and botanicals was observed by measuring mycelial growth of the fungus in millimeters (mm). The mycelia growth was recorded after 7 days of incubation. The linear growth of test fungus was recorded and per cent mycelia growth inhibition was calculated by using formula (Vincent 1947) given below:

Table1. List of phytoextracts used.

Sl. No.	Common name	Botanical name	Plant part used	Family
1.	Neem	*Azadirachta indica*	Leaves	Meliaceae
2.	Garlic	*Alium sativum*	Clove	Amaryllidaceae
3.	Turmeric	*Curcuma longa*	Leaves	Solanacceae
4.	Eucalyptus	*Eucalyptus obliqua*	Leaves	Myrtaceae
5.	Ashok	*Saraca asoca*	Leaves	Fabaceae
6.	Argemone	*Argemone maxicana*	Leaves	Papaveraceae
7.	Bhankatiya	*Solanum torum*	Stem	Solanaceae
8.	Karanja	*Milletia pinnat*	Leaves	Fabaceae
9.	Jasmine	*Jasminum officinarum*	Leaves	Oleaceae

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

Where,

I = Percent inhibition of fungal growth
C = Radial growth of colony in control
T = Radial growth in treated Petri plate

Statistical analysis

The data recorded on radial growth were statistically analyzed using Completely Randomized Block Design (Web Agri Stat Package 1.0).

RESULTS AND DISCUSSION

Effect of phytoextracts on the mycelial growth of *A. alternata*

As plant extracts are cost effective and natural means of management. Therefore, an effort was made to know the efficacy of different phytoextracts against *A. alternata.*

The efficacy of nine plant extracts was tested *in vitro* at three concentrations viz., 10, 20 and 30% against *A. alternata* on PDA media through food poison technique. The results revealed that all the phytoextracts inhibited the growth of pathogen over untreated control. Increase in effectiveness was recorded with increase in concentration. Growth inhibition ranges from 32.69% to 91.12% irrespective of concentrations. Results presented in Table 3 revealed that clove extracts of *Allium sativum* was found highly effective at 5, 10 and 15% in inhibiting the mycelial growth (73.36, 100 and 100%) of *A. alternate*, respectively followed by *Azadirachta indica* leaves extracts (59.25, 67.04 and 75.09%). Extracts of *Millettia pinnata* leaves was found least effective in inhibiting the mycelial growth i.e., 4.27, 36.69 and 57.13% at 10, 20 and 30% concentration, respectively.

Table 2. List of fungicides used.

Sl. No.	Trade name	Fungicide name
1.	Tilt	Propiconazole 25% EC
2.	Bavistin	Carbendazim 50% WP
3.	Ergon	Krosoxim methyl 50% SC
4.	Zymox	Cymoxanil 8%+Mancozeb 64% WP
5.	Mefanoxam	Metalaxyl 8%+Mancozeb 64% WP
6.	Dithane M-45	Mancozeb 75% WP
7.	Folicur	Tebuconazole 25% EC
8.	Contaf	Hexaconazole 5% EC

All the concentrations (10, 20 and 30%) of *Allium sativum* clove extract were found significantly superior over other treatments. There was no significant difference between *Argemone maxicana* and *Millettia pinnata* at 20% concentration.

Our findings were well supported by Sharma *et al.* (2021) as he also found 100% inhibition of mycelial growth *A. alternata* with garlic extract at 10 and 15% concentration. Zade *et al.* (2018) testified 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% mycelial inhibition of *A. alternata.* Mugao *et al*. (2020) reported garlic crude extract was found highly effective against *A. solani*. Allicin is a main antifungal compound present in garlic and presence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins and tannins (Akinmusire *et al.* 2014). The effectiveness of garlic clove extract is

Table 3. Efficacy of phytoextracts on mycelial growth (mm) and inhibition (%) of *A. alternata* on PDA medium after 10 Day after inoculation.

Sl. No.	Common name	Botanical name	Mycelial growth (mm)			Inhibition (%)			Average growth inhibition (%)
			10%	20%	30%	10%	20%	30%	
1.	Argemone	*Argemone maxicana*	73.80	56.56	38.42	17.47	36.64	57.03	37.04
2.	Garlic	*Allium sativum*	23.67	0.00	0.00	73.36	100.00	100.0	91.12
3.	Eucalyptus	*Eucalyptus globules*	40.28	35.55	31.47	55.05	60.24	64.81	60.03
4.	Turmeric	*Curcuma longa*	47.41	40.41	36.45	46.98	54.81	59.24	53.68
5.	Ashok	*Saraca ashoka*	71.47	53.69	46.48	20.08	39.96	48.02	36.02
6.	Karanja	*Millettia pinnata*	85.61	56.61	38.33	4.27	36.69	57.13	32.69
7.	Bhankatiya	*Solanum torvum*	63.44	54.39	48.47	29.06	39.18	45.80	38.01
8.	Jasmine	*Jasminum sambac*	51.54	46.40	42.38	42.36	48.11	52.61	47.69
9.	Neem	*Azadirachta indica*	36.44	29.42	22.47	59.25	67.04	75.09	67.13
10.	Control		89.43	89.27	89.43	-	-	-	-
	CD at (0.01%)		0.12	0.09	0.13				
	CV (%)		0.34	0.24	0.36				
	SEm±		0.30	0.25	0.39				

due to volatile oil which contains diallyl disulphide, diallyl trisulphide and sulphodioxides derived from allicin (Chethana *et al.* 2012).

