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Fungicidal effect of plant extracts against *Colletotrichum gloeosporioides* (Penz.) Sacc. the causal agent of pomegranate anthracnose

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Abstract
Pomegranate anthracnose disease is the major constraint to pomegranate production worldwide. Due to synthetic fungicides' hazardous effects on both humans and the environment, alternative environmentally friendly fungicides are now required to control the disease. The efficacy of aqueous and ethanol extracts of twelve plant extracts were evaluated at the concentrations of 1.25, 2.5, 5 and 10 percent against *Colletotrichum gloeosporioides* (Penz.) Sacc. the causal agent of pomegranate anthracnose. Out of the twelve aqueous plant extracts, *Datura metel* followed by *Allium sativum*, *Moringa oleifera* and *Prosopis julifera* showed the maximum percent inhibition at 10 percent concentration. Ethanol extracts of all tested plant extracts at 5 and 10 percent concentrations showed complete arrestment of the test pathogen. The potential antifungal activity exhibited by these plant extracts makes them suitable candidates for the control of anthracnose disease of pomegranate.

Keywords: Fungicides, plant extracts, *Colletotrichum gloeosporioides*, pomegranate

1. Introduction
Pomegranate (*Punica granatum* L.) is an important ancient fruit crop cultivated in arid and semi-arid regions of the world. It is known as the "fruit of paradise" and possesses powerful ethnomedical properties. It belongs to the family Punicaceae and the genus *Punica* and is commonly known as "Anar" (Chatterjee and Randhawa, 1952) [4]. Its utilization is found in ancient human cultures for food and medicinal use. Though pomegranate consumption helps for the well-being of humans, it is prone to several diseases. Anthracnose, the most important and prevalent disease in all pomegranate growing regions, is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sac, a member of the Fungi imperfectii phylum Ascomycete and Coeleomycetes class (Dean *et al.*, 2012) [5]. In India, anthracnose of the pomegranate disease was first reported by McRac (1924) [10].
The disease is characterized by small circular leaf spots with yellow halos. Infected leaves then become chlorotic and drop from the tree, leading to premature defoliation. Fruit symptoms, on the other hand, are characterized by brown lesions that progress through the rind and arils, resulting in fruit decay. Gray to orange spore masses often become visible on lesions of either green or ripe fruit (Thomidis 2014; Munhuweyi *et al.*, 2016; and Jayalakshmi *et al.*, 2010) [17, 11, 9]. Pomegranate anthracnose is one of the factors contributing to low productivity and large revenue losses. Farmers face huge economic losses due to incidences of this disease, which cause a 10–80 percent reduction in the marketable yield of total crop production (Ashwini and Srividhya, 2012) [2]. As there is a growing demand for eco-friendly products, there is a need to find alternative disease management approaches.
The usage of botanicals is gradually becoming recognized as a crucial technique for combating the widespread issue of pesticide pollution in crops and the environment. Promoting awareness to reduce the use of chemical pesticides by developing an alternative approach for plant disease management is essential. Therefore, there has been a growing interest in research concerning alternatives to chemical pesticides (Pradhanang *et al.*, 2003) [13]. The present investigation has been undertaken to evaluate the fungicidal activity of plant extracts *in vitro* against test pathogen *C. gloeosporioides* causing pomegranate anthracnose.

2. Materials and Methods

2.1 Preparation of plant extracts

Fresh leaves/bulbs/rhizome of 12 different plants were collected locally and washed thoroughly using tap water and air-dried at room temperature for 3 to 4 days and finally dried in a hot-air oven at 45–50 °C for 1 to 2 days depending on the plant species. Dried leaf samples were ground using small grinder, then stored in dry and airtight container for further use at 4 °C (Azwanida., 2015) [3].

The known weight of fresh plant materials was placed in a conical flask with the extracting solvents (aqueous and ethanol) in a ratio of 1: 1 (w/v) and allowed to stand at room temperature for 24 hr with frequent agitation. The mass of a plant part was squeezed through fine muslin cloth and the supernatants were filtered through Whatman filter paper No. 1 and centrifuged at 10,000 rpm for 10 min. The filtrate was collected in 250 ml Erlenmeyer conical flasks. Finally, the extracts were evaporated under reduced pressure at 45 °C. The concentrated extracts were allowed to dry in a hot-air oven, weighed again and kept at 4 °C for further studies (Parekh *et al*., 2005) [12].

