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RESEARCH ARTICLE

In vitro management of anthracnose of pomegranate incited by *Colletotrichum glocosporioides* (Penz.) Penz. and Sacc.

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ABSTRACT

Anthracnose disease caused by *Colletotrichum glocosporioides* (Penz.) Penz. and Sacc. was observed in severe form on pomegranate leaf and fruits. An investigation was carried out to screen the different fungicides, bioagents and botanicals to inhibit the growth of pomegranate anthracnose pathogen. Among the six non-systemic (one combi) and six systemic fungicides tested at different concentrations, carbendizim + mancozeb (0.3 per cent) and propiconazole (0.15 per cent) were found to be effective among the over all the tested fungicides. Among the different botanicals, extract of datura leaves at 30 per cent found to be superior (61.70 %) followed by garlic extract (50.00 %) and among the bioagents, *Trichoderma viride* was found to be superior to all the tested bioagents.

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INTRODUCTION

Pomegranate (*Punica granatum* L.), an ancient and commercially important fruit of both tropical and subtropical countries, is native of Iran. Pomegranate is regarded as "Fruit of paradise". The fruit is prone to many diseases, among which anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is an important destructive disease. Diseases are traditionally managed by chemical fungicides. Hence the present study was undertaken to evaluate efficacy of fungicides, bioagents and plant extracts against *Colletotrichum glocosporioides* causing leaf and fruit spot of pomegranate.

MATERIALS AND METHODS

Poison food technique (Nene and Thapliyal, 1982) was used in present assay. The study was conducted at Plant Pathology Department, Uinversity of Agricultural Sciences, Dharwad during 2009-10. The efficacy of six non-systemic (one combi) and six systemic fungicides were tested against *C. gloeosporioides* for radial growth inhibition on the Potato dextrose agar media using poisoned food technique under *in vitro* conditions. *viz.,* Captan, Carbendizim + Mancozeb, copper oxyxhloride, Chlorothalonil, Mancozeb, Propineb, Azoxystobin, Difenconazole, Carbendazim, Hexaconazole, Iprobenfos, Propiconazole were assyed. The non-systemic fungicides and combi product were tried at 0.1, 0.2 and 0.3 per cent concentrations, whereas systemic fungicides were tried at 0.05, 0.1, 0.15 per cent concentrations.

The quantity of fungicides was calculated for 100 ml medium separately. The requisite quantity of fungicides was added to each flask at 45 °C. the fungicides were thoroughly mixed before solidification and poured into sterilized Petri plates. The mycelia disc of 5 mm diameter of twelve days old culture was cut with the help of sterile cork borer. Each disc was transefered aseptically to the centre of each Petri plate, already poured with poisoned medium.The PDA plates without fungicides were also inoculated and maintained as control.

The plates were incubated at room temperature (27 ±1°C) for 12 days. Five replications per treatment were maintained. The observations on colony growth recorded until Petriplate in control treatment was fully covered with mycelia growth and calculated the per cent inhibition.

Effectiveness of *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride* and *T. harzianum* were assayed aginst pathogen. 12 days old culture of the pathogen and 4 days old culture of biocontrol agents were used for this experiment and these were evaluated for their efficacy through dual culture technique. The bio agents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. Five replications were maintained for each treatment with one control by maintaining only pathogen separately. They were incubated for 12 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions, average was recorded and per cent inhibition of growth of the test fungus was calculated.

Plants were selected for preparation of extract on the basis of their antifungal substance from the plant parts of datura, eucalyptus, neem, garlic, ginger, onion bulb and tulasi. To study the antifungal mechanism of plant extract, the poison food technique was used. Fresh healthy plant parts of 100 g (leaves/bulb/rhizome) as indicated above were collected from field were washed with distilled water and air dried and crushed in 100 ml of sterile water. The crushed product was tied in muslin cloth and collected the filtrate. The prepared solution which gave 100 per cent, was further diluted to required concentrations of 10, 20 and 30 per cent. The extracts were tested against *C. gloeosporioides* on the PDA using poisoned food technique under *in vitro* condition as described earlier. The per cent inhibition of growth of the test fungus was calculated.

RESULTS AND DISCUSSION

Six non-systemic (one combi) and six systemic fungicides were screened against *C. glocosporioides* by poison food technique. The data presented in Table 1a and 1b revealed that fungicides were found significantly superior in reducing the growth of test fungus.

Table 1a : *In vitro* evaluation of non systemic and combi fungicides against *Colletotrichum glocosporioides*

Sr. No.	Fungicides		Per cent inhibition			
			Concentration (%)			
	Common name	Trade name	0.1	0.2	0.3	Mean
1.	Captan	Captaf 50% WP	48.55 (44.15)*	59.88 (50.67)	73.88 (59.49)	60.77 (51.36)
2.	Carbendazim + mancozeb	SAAF 75% WP	68.99 (56.14)	74.44 (59.69)	81.88 (64.79)	75.10 (60.20)
3.	Copper oxychloride	Blue copper 50% WP	0.27 (2.81)	0.88 (5.28)	1.55 (7.26)	0.90 (5.11)
4.	Chlorothalonil	Kavach 75% WP	5.65 (13.68)	9.10 (17.51)	20.32 (26.33)	11.69 (19.17)
5.	Mancozeb	Indofil M- 45 75% WP	9.99 (18.37)	16.19 (23.71)	22.99 (28.63)	16.39 (23.57)
6.	Propineb	Antracol 70% WP	14.83 (22.63)	20.22 (26.70)	29.10 (32.35)	21.33 (27.22)
Mean			24.71 (14.34)	30.12 (43.72)	38.29(35.31)	31.04 (31.12)
S.E.±				0.55		
C.D. @ 1 %				1.46		