Effect of fungicides on the mycelial growth of *A. alternata*

The efficacy of eight fungicides was evaluated *in vitro* against *A. alternata* at five concentrations viz. 250, 500, 1000, 1500 and 2000ppm on a PDA media by food poison technique (Table 4). Among the eight fungicides, Cymoxnil + Mancozeb proved to be the most effective fungicides showing complete average mycelial growth inhibition followed by Hexaconazole (99.35%), Tebuconazole (98.35%) while least average mycelial growth inhibition was found in Kresoxim methyl. There was no significant difference between Cymoxnil + Mancozeb, Hexaconazole and Tebuconazole at 500 and 1000 ppm concentration and Cymoxnil + Mancozeb, Hexaconazole, Tebuconazole and Propiconazole at 1500 ppm concentration whereas in Cymoxnil + Mancozeb, Hexaconazole, Tebuconazole, Propiconazole and Kresoxim methyl at 2000 ppm concentration.

Cymoxnil + Mancozeb was found highly effective at all the concentration and inhibiting 100% mycelial growth followed by Hexaconazole and Tebuconazole which also inhibit the complete mycelial growth at concentration 500 ppm or above while

Table 4. Efficacy of fungicides on mycelial growth and inhibition (%) of *A. alternata* on PDA medium.

Treatment	Mycelia growth (mm) Fungicidal concentration (ppm)					Per cent growth inhibition					Avg growth inhibition (%)
	250	500	1000	1500	2000	250	500	1000	1500	2000	
T_1=Propiconazole 25% EC	16.27	12.37	10.32	0.00	0.00	79.54	84.44	86.77	100.0	100.0	90.15
T_2=Carbendazim 50% WP	22.52	17.29	12.21	10.65	7.49	71.69	78.25	84.35	86.61	90.58	82.29
T_3=Kresoxim methyl 50% SC	55.71	41.42	36.63	21.39	0.00	39.96	47.90	53.06	73.11	100.0	62.81
T_4=Cymoxanil 8%+Mancozeb 64% WP	0.00	0.00	0.00	0.00	0.00	100.0	100.0	100.0	100.0	100.0	100.0
T_5=Metalaxyl 8%+Mancozeb 64% WP	58.38	43.56	0.00	0.00	0.00	26.61	45.21	100.0	100.0	100.0	74.36
T_6=Mancozeb 75% WP	21.60	19.51	16.52	12.63	9.52	72.84	75.46	78.83	84.12	88.03	79.87
T_7=Tebuconazole 25% EC	6.57	0.00	0.00	0.00	0.00	91.76	100.0	100.0	100.0	100.0	98.35
T_8=Hexaconazole 5% EC	2.55	0.00	0.00	0.00	0.00	96.79	100.0	100.0	100.0	100.0	99.35
T_9=Control check	79.55	79.51	78.05	79.55	79.55	-	-	-	-	-	
SEm±	0.11	0.09	0.59	0.07	0.06						
CD at (0.01%)	0.31	0.25	1.69	0.10	0.17						
CV(%)	0.33	0.27	2.30	0.20	0.25						

Metalaxyl +Mancozeb inhibiting the complete mycelial growth at 1000 ppm or above. Propiconazole at 1500 ppm or above inhibit complete mycelial growth and Kresoxim Methyl at 2000 ppm.

Our findings was well corroborated with the findings of Chaudhary *et al*. (2021) as he reported that Hexaconazole 5% EC (97.95%) showed maximum mycelium growth inhibition of *A. alternata* followed by Tebuconazole 25% EC (96.57%). Jewaliya *et al.* (2021) reported that Hexaconazole at 100, 150, 200 and 500 ppm was found highly effective against *A. alternata.* The result was also parallel with that of Prasad *et al*. (2017) showed that combination of fungicides Hexaconazole + Zineb (86.29%) was comparable to Tebuconazole + Trifloxystrobin (84.44%) gave maximum mycelium inhibition at 0.10%, 0.15% and 0.20% concentration against *Alternaria tenuissima.*

CONCLUSION

It is concluded that all the botanicals and fungicides were effectively inhibit the growth over control. Excessive use of chemical fungicides has negative effect on health hazards and environment. Therefore, use of effective chemicals at potential low concentrations can be a safe way to reduce health hazards and environmental pollutions.

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