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In vitro evaluation of fungicides against *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. causing anthracnose of pomegranate (*Punica granatum* L.)

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Abstract: Pomegranate (*Punica granatum* L.) is a widely grown fruit in many regions of the world. Anthracnose of pomegranate is one of the limiting factor for low productivity and also the low market price. Therefore, the management of anthracnose disease is necessary. In this study new fungicide molecules are evaluated under *in vitro* condition against the mycelial growth of *Colletotrichum gloeosporioides*. *In vitro* screening of fungicides against *C. gloeosporioides* showed two combination product Hexaconazole + Zineb, Trifloxystrobin + Tebuconazole and a non-systemic fungicide Captan showed cent percent inhibition at 100, 250, 500 and 1000 ppm concentration. Similarly, systemic fungicides Hexaconazole, Propiconazole, Penconazole, Tebuconazole and Carbendazim showed cent percent mycelial inhibition at 500, 1000 and 2000 ppm concentrations.

Keyword: Anthracnose, *Colletotrichum,* Fungicides, Pomegranate

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient beloved plant and fruit. Pomegranate is regarded as "fruit of paradise". Pomegranate and its usage are deeply embedded in human history, and utilization is found in many ancient human cultures as food and as a medical remedy.As a commercial crop, pomegranate is grown to a limited extent in selected locations in many states of India. Maharashtra accounts for more than two third area, while other states like Karnataka, Andhra Pradesh, Gujarat, Rajasthan and Tamilnadu share the rest. Maharashtra is the leading producer of pomegranate. The estimated area under pomegranate cultivation in India is about 1, 30,750 ha with the production of 13, 45,720 tons during 2013-14. Karnataka accounts 15,100 ha area and 1, 50,300 tons production in 2012-13 (Anonymous, 2014). According to the data published by National Horticulture Board of India there is an undersized decrease in the area of pomegranate cultivation in India from 109 thousand ha in 2008-09 to 107 thousand ha in 2010-11; similarly, the production has decreased from 807 thousand tons to 743 thousand tons during the same period. Export of pomegranate has decreased in quantity from 35175.17 tons in 2007-08 to 30158.59 tons in 2011-12 (APEDA , 2011-12).

Anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. The symptoms on leaves observed as pinhead size of black to brown water soaked spots with circular margin. In advanced stage, these spots enlarged, coalesced and resulted in bigger patches. In severe case, leaves dried up and drooped down. On fruits, brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, these spots coalesced to form necrotic patches over the surface of the fruit (Jayalaxami, 2010).

Anthracnose of pomegranate is one of the contributing factors for this low productivity and large revenue losses. The management of anthracnose disease is necessary because of heavy losses, but there are number of chemicals available in the market as fungicides and their bio efficacy and suitability needs to be verified by *in vitro* and field studies. Therefore, in the present study we tried to find out *in vitro* effectiveness of different systemic and non-systemic chemicals at different concentration against *C. gloeosporioides* fungus. This study provide the suitable chemicals and concentration against *C. gloeosporioides* fungus. However, these chemicals need to be verified under field condition before farmer recommendation.

MATERIALS AND METHODS

The studies were carried out at Department of Plant Pathology, University of Agricultural Sciences, Bengaluru. The pathogen was isolated from the pomegranate (*Punica granatum* L.) leaves and fruits showing typical anthracnose symptoms by standard tissue isolation method and further purified by single spore isolation method (Rangaswami and Mahadevan, 1999) on potato dextrose agar medium (PDA). The pathogen was identified based on its mycelial, conidial characteristics following standard mycological keys (Barnett and

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Hunter, 1972) and were maintained separately on PDA for further experiments.

***In vitro* evaluation of fungicides against *C. gloeosporioides*:** The efficacy of three non-systemic *viz.,* Captaf 50 WP (Captan), Kavach 75 WP (Chlorothalonil) and Dithane M- 45 75 WP (Mancozeb), three combination product *viz.,* Avatar 72 WP (Hexaconazole 4% + Zineb 68%), Nativo 75 WG (Trifloxystrobin + Tebuconazole) and Sectin 60 WG (Fenamidone 10% +Mancozeb 50%) and nine systemic fungicides *viz.,* Amistar 25 SC (Azoxystrobin), Score 25 SC (Difenoconazole), Bavistin 50 WP (Carbendazim), Contaf 5 EC (Hexaconazole), Tilt 25 EC (Propiconazole), Topenco 100 EC (Penconazole), Pulsor 24 SC (Thifluzamide), Compass 50 WG (Trifluoxystrobin) and Folicur 430 SC (Tebuconazole) were tested against *C. gloeosporioides* for radial growth inhibition on the potato dextrose agar media using poisoned food technique under *in vitro* condition (Shravelle, 1961). The percent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947). The non-systemic fungicides were tried at 100, 250, 500 and 1000 ppm, whereas systemic fungicides were tried at 500, 1000, 2000 ppm concentrations. All the data were analysed by ANOVA (CRD 2 factorial design) after transforming the % values to corresponding angular values.

