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BIOEFFICACY OF FUNGICIDES AGAINST POD BLIGHT OF SOYBEAN AND INFECTIVE MORPHOGENETIC PATHWAY INCITED BY *COLLETOTRICHUM TRUNCATUM*

P. Deepthi *et al.,*

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P. DEEPTHI*, C. S. SHUKLA, K.P.VERMA AND A. S. KOTASTHANE
Department of Plant Pathology,
College of Agriculture, Indira Gandhi Krishi Viswavidyalaya, Raipur - 492 012, Chhattisgarh, INDIA
e-mail: deepthireddy03@gmail.com

ABSTRACT

The efficacy of seven different fungicides were evaluated against pod blight of soybean caused by *Colletotrichum truncatum* (Andrus and Moore) employing poisoned food technique, with five different concentrations (write the statement continuously and delete efficacy of ten fungicides). Efficacy of ten fungicides with five different concentrations (100, 200, 300, 400 and 500 ppm), were studied *in vitro* on suppression of radial growth and the most effective fungicide was evaluated for its interference in infective morphogenetic pathway of the against *Colletortricum truncatum* . Results revealed that Tebuconazole was significantly superior to all other fungicides by inhibiting (83.37 %) growth followed by Hexaconazole (67.65 %) and Taquat (31.66 %) over control after 10 days of incubation. Among the fungicides, Tebuconazole was found most effective in suppressing radial growth and inhibited appressorium formation at 400 and 500 ppm, which is most important for the penetration of the pathogen on host surface.

***Corresponding author**

INTRODUCTION

Soybean (*Glycine max* L. Merr.) has received a great deal of attention all over the world as an important source of protein to alleviate the protein deficiency. It is comparatively cheaper than the animal sources of protein such as meat, fish, milk, egg etc. It contains 40-45% protein, 18-20% edible oil, 24-46% carbohydrate and a good amount of vitamins (Kaul and Das, 1986). As legume crop it is capable of utilizing atmospheric nitrogen through biological nitrogen fixation. Soybean fixes about 270 kg N/ha compared to 58 to 157 Kg N/ha by other pulses (Hoque, 1978). As a result the crop is less dependent on chemical nitrogenous fertilizers.

All parts of the soybean plant are susceptible to diseases. More than 100 pathogens are known to affect soybean, of which 35 are of economically important (Sinclair and Backman, 1989). Soybean diseases reduce yield, on an average of 10 to 30% in most production area (Sinclair 1994). Among the most important diseases reported to cause economic losses to soybean, Anthracnose/pod blight of soybean (*Glycine max* L.), incited by *Colletotrichum truncatum* (Schw.) Andrus and Moore, is a serious disease in almost all soybean growing areas of the world including India and thereby causing qualitative as well as quantitative losses to the tune of 30 to 70 % (Lenne, 1992; Backman *et al.*, 1982; Chandrasekaran and Rajappan, 2002). Methods for complete control of soybean seed borne diseases are yet to be developed. Management strategies for these diseases include use of presumed disease free seeds, resistant cultivars and fungicidal sprays. Seed treatment is one of the best methods to manage seed-borne diseases. Few systemic and non systemic fungicides have been recommended to manage this disease successfully (Gopinath *et al.*, 2006 and Jagtap *et al.*, 2012). As the disease is highly influenced by environmental conditions, the results have been inconsistent. Henceforth, assessment of newer fungicides for the management of *Colletotrichum* pod blight disease is extremely imperative to decrease the loss and also to check the unpredicted epidemic of the disease. Therefore, the present study was conducted to find out the effectiveness of fungicides against pod blight of soybean.

