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Invitro Evaluation of Fungicides, Botanicals and Bioagents against *Colletotrichum truncatum*

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A B S T R A C T

Soybean [*Glycine max* (L.) Merrill.] is an important oilseed crop. *Colletotrichum truncatum*, the incitant of anthracnose/ pod blight in soybean is one of the most destructive pathogens. Eight fungicides (@ 500, 1000, 1500, 2000 and 2500ppm each), 10 botanicals@ 10% and four bioagents were evaluated *in vitro* against *C. truncatum*, using PDA as basal medium. The results revealed that all the fungicides, botanicals and bioagents tested were found fungistatic and significantly inhibited the mycelial growth of the test pathogen over untreated control. Among the fungicides, Carbendazim + Mancozeb and Propiconazole recorded the highest mean inhibition (100 %) of mycelial growth of the test pathogen, followed by the fungicide, tebunconazole inhibited 100 per cent inhibition at concentration of 1500 ppm. Least inhibition was observed in hexaconazole at 500 ppm concentration. Among ten botanicals tested, Garlic extract recorded highest mean mycelial growth inhibition (53.22%) of the test pathogen followed by the onion extract (47.11%), neem (46.44%).Vavilaku extract (9.7%) was found to be the least effective in inhibition of mycelial growth of pathogen. Among the bioagents, *T. harzianum* recorded highest mean mycelial growth inhibition of 80.22 followed by *T.viride* (72.55%).

Introduction

Soybean [*Glycine max* (L.) Merrill.] has gained importance in India being rich in oil (20 %) and protein (40 %). In India, it is grown in an area of 10.96 million hectares with a production of 13.45 million tonnes and productivity of 1228 kg/ha (Anonymous, 2018-19). It is cultivated mainly as a kharif crop in the soybean growing states such as Madya pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Chhattisgarh and Telangana state.

However the soybean yields are remarkably low due to various factors of biotic and abiotic nature which take a heavy toll on the crop, of which diseases account for estimated

yield loss of 12 per cent. Among various diseases on soybean anthracnose causes estimated yield losses of 26 per cent (Backman *et al.*, 1982).

In view of this, in vitro studies were undertaken to evaluate the efficacy of fungicides, botanicals and bioagents in inhibiting mycelial growth of *C. truncatum.*

Materials and Methods

The experiment was conducted during 2018 at Department of Plant Pathology, College of Agriculture, Rajendranagar.

In vitro evaluation of fungicides against test pathogen

Eight fungicides viz., mancozeb, carbendazim, tebunconazole, hexaconazole, propiconazole, corboxin + thiram, carbendazim + mancozeb and tebuconazole + triflaxystrobin were tested against *C.truncatum* at concentrations of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, on potato dextrose agar media using poison food technique(Nene and Thapliyal, 1993). under *in vitro* conditions.

The PDA medium was prepared and melted. The fungicidal suspension was added to the melted media to obtain the required concentrations. About 20 ml of poisoned medium was poured in each sterilized petriplates. Suitable check was maintained without addition of fungicides.

Five mm mycelial disc was taken from the periphery of eight days old colony was placed in the centre of petriplates and incubated at 28 ± 2^0C for 15 days. Three replications were maintained for each treatment. The diameter of the colony was measured when maximum growth of the pathogen was observed in control plates.

In vitro evaluation of botanicals against test pathogen

Aqueous extracts of different plant species were used to determine the toxicant properties against *C. truncatum*. Fifty grams of fresh healthy plant parts (leaves/rhizome/bulbs) collected from field were washed with distilled water, air-dried and crushed in 50 ml of sterile water. The crushed product was filtered through muslin cloth and collected the filtrate. The prepared solution gave 100 per cent which was further diluted to required concentrations of 10.0 per cent. The extracts were tested using poisoned food technique (Nene and Thapliyal, 1993) against *C. truncatum* on the potato dextrose agar under *in vitro* conditions.

