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Management of anthracnose in soybean caused by *Colletotrichum truncatum*

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ABSTRACT

A study was conducted in the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemednagar,Maharashtra during 2014 to 2015 to control *Colletotrichum truncatum* causing anthracnose or pod blight of soybean with fungicides and bioagents. All the fungicides and bioagents evaluated *in vitro* were found effective against *C. truncatum* and recorded significant inhibition of the test pathogen over untreated control. However, carbendazim was found most effective and recorded 0.66 mm mean colony diameter and significantly highest growth inhibition (99.26%) of the test pathogen. This was followed by mancozeb (98.88%), hexaconazole (84.44%), chlorothalonil (80.00%), propiconazole (78.15%) and difenconazole (32.22%). Out of the six bioagents evaluated *in vitro T. viride* and *T. harzianum* recorded significantly highest growth inhibition (78.88%) followed by *T. hamatum* (77.04%), yeast (40.37%), *P. fluorescens* (27.77%) and mehandi leaf extract (17.77%). *In vitro* physiological study of pathogen shows that *C. truncatum* grew well at 27^{0}C temperature with 75 per cent relative humidity.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merill] is a species of legume native to East Asia. The plant is classed as an oilseed rather than a pulse. The genus *Glycine* is wild and divided into two subgenera, *Glycine* and *Soja*.Total yield of soybean in India is 12.07 q/ha. Production of soybean in india14.68 million tonns. Soybean growing major states in the country are Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, Gujarat, Uttar Pradesh, Punjab and Haryana (Bhatnagar, 1997). About 80-85 per cent acerage of soybean in India is concentrated in Madhya Pradesh. More than 100 plant pathogens have been reported to affect soybean, but among them very few are economically important causing yield losses to the tons of 12-20 per cent (Mittal *et al.*, 1993). Among the most important diseases reported to cause economic losses to

the soybean anthracnose incited by *Colletotrichum truncatum* (Schw) Andrus and Moore causing yield losses of 16-100 per cent (Sinclair, 1992 and Anonymous, 1999). Among the major fungal diseases of soybean anthracnose (pod blight) caused by *Colletotrichum truncatum* (Schw) Andrus and Moore, has been reported as the major constraint in the successful cultivation of soybean (Khan and Sinclair, 1992 and Mittal *et al.*, 1993).

Objectives :

- To isolate and identify the pathogen causing anthracnose of soybean.
- To study the symptomatology.
- To prove the pathogenicity.
- In *vitro* chemical control of *Colletotrichum truncatum* by poison food technique.
- In *vitro* biological control of *Colletotrichum truncatum* by dual culture technique.
- To study effects of temperature and humidity on growth and sporulation of pathogen.

MATERIAL AND METHODS

***In vitro* evaluation of fungicides :**

A total of six fungicide *viz.*, carbendazim (0.1%), chlorothalonil (0.2%), difenconazole (0.1%) hexaconazole (0.1%), propiconazole (0.1%) and mancozeb (0.1%) were evaluated *in vitro* applying "poison food technique". The requisite quantity of each fungicide was calculated and thoroughly mixed with autoclaved and cooled (40-45^{0}C) PDA in conical flasks to obtain desired concentrations. An appropriate quantity of each fungicide was incorporated separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentrations of each fungicide. The fungicide amended PDA was then poured (15 – 20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. Three plates / treatments where maintained and each treatment was replicated for three times. On solidification of PDA in Petri plates, all treatment plates were inoculated / seeded aseptically by placing in the centre with 5.0 mm uniform mycelial disc obtained from 7 days old culture of *C. truncatum* multiplied on agar plates. Petri plates containing plain PDA without any fungicide were inoculated with 5.0 mm disc of the test pathogen and maintained as suitable untreated control. All the treatment (inoculated) and control Petri plates where then incubated at 24 +2^{0}C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen.

***In vitro* evaluation of bioagents :**

Five bioagents *Trichoderma viride, T. harzianum, T. hamatum* and *P. fluorescens* and yeast were also evaluated *in-vitro* applying dual culture technique and one botanical mehandi leaf extracte valuated *in-vitro* applying poison food technique.