In vitro screening of twelve botanicals with a concentration of 1.25, 2.5, 5 and 10 percent against the isolated pathogen was carried out using Poisoned food technique. An appropriate amount of extract was added to molten PDA to get desired concentrations and shaken well for thorough mixing of the extract. The PDA plates consisting of the plant extracts were inoculated aseptically with a five mm diameter *C. gloeosporioides* mycelial disc from fresh culture. Three replications were maintained for each treatment. The basal medium without any plant extract served as a control. All the inoculated Petri plates were incubated at 28 ± 2 °C. The radial growth of the test fungus was measured after attaining complete growth in the control. The percent inhibition of fungal growth was determined using the following formula given by Vincent (1947) [18].

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

Where,

I = Percent Inhibition (%)

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

T = Growth of the test fungus in treatment (mm)

The data obtained from the *in vitro* studies were subjected to statistical analysis. The differences exhibited by the treatments in the above experiments were tested for their significance using the standard statistical procedure given by Gomez and Gomez (1984). In each experiment, the critical difference CD and SE±m values were calculated to establish the least significant differences among the treatments.

3. Results and Discussion

3.1 *In vitro* efficacy of water extracts of botanicals against *C. gloeosporioides*

The antifungal activity of twelve botanicals was assessed at four concentrations, *viz*., 1.25, 2.5, 5 and 10 percent by using the poison food technique. The results are presented in Table 1. At 1.25 percent concentration, *D. metel* showed the highest inhibition of mycelial growth of 32.98 percent with 60.32 mm of radial growth, which was followed by *A. sativum and M. oleifera* with radial growth of 67.13 and 70.08 mm accounting 25.41 and 22.13 percent inhibition of mycelial growth, respectively. *P. julifera* (19.03%) was on par with *A. indica* (18.98%) with 72.87 and 72.92 mm of mycelial growth. The maximum growth of fungus (78.00 mm) was recorded by *P. guajava* showing least percent inhibition of 13.33.

D. metel showed the maximum antifungal activity even at a concentration of 2.5 percent with 55.85 percent mycelial inhibition, whereas radial growth was 39.74 mm, followed by *A. sativum* with radial growth of 58.27 mm accounting 35.26 percent inhibition. *P. julifera* (59.22 mm) and *M. oleifera* (59.90 mm) showed 34.20 and 33.44 percent inhibition of mycelial growth, respectively. *E. globules* (22.92%) showed the least mycelial growth inhibition with 69.30 mm of radial growth.

At 5 percent, the same trend was followed in the case of *D. metel* with radial growth of 19.99 mm showed 77.79 percent of mycelial growth inhibition. *A. sativum* showed 47.00 percent mycelial inhibition with 47.70 mm of mycelial growth. *M. oleifera, P. julifera* and *C. longa* were showing on par with each other with the inhibition of 40.97, 40.93 and 40.91 percent mycelial growth with 53.17, 53.16 and 53.19 mm of radial growth, respectively.

E. globules and *P. guajava* were on par with each other showing 32.93 and 32.89 percent of the lowest mycelial inhibition with maximum mycelial growth of 60.37 and 60.40 mm, respectively.