In vitro evaluation of biogents against test pathogen

Bioagents *viz., Trichoderma harzianum*, *Trichoderma virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their effectiveness against *C. truncatum* under laboratory conditions. Dual culture technique (Dennis and Webster, 1971) was adopted to evaluate the biocontrol agents under laboratory conditions. The fungal bioagents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. To test the efficacy of bacterium, a 4 cm line was streaked at four corners of the plate. At the centre of the antagonist, a 6mm diameter mycelial disc of test the fungus was placed. The Petri plates with pathogen inoculated at one end alone served as control. Three replications were maintained for each treatment with one control by maintaining only pathogen and bioagents separately. The plates were incubated at 27 ± 2°C and the diameter of the colony of both bioagents and the pathogen was measured when maximum growth of the pathogen was observed in control plates.

Observations on radial mycelial growth of *C. truncatum* were recorded in each treatment and per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) as follows.

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

Where,
I : Per cent reduction in growth of the antagonistic fungus
C : Radial growth of antagonistic fungus in control (mm)
T : Radial growth of antagonistic fungus in treatment (mm)

Results and Discussion

Effect of fungicides

The results (Table 1, Plate. 1 and Fig. 1) revealed that mancozeb inhibited the mycelial growth of the test pathogen to an extent of 76.44 per cent at 2500 ppm concentration. Significant differences were observed among the systemic fungicides concentrations and interactions. Propiconazole inhibited 100 per cent mycelial growth of *C. truncatum* at all the five concentrations tested (500, 1000, 1500, 2000, 2500 ppm). Carbendazim inhibited 84.11 per cent of mycelial growth of *C. truncatum* at concentration of 2500 ppm whereas tebunconazole inhibited 100 per cent inhibition in mycelial growth of *C. truncatum* at concentration of 1500 ppm. Least inhibition in mycelial growth of *C. truncatum* was observed in hexaconazole at 500 ppm concentration.

Among the combi products, carbendazim + mancozeb was the best in inhibiting 100 per cent mycelial growth of *C. truncatum* at all the five concentrations of (500, 1000, 1500, 2000, 2500 ppm) followed by tebuconazole + trifloxystrobin 88.9 per cent at 500 ppm concentration. Among the combi products tested, the least mycelial growth inhibition was observed in carboxin +thiram with inhibition in mycelial growth of 84.04 per cent.

The present results were in accordance with the studies conducted by Nagaraj (2013) who reported that propiconazole and trifloxystrobin +tebuconazole were effective against *C. truncatum* inciting anthracnose of soybean at 500 ppm, 1000 ppm and 1500 ppm concentration. Similarly Kale and Barhate (2016) also reported that propiconazole and hexoconazole at 0.1 per cent concentration inhibited the mycelial growth of the pathogen to an extent of 78.15 per cent and 84.44 per cent respectively. Shovan *et al.*, (2008) also reported that propiconazole completely inhibited the mycelial growth of *C. truncatum*. Gawade *et al*., (2009) reported that carbendazim recorded the highest mean inhibition in mycelial growth of the test pathogen followed propiconazole, hexaconazole, difenconazole and chlorothalonil at 100 ppm, 150 ppm and 200 ppm concentrations.

Effect of bioagent

It was observed *T. harzianum* gave highest mycelial growth inhibition of the pathogen (80.22%) which was followed by *T.viridae*(72.55%). The least inhibition of the fungus was observed in *P. fluorescens* (46.44%) and *Bacillus subtilis* (36.00%). (Table 2, Fig. 2 and Plate 2).

The present findings are in agreement with the studies conducted by Medereiros and Menezas. 1994, Pathania *et al.*, (2004) and Laxman (2006). Kale and Barhate (2016) reported that *T. viride* was effective against *C. truncatum* inciting anthracnose of soybean.