Healthy and disease free fresh leaf sample of mehandi plant species were brought to the laboratory and washed with sterile distilled water and then chopped into small bits with sterilized sharp knife. Leaf sample was then separately grind and homogenized in mechanical grinder with equal quantity of sterile distilled water (1:1, w:v). The homogenate obtained was then strained through double layered muslin cloth and filtrate collected was then filtered through Whatman number l filter paper using volumetric flasks (50 ml capacity). The clear leaf extracts obtained formed the stock solution of 100 per cent. An appropriate quantity of mehandi extract was incorporated separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentrations (10%) of extract and autoclaved at 15 Lbs pressure for 15 min. The leaf extracts added PDA was then poured (15 – 20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. Disc (5 mm) of *Colletotrichum truncatum* was placed at the center of one corner of different Petri plates containing solidified PDA medium and disc of *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum* was placed at other corner of different Petri plates. The disc of each bio-agent kept with *Colletotrichum truncatum* disc in three Petri plates. A loopful of 24 hour old culture of *Pseudomonas fluorescens* and *Yeast* was inoculated at 2 cm just opposite to the pathogen on each plate (Dennis and Webster, 1971).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.

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

where,

C = Growth of test fungus (mm) in control plate,

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

***In vitro* evaluation of effect of temperature and humidity on growth and sporulation of pathogen :**

Mycelial growth :

Experiment of effect of temperature and humidity on growth and sporulation of *C. truncatum* were conducted *in vitro* on potato dextrose agar medium. The Petri plate were inoculated with fresh culture of effective *C. truncatum* isolates and incubated at 5, 20, 27, 34, 40, 45^0C temperature with humidity 95, 85, 75, 65, 55, 50 per cent, respectively. Each treatment replicated for four times. Growth of each isolates in each Petri plate was recorded at every alternate day upto 7^{th} day after inoculation.

Sporulation :

The cavity slides containing 0.1 ml of conidial suspension (10^6 conidia/ml) were placed in Petri plates containing moist filter papers at the bottom were incubated for 24 hrs. at the different temperature and humidity as mentioned earlier. Each treatment was replicated for four times and in each replication 100 conidia were counted for the germination.

All Observations on radial mycelial growth and sporulation of *C. truncatum* recorded in each treatment and replication and mean colony diameter is calculated.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

***In vitro* evaluation of fungicides :**

Results (Table 1) indicated that, all the fungicide tested significantly inhibited the mycelial growth of *C. truncatum* over untreated control Among the six fungicides, tested Carbendazim recorded least mean colony diameter (0.66 mm) and highest inhibition (99.26 %) of mycelial growth of the test pathogen over untreated control followed by mancozeb (98.44%), hexaconazole (84.44%), chlorothalonil (80.00%), propiconazole (78.15 %) and difenconazole (32.22%) which recorded mean colony diameter of test pathogen 1.00, 14.00, 18.00, 19.66 and 61.00 mm, respectively. Fungicide Difenconazole was found comparatively least effective than other fungicides.Carbendazim was reported

Table 1 : *In vitro* evaluation of fungicides on radial growth of *C. truncatum*

Treat. No.	Treatments	Concentration (%) used	Mean colony diameter (mm)*	Inhibition(%)
T_1	Carbendazim	0.1%	0.66	99.26
T_2	Chlorothalonil	0.2%	18.00	80.00
T_3	Difenconazole	0.1%	61.00	32.22
T_4	Hexaconazole	0.1%	14.00	84.44
T_5	Propiconazole	0.1%	19.66	78.15
T_6	Mancozeb	0.1%	1.00	98.88
T_7	Control	-	90.00	0.00
	S.E.±	1.17		
	C.D. (P=0.05)	3.63		

* Mean of three replications

Table 2 : *In vitro* evaluation of effect of bio-agents on radial growth of *C. truncatum*

Sr. No.	Biological agents	Mean colony diameter (mm)*	Inhibition(%)
T_1	*Trichoderma viride*	19.00	78.88
T_2	*Trichoderma harzianum*	19.00	78.88
T_3	*Trichoderma hamatum*	20.66	77.04
T_4	*Pseudomonas fluorescens*	65.00	27.77
T_5	*Yeast* (*S. cervisiae*)	53.66	40.37
T_6	Mehandileaf extract (10%)	74.00	17.77
T_7	Control	90.00	00.00
	S.E.±	1.28	
	C.D. (P=0.05)	3.94	

* Mean of three replications

inhibitory to the *C. lindemuthianum* causing anthracnose of French bean (Chakraborty and Shyam, 1988), *C. truncatum* causing anthracnose of soybean (Ghawde *et al.*, 1996), *C. graminicola* (Singh and Dwivedi, 2002), *C. capsici* causing blight of bitter gourd (Dubey and Ekka, 2003), *C. gloeosporioides* causing anthracnose of mango (Kumar *et al*., 2003).

