Doi: HTTPS://DOI.ORG/10.23910/2/2021.0407

In-vitro Evaluation of Different Botanicals Against *Alternaria alternata* Causing Alternaria Leaf Spot of Ber (*Zizyphus mauritiana* Lamk.)

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Article History

Article ID: IJEP0407
Received in 10th December, 2020
Received in revised form 17th February, 2021
Accepted in final form 27th February, 2021

Abstract

Evaluation of different botanicals against alternaria leaf spot of ber (*Zizyphus mauritiana* Lamk.) was done under *in-vitro* at the NDUAT, Kumarganj main experiment station of Horticulture and Plant Pathology laboratory during 2018 to 2020. The fungus was isolated from the infected leaves of ber plant and cultural and morphological study was done through Poison Food Technique to confirm that the fungus was *Alternaria alternata* having branched and septate mycelium with light brown to blackish conidiophores in acropetal chain and the conidia having olive green to dark brown colour with transverse and longitudinal septa. The growth of the fungus was tested with botanicals. The nine different botanicals *ie. Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Parthenium hysterophorus* (Parthenium), *Ocimum sanctum* (Tulsi), *Datura stramonium* (Dhatura), *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allium cepa* (Onion), *Eucalyptus oblique* (Eucalyptus) were tested against *A. alternata* infection in ber leaves. 100-gram leaves or cloves or rhizomes from each plant were collected and after washing extract was prepared in 100 ml distilled water. The extract was filtered through muslin cloth and then sterilized by passing them through a Millipore filter using a swimming filter adapter. All the botanicals 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.

Keywords: *Alternaria alternata*, ber, botanicals, leaf spot disease, phytochemical

1. 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 and spread over more than 50,000 ha of land with a production of 513,000 MT (Pareek, 1983., Singh et al., 2016; NHB, 2018., Kaur et al., 2020). "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, 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. 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 powdery mildew (*Oidium erysiphoides* f.sp. *ziziphi*), rust (*Phakospora zizyphus 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 yield of the ber (Bai., et al., 1915; Kumar et al., 2015; Manjot et al., 2019; Kaur et al., 2020; Wang et al., 2021: Ajayabhai et al., 2018). The leaf spot disease caused by *Alternaria alternata* is an important disease in the major crop growing areas of the country. The disease leads to formation of irregular brown spots on the upper surface of leaves followed by dark brown to black spots on the lower surface. Later on, the spots coalesce and leaves drop off. The disease occurs widely in crop grown in deep sandy loam to loamy soils. Alternaria leaf spot was a minor disease, but due to climatic changes, it emerged moderately to severe form nowadays.

Therefore, keeping in view the importance of the orchard and seriousness of the disease the present study was conducted during 2018-2020 to evaluate *in vitro* efficacy of some plant extracts such as *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Parthenium hysterophorus* (Parthenium), *Ocimum sanctum* (Tulsi), *Datura stramonium* (Dhatura), *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allium cepa* (Onion), *Eucalyptus oblique* (Eucalyptus) against *Alternaria alternata* infection in Ber.

2. Materials and Methods

The experiment was conducted during 2018-2020 at Main Experimental Station, Horticulture, and Department of Plant Pathology Laboratory, A.N.D.U.A.T., Kumarganj, Ayodhya, U.P., India. Laboratory experiment was carried out to find out the fungitoxicity of following nine plant extracts (Table 1) with four replications under *in vitro* conditions by using Poison Food Technique to study the inhibitory effect of these botanicals on the mycelial growth against *Alternaria alternata* in completely randomized design (CRD).

The 100-gram leaves or cloves or rhizomes from each plant were collected and washed 2-3 times with distilled water and allowed to dry at room temperature (25±1°C) for six hours. Before extraction from leaves or cloves or rhoizome of each plant (100 g) were crushed separately with 100 ml distilled water. The extract was filtered through muslin cloth and then the extract was sterilized by passing them through a Millipore filter using a swimming filter adapter. The extract of each plant species was diluted in order to achieve three concentrations viz., 10, 15 and 20%. Petri-plates containing PDA supplemented with different plant extracts, each with three concentrations and replicated four times were inoculated with seven days old culture (5 mm diameter disc). A suitable check (without plant extract) was also maintained. Fungal colony was measured after 7 days of inoculation at 25±1°C. The efficacy of botanicals in each treatment and average of four replications were calculated.