RESULTS

In vitro* evaluation of non-systemic/combination fungicides against *C. gloeosporioides: The inhibition of mycelial growth of *C. gloeosporioides* at four different concentration (100 ppm, 250 ppm, 500 ppm and 1000 ppm) of three non-systemic fungicides and three combination product was recorded and presented in Table 1a.

Cent percent growth inhibition of *C. gloeosporioides* was recorded in combination product Hexaconazole + Zineb, Trifloxystrobin + Tebuconazole and non-systemic fungicide Captan at all four concentrations. Least percent inhibition was noticed in Chlorothalonil (37.71%). However, maximum percent inhibition of mycelial growth was at 1000 ppm concentration irrespective of fungicides (Table 1a).

At 1000 ppm concentration all combination product *viz.*, Hexaconazole + Zineb, Trifloxystrobin + Tebuconazole and Fenamidone + Mancozeb showed cent percent mycelial growth inhibition. However, non-systemic fungicides, Captan showed cent percent mycelial inhibition followed by Mancozeb (47.48%) and Chlorothalonil (55.88%)

***In vitro* evaluation of systemic fungicides against *C. gloeosporioides*:** The inhibition of mycelial growth of *C. gloeosporioides* at three different concentrations (500 ppm, 1000 ppm and 2000 ppm) of nine systemic fungicides was recorded and % inhibition is presented in Table 1b.

It was observed that, fungicides concentrations and their interaction differed significantly (at 1% level of significance) with respect to inhibition of the mycelial growth of *C. gloeosporioides*.

Among nine systemic fungicides tested, maximum inhibition of growth of *C. gloeosporioides* was observed cent percent in Carbendazim, Hexaconazole, Propiconazole, Penconazole and Tebuconazole treated plates at all concentration (500 ppm, 1000ppm and 2000 ppm), Which were significantly (at 1% level of significance) superior to all other fungicides followed by Difenoconazole (84.84%), Trifluoxystrobin

Table 1a. *In vitro* evaluation of non-systemic/ combi fungicides against *C. gloeosporioides.*

S. N.	Fungicides	Concentration/ % inhibition				Mean
		100ppm	250ppm	500ppm	1000ppm	
1.	Hexaconazole + Zineb72 WP	100 (90.00)*	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
2.	Captan 50 WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3.	Chlorothalonil 75 WP	15.54 (23.18)	30.67 (33.62)	48.74 (44.27)	55.88 (48.13)	37.71 (37.31)
4.	Mancozeb 75 WP	13.86 (21.74)	28.57 (32.28)	47.48 (43.55)	100 (90.00)	47.48 (46.81)
5.	Trifloxystrobin + Tebuconazole 75 WG	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Fenamidone + Mancozeb 60 WG	49.58 (44.76)	57.56 (49.36)	73.95 (59.32)	100 (90.00)	70.27 (60.78)
	Mean	59.77	64.05	69.52	82.75	
			Fungicides(F)		Concentration (C)	F×C
	CD@1 %	1.21		0.99		2.43
	CV (%)	1.61				

*Arcsine transformed values of percent inhibition. All values are mean of three replicates.

Table 1b. *In vitro* evaluation of systemic fungicides against *C. gloeosporioides.*

Sl. No.	Fungicides	Concentration/% inhibition			Mean
		500ppm	1000ppm	2000ppm	
1.	Azoxystrobin 25 SC	52.64 (46.51) *	52.64 (46.51)	50.64 (45.54)	51.97 (46.18)
2.	Carbendazim 50 WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3.	Difenoconazole 25 SC	79.24 (62.91)	85.85 (67.91)	89.43 (71.03)	84.84 (67.28)
4.	Hexaconazole 5 EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Propiconazole 25 EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Penconazole 100 EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7.	Thifluzamide 24 SC	30.19 (33.32)	34.90 (36.21)	40.75 (39.67)	35.28 (36.40)
8.	Trifluoxystrobin 50 WG	78.11 (62.11)	76.23 (60.82)	66.60 (54.70)	73.65 (59.21)
9.	Tebuconazole 430 SC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
	Mean	82.24 (72.59)	83.29 (73.32)	83.04 (73.26)	
		Fungicides(F)	Concentration(C)		F×C
	CD@1%	0.66	0.38		1.14
	CV (%)	0.72			

*Arcsine transformed values of percent inhibition. All values are mean of three replicates.

(73.65%) and Azoxystrobin (51.97%). The least percent inhibition of fungus was recorded in Thifluzamide (35.28%).

Among the three tested concentrations (500ppm, 1000ppm and 2000ppm), 2000 ppm concentration (83.04%) was on par with 1000 ppm (83.29%) concentration followed by 500 ppm (82.24%). At 2000 ppm concentration, Carbendazim, Hexaconazole, Propiconazole, Penconazole and Tebuconazole showed cent percent mycelial growth inhibiton of *C. gloeosporioides* followed by Difenoconazole (89.43%), Trifluoxystrobin (66.60%), Azoxystrobin (50.64%) and Thifluzamide (40.75%) (Table 1b).