MATERIALS AND METHODS

The sensitivity of C. *truncatum* to seven fungicides saff, Mancozeb, Hexaconazole, Sulphur, Tebuconazole, Ridomil, and Taquat were evaluated by poisoned food technique (Nene and Thapliyal, 1993). Required quantity of fungicides were added to sterilized PDA medium, sterilized and poured into 9 cm diameter Petridishes. Each fungicide was evaluated at 100, 200,300, 400 and 500 ppm concentrations. PDA alone served as control. After the media had solidified the plates were inoculated with 5 mm diameter agar plugs cut from the margin of 7 days old culture of the pathogen. Three replicates of each concentration were made. After 7 days of incubation at (28 $\pm$ 2ºC), the diameter of the colony was measured for each plate and inhibition of growth was calculated. Observations on radial growth of the test pathogen was recorded and percent growth inhibition of the test pathogen over control was calculated by the formula of Vincent (1947).

$I = (C-T)/C \times 100$

Where,

I : Per cent inhibition

C : Mycelial growth in control

T : Mycelial growth in treatment

Effect of fungicides on the infective morphogenetic pathway of *C. truncatum*

The most effective fungicide (Tebuconazole) as determined by 100% inhibition in radial growth was tested for its efficacy on the infective morphogenetic pathway of *C. truncatum*. Conidia suspension of *C. truncatum* were taken from 10 days old culture on PDA. Ten µL of fungicide suspension at different concentrations (100, 200, 300, 400 and 500 ppm) and 10 µL of the conidial suspension were mixed and the mixtures were added to the surface of dried depression slides and kept in moisture chamber at 25°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed over conidial suspension on the slides. The slides were examined under the microscope of high power (100 X) for recording the inhibition in the morphogenetic pathway of *C. truncatum*. The experiment was laid out in CRD with three replications.

Statistical analyses

Replications did not serve as sources of variation and the mean values and standard error of the mean for all experiments were reported. All percentages were arcsine-transformed and data were compared by analysis of variance. Means were separated by Fisher's least significant difference test with a significance threshold of $p \leq 0.05$. The design adopted for the analysis is CRD with additional treatment as control.

RESULTS AND DISCUSSION

Effect of different fungicides on radial growth of *C. truncatum*

Results (Table 1) revealed that all the fungicides tested were significantly effective in inhibiting the mycelial growth of the test pathogen as compared to the control (untreated). Among the fungicides, Hexaconazole and Tebuconazole were found to be most effective at all concentrations, showing 67.65% (55.90) and 83.37% (71.17) mean inhibition respectively.

The effect of Tebuconazole was higher than all others; it completely inhibited the fungal growth at concentrations 30, 40 and 50 ppm. The Hexaconazole was second with 46.08 (42.75), 57.78 (49.47), 72.07 (58.09), 74.67 (59.78) and 87.65 % (69.43) inhibition at 100, 200, 300, 400 and 500 ppm (change 10, 20, 30, 40 and 50 ppm to 100, 200, 300, 400 and 500 ppm)10, 20, 30,40 and 50 ppm, respectively, followed by Taquat and Mancozeb. The least control of the pathogen growth was seen in Saff: 11.01 (19.20), 12.96 (20.91), 15.57 (23.23), 24 (29.29) and 33.09% (35.09) inhibition at 10, 20, 30, 40 and 50 ppm to 100, 200, 300, 400 and 500 ppm, concentrations, respectively.

The mean colony diameter of Tebuconazole was observed to be lowest, that is, 12.8 mm followed by Hexaconazole (24.9 mm), Taquat (52.6 mm) and Mancozeb (53.3 mm). The highest colony diameter was observed by Sulphur (62.6 mm). The mean percent inhibition was highest by Tebuconazole followed by Hexaconazole, Taquat and Mancozeb , that is, 83.37 (71.17), 67.65 (55.90), 31.66 (34.12) and 30.76

Table 1:

S.No	Fungicide	Radial growth(mm) Concentration(ppm)					Average Growth(mm)	Inhibition % Concentration(ppm)					AverageInhibition(%)
		100	200	300	400	500		100	200	300	400	500	
1	Saff	68.5	67	65	58.5	51.5	62.1	11.01(19.20)	12.96 (20.91)	15.57 (23.23)	24 (29.29)	33.09 (35.09)	19.33(25.54)
2	Hexaconazole	41.5	32.5	21.5	19.5	9.5	24.9	46.08 (42.75)	57.78 (49.47)	72.07 (58.09)	74.67 (59.78)	87.65 (69.43)	67.65(55.90)
3	Ridomil	66.5	63	52.5	46	44	54.4	13.62 (21.65)	18.16 (25.20)	31.8 (34.31)	40.25 (39.37)	42.83 (40.87)	29.33(32.38)
4	Taquat	60	55.5	50	51	46.5	52.6	22.05 (27.97)	27.91 (31.89)	35.04 (36.28)	33.75 (35.52)	39.57 (38.93)	31.66(34.12)
5	Tebuconazole	43.5	20.5	0	0	0	12.8	43.48 (41.25)	73.37 (58.93)	100.00(85.22)	100.00 (85.22)	100.00 (85.22)	83.37(71.17)
6	Mancozeb	56.5	55.5	54.5	54.5	45.5	53.3	26.62 (31.05)	27.9 (31.85)	29.19 (32.67)	29.21 (32.71)	40.88 (39.73)	30.76(33.60)
7	Sulphur	63.5	63.5	63	62	61	62.6	17.5 (24.65)	17.53 (24.71)	18.16 (25.17)	19.46 (26.16)	20.75 (27.06)	18.68(25.55)
8	Control	77	78	76			77	0 (0.694)	0 (0.694)	0 (0.694)			0(0.694)
	CD{Control Vs Rest}	2.28											
	CD{Comparing level of C}	0.93											

(33.60), respectively. The lowest mean percent inhibition was observed by Sulphur, that is, 18.68 (25.55). Similar study was conducted by many workers Gud and Raut (2008), Jadhav *et al.* (2008), Patel (2009), Watve *et al.* (2009), Jagtap *et al.* (2013) and Ingle *et al.* (2014), who reported that triazole fungicides like tebuconazole, Hexaconazole and Propiconazole exhibited 100% inhibition in the growth of fungicide. Basha *et al.*, also reported that the triazole fungicides like Hexaconazole and propiconazole were effective in managing the anthracnose of mango.

Effect of fungicides on the infective morphogenetic pathway of *C. truncatum*

It is evident from results that tebuconazole was effective at all the concentrations in suppression of germination and interfering in the morphogenetic pathway of *C. truncatum* at variable concentrations. Among the different concentrations of fungicide, at 100, 200 and 300 ppm spore germination, germ tube elongation and appressorium formation was observed, at 400 and 500 ppm germination and germ tube elongation was observed but formation of appressoria was inhibited.

Tebuconazole, a triazole fungicide inhibit one specific enzyme, C14-demethylase, which plays a role in sterol production. Sterols, such as ergosterol, are needed for membrane structure and function, making them essential for the development of functional cell walls. Therefore, these fungicides result in abnormal fungal growth and eventually death. Each triazole compound may act in a slightly different part of the biochemical sterol-producing pathway. While the results are similar in various fungi-abnormal fungal growth and death-there are great differences in the activity spectra of these fungicides. Triazoles have no effect against spore germination because spores contain enough sterol for the formation of germ tubes. Some spores even have enough sterol to produce infection structures so, in some cases, triazoles may not be effective against infection of the host tissue.

Triazoles may be applied preventively or as early-infection treatments. When applied as an early-infection treatment, applications must be made early in the fungal infection process. Some triazole fungicides have anti-sporulant properties, which means they inhibit spore production and therefore help to slow disease development. Similar study was conducted by Gopinath *et al.* (2006) evaluated the efficacy of propiconazole, difenoconazole and carbendazim at different concentrations and stated that propiconazole, a triazole fungicide as superior to inhibit mycelial growth, biomass production, sporulation and spore germination at 0.1µ/mL.

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