Table 1: List of botanicals used in vitro during study against *Alternaria* leaf spot of *ber*

Sl. No.	Common name of plant	Botanical name	Plant part use	Concentrations in vitro
1.	Neem	*Azadirachta indica*	Leaves	10%, 15% and 20%
2.	Garlic	*Allium sativum*	Clove	10%, 15% and 20%
3.	Parthenium	*Parthenium ysterophorus*	Leaves	10%, 15% and 20%
4.	Tulsi	*Ocimum sanctum*	Leaves	10%, 15% and 20%
5.	Dhatura	*Datura stramonium*	Leaves	10%, 15% and 20%
6.	Turmeric	*Curcuma longa*	Leaves	10%, 15% and 20%
7.	Ginger	*Zingiber officinale*	Rhizome	10%, 15% and 20%
8.	Onion	*Allium cepa*	Bulb	10%, 15% and 20%
9.	Eucalyptus	*Eucalyptus obliqua*	Leaves	10%, 15% and 20%
10.	Control			

The botanical the linear growth of test fungus was recorded and mycelial growth inhibition (%) was calculated by using formula (Vincent, 1947) given below:

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

Where,

I = % inhibition of fungal growth

C = Radial growth of colony

T = Radial growth in treated petri plates

2.1. Statistical analysis

Data was analyzed using analysis of variance through factorial arrangements under completely randomized design (CRD). The analysis was performed using SPSS 20.0 software. The statistical analysis was done by the method "Analysis of variance" described by Fisher and Yates (1963). Actual data in percentage were converted to angular values, before analysis according to the table given by Snedecor and Cochran (1967).

3. Results and Discussion

As plant extracts are cost effective and natural means of management therefore an effort was made to know the efficacy of different plant extracts against *A. alternata*. The efficacy of nine plant extracts was tested in vitro at three concentrations (Table 2 and 3) viz., 10, 15 and 20% against *A. alternata* on PDA media by Poison Food Technique. The results revealed that among the botanicals highest average mycelium growth inhibition was obtained by garlic (74.88%), which was significantly superior over ginger (73.51%), Neem (69.25%), Onion (69.19%), Eucalyptus (67.26%), Turmeric (60.62%) while the least effective in inhibiting mycelial growth was Parthenium (34.64%) followed by Dhatura (53.37%) and Tulsi (56.8%) while the maximum average mycelial growth was seen in Parthenium (56.20 mm) followed by Dhatura (40.09 mm) and Tulsi (37.14 mm) and least was seen in Garlic (21.67 mm) followed by Ginger (22. 78 mm).

Among the different concentrations significantly highest

Table 2: Efficacy of botanicals against percent growth inhibition of *Alternaria alternata* on 7th day of incubation at 25+1°C (pooled data)

Sl. No.	Treatment	Botanical name	Percent growth inhibition			Average percentage inhibition
1.	Neem	*Azadirachta indica*	62.34 (52.13)	70.49 (57.15)	74.92 (59.95)	69.25 (56.41)
2.	Dhatura	*Calotropis gigantia*	49.08 (44.45)	52.91 (46.65)	58.14 (49.68)	53.37 (46.92)
3.	Turmeric	*Curcuma longa*	54.29 (47.47)	61.02 (51.36)	66.55 (54.68)	60.62 (51.17)
4.	Garlic	*Allium sativa*	72.10 (58.15)	75.29 (60.20)	77.25 (61.54)	74.88 (59.96)
5.	Ginger	*Zingiber officinale*	70.29 (57.03)	73.24 (58.90)	77.00 (61.38)	73.51 (59.10)
6.	Tulsi	*Ocimum sanctum*	50.55 (45.30)	57.88 (49.55)	61.97 (51.91)	56.8 (48.92)
7.	Onion	*Allium cepa*	64.18 (53.23)	69.22 (56.33)	74.19 (59.45)	69.19 (56.33)
8.	Parthenium	*Parthenium hysterophorus*	24.93 (29.81)	35.30 (36.37)	43.71 (41.31)	34.64 (35.87)
9.	Eucalyptus	*Eucalyptus obliqua*	61.23 (51.49)	67.75 (55.40)	72.82 (58.57)	67.26 (55.15)
10.	Control		0 (0)	0 (0)	0 (0)	0 (0)
	SEm±		2.06			
	CD (*p*=0.05)		5.82			
	Plant extract (P)		0.15			
	Concentration (C)		0.09			
	P×C		13.50			

Note: Average of four replications; Figures in parentheses are angular transformed value

Table 3: Efficacy of botanicals on mycelium growth of *Alternaria alternata* on solid media (pooled data)