Table 1: Water extracts of botanicals on *C. gloeosporioides*

Sl. No.	Botanical	Concentrations							
		1.25%		2.5%		5%		10%	
		MG	MIOC	MG	MIOC	MG	MIOC	MG	MIOC
1	*Prosopis julifera*	*72.87 **(58.59)	19.03	59.22 (50.29)	34.20	53.16 (46.80)	40.93	30.58 (33.56)	66.03
2	*Eucalyptus globules*	75.59 (60.37)	16.01	69.30 (56.33)	23.00	60.37 (50.96)	32.93	47.94 (43.80)	46.73
3	*Lantana camera*	74.99 (59.97)	16.68	63.00 (52.52)	30.00	60.40 (50.98)	32.89	53.76 (47.14)	40.27
4	*Azadirachta indica*	72.92 (58.62)	18.98	64.74 (53.55)	28.06	60.11 (50.81)	33.22	53.11 (46.77)	40.99
5	*Ocimum tenuiflorum*	76.91 (61.25)	14.55	64.00 (53.11)	28.89	58.10 (49.64)	35.44	49.82 (44.88)	44.65
6	*Moringa oleifera*	70.08 (56.82)	22.13	59.90 (50.69)	33.44	53.12 (46.77)	40.97	43.28 (41.12)	51.91
7	*Curcuma longa*	75.58 (60.36)	16.02	60.05 (50.78)	33.28	53.19 (46.81)	40.91	48.07 (43.87)	46.59
8	*Allium sativum*	67.13 (55.00)	25.41	58.27 (49.74)	35.26	47.70 (43.66)	47.00	33.40 (35.29)	62.89
9	*Calotropis gigantica*	77.41 (61.60)	13.99	66.72 (54.75)	25.87	59.43 (50.42)	33.97	48.59 (44.17)	46.01
10	*Psidium guajava*	78.00 (62.01)	13.33	61.94 (51.89)	31.17	55.78 (48.30)	38.02	52.01 (46.13)	42.21
11	*Datura metel*	60.32 (50.89)	32.98	39.74 (39.06)	55.85	19.99 (26.55)	77.79	10.88 (19.26)	87.91
12	*Nerium oleander*	74.87 (59.89)	16.81	64.87 (53.63)	27.92	57.58 (49.34)	36.02	44.09 (41.59)	51.01
	Control	90.00			90.00	90.00		90.00	
		SE± m				CD (P=0.01)			
	Botanical	0.04				0.11			

	Concentration	0.02	0.06
	Botanical x Concentration	0.08	0.22

* Mean of three replications, ** Angular transformed values, MG- Mycelial growth (mm), MIOC- Mycelial inhibition over control (%)

At 10 percent concentration, *D. metel* with 87.91 percent showed the highest mycelial growth inhibition, whereas radial growth was 10.88 mm, followed by *A. sativum* with radial growth of 30.58 accounting 66.03 percent inhibition. *M. oleifera* and *P. julifera* with radial growth of 33.40 and 43.23 mm accounting percent inhibition of 62.89 and 51.91, respectively. The maximum growth of the fungus was recorded in *P. guajava* (53.76 mm) with least inhibition of 40.27 percent.

Jayalakshmi *et al*. (2013) [8] reported that Datura leaf extract showed the maximum inhibition of 61.70 percent against anthracnose of pomegranate, followed by garlic bulb extract (50.00%). Similarly, Sataraddi *et al*. (2011) [15] revealed that among all the seven evaluated plant extracts, Datura leaf extract and garlic bulb extract showed more than 70 percent inhibition of mycelial growth at both 7.5 and 5 percent against *C. gloeosporioides* which causes pomegranate anthracnose.

Gomathi and Kannabiran (2000) [7] found that the leaf extracts of *D. metel* and P. *juliflora* significantly inhibited the radial growth of mycelium. The percent inhibition of mycelial growth in *C. capsica* over the control was 69.3 and 43.8, respectively. According to Rafael *et al*. (2018) [14], *M. oleifera* at 10 and 15 percent concentrations inhibited radial growth of mycelium by 32.56 and 35.60 percent, respectively. As the concentration increased, a slightly greater effect was observed.

3.2 *In vitro* efficacy of ethanol extracts of botanicals against *C. gloeosporioides*

In ethanol extraction, all the botanicals showed inhibition of mycelium at the different concentrations tested.

The data in Table 2 revealed that *D. metel* was showing significantly lower mycelial growth of 43.10 mm with 52.12 percent of mycelial inhibition. This was followed by *M. oleifera* with radial growth of 58.53 mm accounting percent inhibition of 34.96. *A. sativum* and *P. julifera* showed the radial growth of 61.93 and 62.63 mm accounting percent inhibition of 31.19 and 30.42. Mycelial inhibition was found to be lowest in *P. guajava* (16.80%) and *L. camera* (16.90%) with radial growth of 74.98 and 74.76 mm, which are on par with each other.

At the concentration of 2.5 percent, concerning *D. metel* (68.97%) and *M. oleifera* (62.15%), the same trend was followed, by the first and second best botanicals in mycelial growth inhibition rate with 27.93 and 34.07 mm of radial growth, respectively.

