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In-vitro Evaluation of Fungicides, Botanicals and Bioagents Against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., the Causal Agent of Anthracnose Disease of Pomegranate

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Abstract Anthracnose of pomegranate is a very serious disease which affects quality of fruit. Five non-systemic, seven systemic and combi products and bioagents along with ten botanicals were evaluated *in vitro* against *Colletotrichum gloeosporioides*, the causal agent of anthracnose of pomegranate. Non systemic fungicides Captan, Antracol at 0.3%, systemic fungicide Iprobenfos at 0.1% and a combi-products, Carboxin + Thiram, Hexaconazol + Zineb, Hexaconazole + Captan and Tebuconazole + Trifloxystrobin (0.1 %) gave cent per cent inhibition of pathogen. Among fungal antagonists, *Trichoderma viride* and bacterial antagonists, *Pseudomonas fluorescens* inhibited the maximum mycelial growth of *C. gloeosporioides*. Bulb of garlic, rhizome of turmeric and root powder of asafoetida extract at 15% concentration gave higher level of inhibition.

Keywords Pomegranate Anthracnose, *Colletotrichum gloeosporioides*, Bioagents, Botanicals.

Introduction

Pomegranate (*Punica granatum* L.), an ancient and commercially important fruit crop of both tropical and subtropical countries ; belongs to the family: *Punicaceae*. Pomegranate is regarded as the "Fruit of Paradise". It is one of the most adaptable subtropical minor fruit crops and its cultivation increasing very rapidly.

Successful cultivation of pomegranate in recent years suffers from various diseases such as, fruit rot, bacterial spot / canker and anthracnose causing significant loss in the recent years [1]. Among these leaf / fruit spot caused by various organisms such as, *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Sphaceloma punicae*, *Cercospora punicae*, and *Phomopsis* sp., take a heavy toll on the crop. This results in drastic reduction in the yield as well as ultimate marketability by way of severe spotting of the produce. It is important to generate information on the efficacy of available and new fungicides, botanicals and bio agent for managing disease. Hence, the present study was undertaken to screen various fungicides, botanicals and bio agent *in-vitro* to manage fruit spot within reasonable limit of fungicidal residues permitted by importing countries.

Materials and Methods

The fungus was isolated by tissue isolation technique. The infected portion of pomegranate fruit along with some healthy portion was cut and surface sterilized using 1 : 1000 mercuric chloride solution for

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Table 1. *In vitro* evaluation of non systemic fungicides against *Colletotrichum gloeosporioides*. # Mean of four replications. * Arcsine transformed values.

Sl. No.	Fungicides Common name	Trade name	Percent inhibition 0.1	0.2	0.3#	Mean
1	Antracol	Antracol 70% WP	29.63 (32.96)	64.44 (53.38)	67.60 (55.38)*	53.89 (47.24)
2	Captan	Captaf 50% WP	61.11 (51.40)	66.30 (54.49)	67.80 (55.43)	65.06 (53.76)
3	COC	Blue copper 50% WP	31.11 (33.88)	35.19 (36.37)	42.59 (40.72)	36.30 (36.99)
4	Kavach	Kavach 75% WP	31.48 (34.11)	55.56 (48.17)	70.74 (57.24)	52.59 (46.51)
5	Mancozeb	Dithane M-45 75% WP	33.70 (35.47)	47.04 (43.28)	50.37 (45.19)	43.70 (41.32)
	Mean		37.41 (37.57)	53.70 (47.14)	59.85 (50.79)	50.32 (45.16)
			Fungicides (F)	Concen-tration (C)	F×C	
	SEm±		0.21	0.28	0.48	
	CD@ 1%		0.83	1.08	1.86	

60 seconds, washed in sterile water to remove the traces of mercuric chloride, if any and then aseptically transferred to potato dextrose agar (PDA) slants and incubated at room temperature (27 ± 1°C) for fungal growth and sporulation. The fungal colonies, on development were identified using compound micro-

Table 2. *In vitro* evaluation of systemic fungicides against *Colletotrichum gloeosporioides*. # Mean of four replications. *Arcsine transformed values.