In vitro evaluation of bioagents :

Results (Table 2) revealed that among the six bioagents, *T. viride* and *T. harzianum* was found most effective and recorded 19.00 mm mean colony diameter and 78.88 per cent inhibition of the test pathogen followed by *T. hamatum* (77.045%), yeast (40.37%), *P. fluorescens* (27.77%) and *Mehandi* leaf extract (17.77%) with mean colony diameter 20.66, 53.66, 65, 74 mm, respectively. The bioagents, *T. viride* and *T. Harzianum* were reported as effective antagonists against *Colletotrichum* species by several workers (Barros *et al.*, 1995; Jayalakshmi *et al.*, 1998; Ingle *et al.*, 2002; Devis *et al.*, 2003; Raheja and Thakur, 2002; Rao and Narayana, 2005 and Kaur *et al*., 2006).

Fig. 1 : ***In vitro*** **evaluation of fungicides against** ***C. truncatum***

Fig. 2 : ***In vitro*** **evaluation of bioagents against** ***C. truncatum***

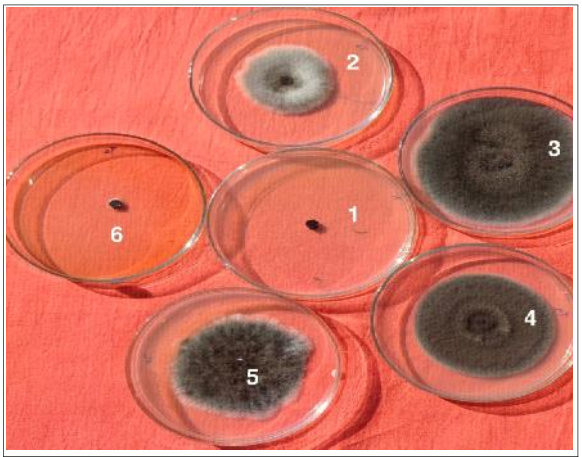

Fig. 3 : **Effect of temperature and humidity on** ***C. truncatum***

In vitro evaluation of effect of temperature and humidity on *C. truncatum* :

The results presented in Table 3 reveals that the

Table 3 : ***In vitro*** **evaluation of effect of temperature and humidity on radial growth and sporulation of** ***C. truncatum***

Sr. No.	Temperature (°C)	Humidity (%)	Mean colony diameter (mm)*	Sporulation
1.	5	95	00.00	-
2.	15	85	45.25	+
3.	27	75	84.00	+++
4.	34	65	74.25	++
5.	40	55	63.75	+
6.	45	50	00.00	-
	S.E.±	0.39		
	C.D. (P=0.05)	1.12		

* Mean of four replications

growth of pathogen was maximum at 27^{0}C temperature with humidity 75 per cent. Below 5^{0}C and above 40^{0}C were inhibitary to the test pathogen. There was no growth observed at 5^{0}C and 45^{0}C temperature. As regards to sporulation the good sporulation was observed at 27^{0}C temperature with humidity 75 per cent and moderate sporulation at 34^{0}C with humidity 65 per cent. Scanty sporulation was recorded at 15^{0}C and 40^{0}C temperature with humidity 85 per cent and 55 per cent, respectively. There was no sporulation at 5^{0}C and 45^{0}C temperature. Results are more or less similar with several workers Doornik (1982); Leng *et al.* (1984); Muniz *et al*. (1998) and Prabhakar *et al*. (2003).

Conclusion :

The fungal pathogen *Colletotrichum truncatum* caused anthracnose of soybean. From this experiment it was conclude that among the six fungicides tested *in vitro*. Carbendazim (0.1%) found most effective in inhibiting growth of test pathogen, followed by mancozeb (0.1%), hexaconazole (0.1%), chlorothalonil (0.2%) propiconazole (0.1%) and difenconazole (0.1%) in order of merit *in vitro.* In biological control the *Trichoderma viride* and *Trichoderma harzianum* found most effective in inhibiting growth of test pathogen followed by *Trichoderma hamatum*, yeast, *Pseudomonas fluorescens* and mehandi leaf extract in order of merit *in vitro*. Test pathogen grew well with maximum sporulation at optimum temperature of 27^{0}C with 75 per cent relative humidity.

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