Table.1 Efficacy of different fungicides on radial growth of *Colletotrichum truncatum in vitro*

Sl.No.	Fungicides	Percent inhibition of the mycelial growth of fungus					
		Concentrations (ppm)					Mean
		500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	
1.	Mancozeb	56.44 (48.70)*	59.33 (50.38)	67.00 (54.94)	70.88 (57.35)	76.44 (60.98)	66.01 (54.12)
2.	Carbendazim	65.33 (53.93)	68.00 (55.56)	70.77 (57.28)	77.44 (61.66)	84.11 (66.62)	73.13 (62.52)
3.	Tebuconazole	72.66 (58.48)	78.88 (62.66)	100 (85.95)	100 (85.95)	100 (85.95)	90.30 (72.56)
4.	Hexaconazole	57.11 (49.09)	62.88 (52.47)	67.44 (55.21)	70.55 (61.76)	77.44 (61.66)	67.08 (55.02)
5.	Propiconazole	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)
6.	Carboxin + Thiram	84.04 (66.48)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	96.80 (82.36)
7.	Carbendazim+ Mancozeb	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)
8.	Tebuconazole+ Trifloxystrobin	88.9 (70.59)	100 (85.95)	100 (85.95)	100 (85.95)	100 (85.95)	97.78 (83.18)
9.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	69.38 (58.13)	74.34 (63.10)	78.35 (66.80)	77.36 (65.07)	82.01 (69.22)	
	S. Em ±	0.65	0.48	0.41	0.50	0.46	
	C D @ 5%	1.94	1.42	1.22	1.50	1.37	

All the figures are means of three replications
*Figures in parenthesis are angular transformed values

Table.2 Evaluation of bioagents on radial growth of *Colletotrichum truncatum in vitro*

Sl.No.	Bio-agents	Per cent inhibition of the mycelial growth of fungus
1.	*Trichoderma harzianum*	80.22 (63.61) *
2.	*Trichoderma viride*	72.55 (58.41)
3.	*Pseudomonas fluorescens*	46.44 (42.96)
4.	*Bacillus subtilis*	36.00 (34.87)
5.	Control	0.00 (0.00)
	Mean	47.04 (41.18)
	S.E m ±	0.65
	CD at 5%	2.06

All the figures are means of three replications
*Figures in parenthesis are angular transformed values

Table.3 Evaluation of botanicals on the radial growth of *Colletotrichum truncatum in vitro*

Sl. No.	Botanicals	Plant parts used	Per cent inhibition of the mycelial growth of fungus
1.	Mehandi	Leaves	39.66 (39.03)*
2.	Onion	Bulb	47.11 (43.34)
3.	Garlic	Bulb	53.22 (46.85)
4.	Ginger	Rhizome	37.55 (37.79)
5.	Neem	Leaves	46.44 (42.96)
6.	Prosopis	Leaves	34.33 (35.86)
7.	Bougainvillia	Leaves	39.66 (39.03)
8.	*Vincarosea*	Leaves	44.66 (41.93)
9.	Pongamia	Leaves	37.66 (37.85)
10.	Vavilaku	Leaves	9.70 (18.09)
	Control		0.00 (0.00)
	Mean		35.45 (35.16)
	S.E m ±		0.69
	CD at 5%		2.04

All the figures are means of three replications
All botanicals were tested against *C. truncatum* at 10 per cent concentration
*Figures in parenthesis are angular transformed values

Effect of botanicals

The results (Table 3, Fig. 3 and Plate 3) revealed that, among the ten botanicals evaluated *in vitro* against *C. truncatum*, significantly the highest inhibition was obtained by garlic extract (53.22%) and it was followed by onion extract (47.11%), Neem extract (46.44%) and Vincarosea extract (44.66%) and were significantly superior over Mehindi extact (39.66%), Bougainvillia extract (39.66%), Pongamia extract (37.66%), ginger extract (37.55%) and prosopis (34.33%). Vavilaku extract (9.7%) was found to be the least effective in inhibition of mycelial growth of pathogen. The present findings are in agreement with the studies conducted by earlier workers Kulkarni (2009), Gawade *et al*., (2009).

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