The mycelial growth inhibition of Azoxystrobin fungicides was found on par at 500ppm, 1000 ppm and 2000 ppm concentration. Although, Trifluoxystrobin was also found on par at 500 ppm and 1000 ppm concentration.

DISCUSSION

***In vitro* evaluation of fungicides:** There are lacks of resistant cultivars against various diseases. Therefore, the use of fungicides is one of the disease management practices. When there is outbreak of epidemic for any reason perhaps the use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need. Availability of various new fungicides in the market necessitates its evaluation under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions (Jayalaxami, 2010).

In vitro evaluation of new synthetic molecules of fungicides is very much necessary before they are tried under field condition. Among the tested fungicides maximum percent inhibition (100%) of *C. gloeosporioides* was recorded in combination product Hexaconazole + Zineb, Trifloxystrobin + Tebuconazole and non-systemic fungicide Captan at all four concentration. Least % inhibition was noticed in Chlorothalonil (37.71%). Ekbote *et al.* (1996) reported that among the four fungicides tested against mango anthracnose caused by *C. gloeosporioides* Carbendazim + Mancozeb gave cent percent inhibition of mycelial growth at 0.1 % concentration. Least percent inhibition of mycelial growth was observed in Chlorothalonil at all the tested concentration.

Among the nine systemic fungicides tested, maximum percent inhibition of growth of *C. gloeosporioides* was observed cent percent in Carbendazim, Hexaconazole, Propiconazole, Penconazole and Tebuconazole treated plates at all concentration. However, the least percent inhibition of fungus was recorded in Thifluzamide (35.28%). The effectiveness of the Triazoles fungicides may be attributed to their interference with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergo-sterol is essential to the structure of cell wall and its absence cause irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of Triazoles, which inhibit the sterol biosynthesis pathway in fungi (Nene and Thapliyal, 1973). Prashanth *et al.* (2008) reported that among four sys-

temic fungicides maximum % inhibition of growth of *C. gloeosporioides* was observed in Difenconazole (90.78 %) and Propiconazole (90.78 %), Gud and Raut (2008) reported that Thiophenate-methyl and Propiconazole were most effective against *C. gloeosporioides* followed by Hexaconazole and Carbendazim. Patel (2009) and Pandey *et al.* (2012) studied that among the tested fungicides Tricyclazoles were found to be superior for controlling *C. gloeosporioides* causes anthracnose of mango.

At higher concentration (2000 ppm) most of the fungicides viz. Hexaconazole, Propiconazole, Penconazole, Tebuconazole, Carbendazim, Azoxystrobin, Difenoconazole, Thifluzamide and Trifluoxystrobin inhibited maximum mycelial growth but decreased with reduced concentration (500 ppm and 1000 ppm). These results are in agreement with that of Sudhakar (2000); Prashanth (2007); Patel (2009); Devamma *et al.* (2012) reported that among all the six fungicides evaluated against *C. gloeosporioides* the cause of mango anthracnose, the systemic fungicide Thiophanate-methyl (100 %) and the non-systemic fungicide Mancozeb (100 %) proved to be effective in inhibiting the mycelial growth of the highly virulent pathogen at 50 ppm and 500 ppm concentrations, respectively. Similarly, Pandey *et al.* (2012) studied the effect of different fungicides on the control of *C. gloeosporioides* causes anthracnose of mango. Among the tested fungicides Tricyclazoles were found to be superior for controlling the incidence of pathogen and Saju *et al.* (2012) reported the effectiveness of different fungicides against *C. gloeosporioides* infecting large cardamom, the *in vitro* tests showed that, the pathogen was highly sensitive to Copper oxychloride 50 WP (0.3 %) followed by Mancozeb 75 WP (0.3 %) and combined formulation of Carbendazim + Mancozeb (12 + 63) WP (0.3 %).

Among the tested fungicides viz. contact/ combination product and systemic, most of the Triazoles and combination product fungicides given cent percent mycelial growth inhibition at all tested concentration. Therefore, this indicates that the systemic fungicides were found more effective as compare to contact fungicides in controlling fungal mycelial growth. Among the tested concentrations, higher concentrations of contact/ combination and systemic fungicides (1000 and 2000ppm) were found to be more effective as compared to lower concentrations.

Conclusion

Chemical control is one of the indispensable approach of integrated disease management. However, there is lack of effective chemicals with suitable concentration against *C. gloeosporioides* causing anthracnose of pomegranate. Therefore, in this paper some new fungicide molecules (Systemics and non-systemics) has been tested under *in vitro* condition at different concentration (100, 250, 500, 1000 and 2000ppm). Among tested fungicides some viz. Hexaconazole + Zineb, Trifloxystrobin + Tebuconazole and non-systemic fungicide Captan and systemic fungicides viz. Carbendazim, Hexaconazole, Propiconazole, Penconazole and Tebuconazole shown cent percent mycelial growth inhibition of the fungus. Therefore, present studies provide some new chemical molecules which have been found effective in controlling *C. gloeosporioides* causing anthracnose of pomegranate. However, further evaluation of effective fungicides are needed in field for better recommendation for management of anthracnose of pomegranate.

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