*Arcsine transformed values

Table 1b : *In vitro* evaluation of systemic fungicides against *Colletotrichum glocosporioides*

Sr. No.	Fungicides		Per cent inhibition			
			Concentration (%)			
	Common name	Trade name	0.05	0.1	0.15	Mean
1.	Azoxystrobin	Amistar 25%SC	55.01 (47.85)*	57.88 (49.51)	62.77 (52.43)	58.55 (49.93)
2.	Carbendazim	Bavistin 50% WP	19.81 (26.53)	50.55 (45.29)	62.09 (51.98)	44.15 (41.27)
3.	Difenconazole	Score 25 %EC	63.75 (52.84)	64.43 (53.37)	67.21 (55.05)	65.13 (53.72)
4.	Hexaconazole	Contaf 5% EC	54.55 (47.58)	65.77 (54.15)	64.55 (53.42)	61.62 (51.72)
5.	Iprobenfos	Kitazin 48%EC	80.33 (63.63)	84.12 (66.50)	87.99 (69.67)	84.14 (66.60)
6.	Propiconazole	Tilt 25% EC	84.06 (66.39)	86.37 (68.28)	87.10 (69.47)	85.84 (68.05)
Mean			59.58 (50.80)	68.18 (56.18)	71.95 (58.67)	66.57 (55.21)
S.E.±				0.38		
C.D. at 1 %				1.01		

*Arcsine transformed values

Among non-systemic (one combi) fungicides, carbendazim + mancozeb at 0.3 per cent concentration showed 75.10 per cent inhibition of mycelial growth of fungus followed by captan with 60.77 per cent and least inhibition of mycelial growth was recorded in copper oxychloride with 0.9 per cent. Systemic fungicides, iprobenfos showed 87.99 per cent inhibition of myceliall growth of fungus and was followed by propiconazole (87.10%) at 0.15 per cent concentration while, least per cent inhibition of mycelial growth was recorded in carbendazim (62.09). The effectiveness of the triazole fungicides like propiconazole may be attributed to their interfeance with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. These results are in conformity with findings of Ekbote *et al*. (1996) Sudhakar (2000) and Prashanth *et al,* (2008).

Bioagents *viz., Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride* and *T. harzianum* were tested against *C. glocosporioides* and the results are presented in Table 2. Among the all bioagents tried, *Trichoderma viride* was found to be best in inhibiting the mycelial growth of *Colletotrichum glocosporioides* (86.82%) followed by *Trichoderma harzianum* (72.47%) and *Pseudomonas fluorescens* (67.00%) and least per cent inhibition of myceliall growth was observed in *Bacillus subtillis* (53.88). Present studies recorded significant mycoparasitism of *Trichoderma viride* and *Trichoderma harzianum* on anthracnose fungus that caused lysis of the hyphae and the spores *in vitro.* Similar results were obtained by Mandhare *et al.* (1996).

Effect of plant extracts is presented in Table 3. Among seven plant extracts, most of the plant extracts showed fungistatic nature at higher concentration (30%). Two plant extracts *viz.* datura leaf extract (61.70%), garlic extract (50.00%) showed =50 per cent inhibition of mycelial growth, while least inhibition of mycelial growth was noticed in tulasi leaf extract (0.70%). At 20 per cent concentration, three plant extracts namely, garlic leaf extract, onion bulb extract and garlic bulb extract showed more than 30 per cent inhibition of mycelial growth. The extracts of datura leaf showed maximum inhibition of myceliall growth of *Colletotrichum glocosporioides* even at 10 per cent concentration. Present results are tune with Shivapuri *et al*. (1997).

Table 2 : *In vitro* evaluation of bioagents against *Colletotrichum glocosporioides*

Sr. No.	Bioagents	Per cent inhibition[#]
1.	*Bacillus subtilis*	53.88 (46.63)*
2.	*Pseudomonas fluorescens*	67.0 (54.64)
3.	*Trichoderma harzianum*	72.47 (57.67)
4.	*Trichoderma viride*	86.82 (67.85)
S.E.±		1.32
C.D. at 1%		4.04

Table 3 : *In vitro* evaluation of botanicals against *Colletotrichum glocosporioides*

Sr. No.	Botanicals	Per cent inhibition			
		Concentration (%)			
		10	20	30	Mean
1.	Datura leaf extract	54.58 (47.25)*	59.02 (50.17)	61.70 (51.72)	58.42 (49.84)
2.	Eucalyptus leaf extract	0.45 (3.83)	1.38 (6.67)	5.27 (13.17)	2.37 (7.89)
3.	Garlic bulb extract	7.80 (16.08)	21.66 (27.72)	50.00 (44.98)	20.92 (29.59)
4.	Ginger rhizome extract	15.94 (19.27)	27.8 (31.78)	33.33 (35.23)	31.23 (31.35)
5.	Neem leaf extract	0.83 (5.14)	4.70 (12.41)	10.83 (19.19)	5.46 (12.27)
6.	Onion bulb extract	28.18 (32.05)	38.23 (38.88)	43.32 (41.14)	36.58 (37.36)
7.	Tulsi leaf extract	0.35 (3.09)	0.65 (4.61)	0.70 (4.78)	0.56 (4.16)
Mean		15.44 (19.27)	21.92 (24.62)	29.30 (30.04)	22.22 (24.64)
SEm.±			0.55		
CD at1%			1.46		

*Arcsine transformed value

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