Sl. No.	Common name	Fungicides Trade name	Percent inhibition 0.05	0.075	0.1#	Mean
1	Azoxystrobin	Amitsar 25% SC	54.07 (47.32)	55.19 (47.96)	55.56 (48.17)*	54.94 (47.82)
2	Carbendazim	Bavistin 50% WP	35.56 (36.59)	38.15 (38.13)	41.11 (39.86)	38.27 (38.19)
3	Difenconazole	Score 25% EC	60.74 (51.18)	61.11 (51.40)	65.19 (53.82)	62.35 (52.13)
4	Hexaconazole	Contaf 5% EC	48.52 (44.13)	52.59 (46.47)	54.07 (47.32)	51.73 (45.97)
5	Iprobenfos	Kitazin 48% EC	75.93 (60.59)	77.41 (61.60)	77.90 (61.80)	76.91 (61.26)
6	Propiconazole	Tilt 25% EC	73.33 (58.89)	75.93 (60.59)	77.04 (61.34)	75.43 (60.28)
7	Tebuconazole	Folicure 250 EC	70.74 (57.23)	72.96 (58.65)	74.81 (59.85)	72.84 (58.58)
	Mean		59.84 (50.85)	61.90 (52.11)	63.60 (53.14)	61.78 (52.03)
			Fungicides (F)	Concentration (C)		F × C
	SEm±		0.17	0.11		0.29
	CD @ 1%		0.63	0.41		1.10

Table 3. *In vitro* evaluation of combi product fungicides against *Colletotrichum gloeosporioides*. # Mean of four replications. *Arcsine transformed values.

Sl. No.	Fungicides Common name	Trade name	Percent inhibition 0.05	0.075	0.1#	Mean
1	Avatar	Hexaconazol 4% WP+Zineb 68%	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)*	100.00 (89.96)
2	Cabrio-top	Metiram complex 55%+Pyraclos-trobin 5% WG	88.15 (69.89)	89.63 (71.21)	92.59 (74.39)	90.12 (71.83)
3	Merger	Tricyclazole 18%+Mancozeb 62% WG	87.04 (68.90)	97.78 (84.98)	98.15 (85.42)	94.32 (79.77)
4	Nativo	Tebuconazole 50%+Trifloxystrobin 25% WG	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
5	SAAF	Carbendazim (12%)+ Mancozeb (63%)	60.00 (50.76)	76.30 (60.85)	76.30 (60.85)	70.86 (57.49)
6	Taqat	Hexaconazole 5% WP+ Captan 70%	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
7	Vitavax Power	Carboxin (37.5% WP)+Thiram (37.5% WP)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
	Mean		89.84 (78.49)	94.82 (82.41)	95.29 (82.93)	93.32 (81.27)
			Fungicides (F)	Concentration (C)		F × C
	SEm±		0.93	0.61		1.60
	CD @ 1%		3.53	2.31		6.12

scope and recorded the mycelial and spore characters.

Purification of fungal culture

Ten ml of clear filtered two percent water agar was poured into the sterile petri plates and allowed to solidify. Dilute spore suspension was prepared in sterile distilled water from 12 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at 27 ± 1°C for 12 hours and examined under microscope so as to locate germinated conidia. Single germinated conidium was picked up from the culture using cork borer and transferred to fresh PDA slants under aseptic conditions and incubated at 27 ± 1°C. The pure culture thus obtained was used for further studies.

In vitro evaluation of fungicides

Five non-systemic, seven systemic and combi product were tested against *Colletotrichum gloeosporioides* for inhibition of the fungal radial growth on potato dextrose agar by using poisoned food technique *in vitro* condition. Non-systemic fungicides were tested at 0.1, 0.2 and 0.3%, systemic and combi products were tested at 0.05, 0.075 and 0.1% concentrations.

In vitro evaluation of bioagents

Two each of fungal antagonists viz., *Trichoderma viride* and *T. harzianum* and bacterial antagonists viz., *Pseudomonas fluorescens* and *Bacillus subtillis* were collected from Institute of organic farming, UAS, Dharwad were evaluated for their efficacy through dual culture technique. The bioagents and the test fungus were inoculated side by side on a single petri dish containing solidified PDA medium. Four replications were maintained for each treatment with one control by maintaining only pathogen and bioagent separately. The plates were incubated at 27 ± 1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula [2].

Table 4. *In vitro* evaluation of botanicals against *Colletotrichum gloeosporioides*. # Mean of four replications. * Arcsine transformed values.