Sl. No.	Treatment	Botanical Name	Percent Growth Inhibition			Average percentage inhibition
			10%	15%	20%	
1.	Neem	*Azadirachta indica*	32.38 (34.67)	25.37 (30.12)	21.57 (27.64)	26.44 (30.81)
2.	Dhatura	*Calotropis gigantia*	43.78 (41.41)	40.49 (39.49)	36.00 (36.84)	40.09 (39.24)
3.	Turmeric	*Curcuma longa*	39.31 (39.54)	33.52 (35.33)	28.76 (32.39)	33.86 (35.75)
4.	Garlic	*Allium sativa*	23.99 (29.27)	21.25 (27.39)	19.78 (26.19)	21.67 (27.61)
5.	Ginger	*Zingiber officinale*	25.55 (30.28)	23.01 (28.59)	19.78 (26.34)	22.78 (28.40)
6.	Tulsi	*Ocimum sanctum*	42.52 (50.65)	36.22 (36.91)	32.70 (34.85)	37.14 (40.80)
7.	Onion	*Allium cepa*	30.80 (33.67)	26.47 (30.90)	22.19 (28.08)	26.48 (30.88)
8.	Parthenium	*Parthenium hysterophorus*	64.56 (53.52)	55.64 (48.27)	48.41 (44.11)	56.20 (48.63)
9.	Eucalyptus	*Eucalyptus obliqua*	33.34 (35.21)	27.73 (71.74)	23.37 (28.89)	28.14 (45.28)
10.	Control		86	86	86	86
	SEm±		1.74			
	CD (*p*=0.05)		4.91			
	Plant extract (P)		0.16			
	Concentration (C)		0.11			
	P×C		17.60			

Note: Average of four replications; Figures in parentheses are angular transformed value

inhibition was recorded at 20% (60.65%) followed by 15% (56.31%) and 10% (50.89%) concentrations of botanicals. Significantly highest mycelium growth inhibition was observed in Garlic at 20% (77.25%) followed by Ginger at 20% (77.00%), Garlic at 15% (75.29%), Neem at 20% (74.92%), Onion at 20% (74.19%), Ginger at 15% (73.24%), Eucalyptus at 20% (72.82%), Garlic at 10% (72.10%) Neem at 15% (70.49%) and Ginger at 10% (70.29%) while Onion at 15% (69.22%), Eucalyptus at 15%

(67.75%), Turmeric at 20% (66.55%), Onion at 10% (64.18%), Neem at 10% (62.34%), Tulsi at 20% (61.97%), Eucalyptus at 10% (61.23%), Turmeric at 15% (61.02%), Dhatura at 20% (58.14%) and Tulsi at 15% (57.88%) respectively were found moderate inhibiting the growth of test fungus *A. alternata in vitro* bioassay.

The least fungal inhibition was observed in Parthenium at 10%, 15% and 20% (24.93%, 35.30% and 43.71%) respectively followed by Dhatura at 10% (49.08%), Tulsi at 10% (50.55%), Dhatura at 15% (52.91%) and Turmeric at 10% (54.29%). The present findings are similar with the result of *Kadam et al.* (2018), reported that the mycelial growth inhibition at 10%, 15% and 20% concentration was ranged from 19.81 % (*Bougain A. indica* (71.17 %) whereas *P. pinnata*, *P. hysterophorus* and *Bougainveilliea* spp. recorded comparatively maximum mycelial growth of 40.37, 33.33 and 19.81 %, respectively. Similar type of result was also obtained on *Alternaria alternata* or another species of *Alternaria* by Kantwa et al. (2014), reported that irrespective of the concentration, garlic clove extract was found most effective in inhibiting the mycelial growth (46.60 %) of *A. alternata* followed by neem (43.30%) and datura (40.30%) leaf extract. The same was obtained by Nandagopal and Ghewande (2004) and Shingne et al. (2020), plant extract of neem was found to be most effective against *A. alternata* causing leaf blight of groundnut and niger. 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*. The findings of Kantwa et al. (2014), are on the similar line of the present results; while testing the different efficacy of botanicals they reported that garlic clove extract @ 10% concentrations exhibited the maximum inhibition against *A. alternata* whereas less inhibition of mycelial growth was observed in case of *A. tenuissima* followed by onion and neem. The finding of Biswas and Ghosh, (2018) and Kutawa et al., (2018) are similar to the above findings. Pun et al., (2020) also reported that seet flag, neem and garlic show inhibition of mycelium growth in Alternaria brassicola causing leaf spot in cabbage.

4. Conclusion

It is concluded that the botanicals tested in present study have shown potential in controlling *A. alternata*. The garlic clove extract and ginger rhizome extract were found most effective while the least effective was parthenium leaf extract against *A. alternata* infection in ber. Therefore, botanicals could be a potentially used as novel fungicides alternative to harmful chemical fungicides.

5. Acknowledgement

The facility for doing the present study provided by Vice Chancellor of Acharya Narendra Deva University of Agriculture and Technology, Ayodhya Uttar Pradesh is gratefully acknowledged.

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