Followed by *P. julifera* (58.05%) and *A. sativum* (57.74%) with radial growth of 37.73 and 38.03 mm, respectively. Maximum growth of fungus was recorded in *L. camera* (50.41 mm) showing the least inhibition of 43.99 percent. All the botanicals were cent percent effective at 5 and 10 percent concentrations in the ethanol extraction which completely arrested the growth of test fungus.

An ethanolic extract of *D. metel* has demonstrated strong antifungal activity against plant pathogenic fungi and thus could be used as an alternative to chemical fungicides for fungal infection management in plants (Sharma and Sharma, 2013) [16]. Yasmin and Shamsi (2019) [19] reported that among the ten ethanolic plant extracts, four plant extracts including *M. oleifera* at 20 percent concentration completely inhibited the radial growth of *C. gloeosporioides* anthracnose of *Rauwolfia serpentina*. Alvindia and Mangoba (2020) [1] reported that *A. longicuspis* extracted from ethanol solvent at 0.75 to 2.5 g/l completely suppressed mango anthracnose caused by C. *gloeosporioides.* Deressa *et al.* (2015) [6] demonstrated that *P. juliflora* extract against *C. gloeosporioides* showed radial growth inhibition of 79.60 percent and these results are in agreement with the present experimental study.

Table 2: Ethanol extracts of botanicals on *C. gloeosporioides*

Sl. No.	Botanical	Concentrations							
		1.25%		2.5%		5%		10%	
		MG	MIOC	MG	MIOC	MG	MIOC	MG	MIOC
1	*Prosopis julifera*	*62.63 **(52.29)	30.42	37.73 (37.88)	58.08	0.00 (0.54)	100	0.00 (0.54)	100
2	*Eucalyptus globules*	72.07 (58.07)	19.92	39.64 (39.01)	55.95	0.00 (0.54)	100	0.00 (0.54)	100
3	*Lantana camera*	74.76 (59.82)	16.93	50.41 (45.22)	43.99	0.00 (0.54)	100	0.00 (0.54)	100
4	*Azadirachta indica*	73.90 (59.25)	17.89	47.55 (43.58)	47.17	0.00 (0.54)	100	0.00 (0.54)	100
5	*Ocimum tenuiflorum*	72.92 (58.62)	18.98	44.18 (41.64)	50.92	0.00 (0.54)	100	0.00 (0.54)	100
6	*Moringa oleifera*	58.53 (49.89)	34.96	34.07 (35.70)	62.15	0.00 (0.54)	100	0.00 (0.54)	100
7	*Curcuma longa*	69.47 (56.43)	22.82	44.98 (42.10)	50.03	0.00 (0.54)	100	0.00 (0.54)	100
8	*Allium sativum*	61.93 (51.88)	31.19	38.03 (38.06)	57.74	0.00 (0.54)	100	0.00 (0.54)	100
9	*Calotropis gigantica*	65.39 (53.94)	27.34	45.00 (42.11)	50.00	0.00 (0.54)	100	0.00 (0.54)	100
10	*Psidium guajava*	74.98 (59.97)	16.69	48.46 (44.10)	46.16	0.00 (0.54)	100	0.00 (0.54)	100
11	*Datura metel*	43.10 (41.02)	52.12	27.93 (31.89)	68.97	0.00 (0.54)	100	0.00 (0.54)	100
12	*Nerium oleander*	72.91 (58.61)	18.99	46.78 (43.14)	48.02	0.00 (0.54)	100	0.00 (0.54)	100
	Control	90.00		90.00		90.00		90.00	
		SE± m				CD (P=0.01)			
	Botanical	0.04				0.12			
	Concentration	0.02				0.07			
	Botanical x oncentration	0.08				0.24			

* Mean of three replications, ** Angular transformed values, MG- Mycelial growth (mm), MIOC- Mycelial inhibition over control (%)

4. Conclusion

The plant extracts used in this study inhibited mycelia growth of *C. gloeosporioides*. The antifungal activity of the plant extracts differed according to solvent type and concentration. Among the plant extracts, *D. metel*, *A. sativum*, *M. oleifera* and *P. julifera* showed promising prospect in controlling radial growth of *C. gloeosporioides* the causal agent of pomegranate anthracnose.

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