Sl. No.	Botanicals	Part's used	Percent inhibition 5%	10%	15% #	Mean
1	Asafoetida	Ready powder	16.67 (24.09)	24.44 (29.62)	40.00 (39.21)*	27.04 (30.97)
2	Eucalyptus	Leaves	26.11 (30.71)	36.67 (37.25)	50.00 (44.98)	37.59 (37.65)
3	Eupatorium	Leaves	22.78 (28.49)	25.00 (29.99)	30.56 (33.54)	26.11 (30.67)
4	Garlic	Bulb	39.44 (38.86)	47.22 (43.39)	62.22 (52.06)	49.63 (44.77)
5	Garlic+Turmeric + Asafoetida	Bulb+Rhizome	40.56 (39.54)	50.56 (45.30)	67.22 (55.05)	52.78 (46.63)
6	Neem	Seeds	38.33 (38.24)	48.33 (44.03)	59.44 (50.43)	48.70 (44.23)
7	Parthenium	Leaves	18.33 (25.32)	32.22 (34.57)	47.22 (43.39)	32.59 (34.43)
8	Soapnut	Fruit	27.78 (31.79)	37.22 (37.58)	45.56 (42.43)	36.85 (37.27)
9	Tulsi	Leaves	23.33 (28.87)	41.11 (39.86)	56.67 (48.81)	40.37 (39.18)
10	Turmeric	Rhizome	12.78 (20.93)	21.11 (27.33)	32.22 (34.57)	22.04 (27.61)
		Mean	26.61 (30.68)	36.39 (36.89)	49.11 (44.45)	37.37 (37.34)
			Fungi-cides (F)	Concentra-tion (C)	F × C	
		SEm±	0.47	0.26	0.81	
		CD @ 1%	1.82	1.00	3.15	

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

Where, I = Per cent inhibition of mycelial growth, C = Growth of mycelium in control, T=Growth of mycelium in treatment.

In vitro evaluation of botanicals

Ten plant extracts were tested against the growth of anthracnose pathogen, *C. gloeosporioides in vitro* by poisoned food technique. Fresh healthy plant parts of 50 g (leaves / root / bulb) collected from field were washed with distilled water and air dried and crushed in 50 ml of sterile water. The crushed product was tied in muslin cloth and the filtrate was extracted. The stock solution was further diluted in to desired concentrations of 5.0, 10 and 15%. The results were expressed in terms of per cent inhibition of mycelial growth over control. Further, angular transformations were made for data and analyzed statistically.

Results and Discussion

Among the non-systemic fungicides, Kavach 75% WP showed 70.74% inhibition followed by Captan 50% WP at 0.3% concentration showed 67.80% inhibition. Least inhibition of mycelial growth was recorded in Copper oxychloride with 0.3% (Table 1).

Systemic fungicides, Iprobenfos showed 77.90% inhibition of mycelial growth of fungus and was followed by Propiconazole (77.04%) at 0.1% concentration while, least per cent inhibition of mycelial growth was recorded in carbendazim (41.11%) (Table 2). The effectiveness of the triazole fungicides like Propiconazole may be attributed to their interferance

Table 5. *In vitro* evaluation of bio-agents against *Colletotrichum gloeosporioides*. # Mean of five replications. *Arcsine transformed values.

Sl. No.	Bioagents	Per cent inhibition #
1	*Trichoderma harzianum*	77.11 (61.40)*
2	*Trichoderma viride*	80.22 (63.59)
3	*Pseudomonas fluorescens*	70.89 (57.33)
4	*Bacillus subtilis*	64.00 (53.12)
	SEm±	0.59
	CD at 1%	2.44

with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. Among the combi products tested Hexaconazol 4% WP + Zineb 68%, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 5% WP + Captan 70%, Carboxin 37.5% WP+Thiram 37.5% WP showed cent percent inhibition (Table 3). These results are in conformity with findings earlier report [3, 4].

Among ten plant extracts, most of the plant extracts showed fungistatic nature at higher concentration (15%) (Table 4). Four plant extracts showed more than 50% inhibition at 15%. Garlic bulb extract, rhizome of turmeric and root powder of asafoetida at 15% concentration gave higher level of inhibition of *C. gloeosporioides* (67.22). The extracts of garlic bulb showed maximum inhibition of mycelial growth of *C. gloeosporioides* even at 5% concentration (39.44). Present results are in tune with earlier report [5].

Bioagents viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum* were tested against *C. gloeosporioides* and the results are presented in Table 5. Among the all bioagents tried, *Trichoderma viride* was found to be best in inhibiting the mycelial growth of *C. gloeosporioides* (80.22%) followed by *Trichoderma harzianum* (77.11%) and *Pseudomonas fluorescens* (70.89%) and least per cent inhibition of mycelial growth was observed in *Bacillus subtillis* (64.00%). 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* [6].

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