In vitro Evaluation of Fungicides, Botanicals and Bioagents Against Soybean Anthracnose Incited by *Colletotrichum truncatum*

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SUMMARY

Colletotrichum. truncatum*, the incitant of anthracnose/ pod blight in soybean is one of the most destructive pathogens. Five fungicides *viz.* Carbendazim 50 WP, Chlorothalonil 75 WP, Difenconazole 25 EC, Hexaconazole 5 EC and Propiconazole 25 EC were evaluated (@ 100, 150 and 200 ppm each), five botanicals *viz.*, Neem, Mehandi, Eucalyptus, Bougainnveilia and Parthenium (each @ 10 and 15%) and ready made formulations of two bioagents *viz.*, *Trichoderma viride* (Tricho-Action, 100% w/w) and *Verticillium lecanii* (Viro-Action, 100 w/w) were evaluated *in vitro* against *C. truncatum*, using PDA as basal medium applying poisoned food technique. The results revealed that all the fungicides, botanicals / plant leaf extracts and bioagents tested were found fungistatic and significantly inhibited mycelial growth of the test pathogen over untreated control. Among the fungicides, Carbendazim recorded the highest mean inhibition (90.59 %) of mycelial growth of the test pathogen, followed by the fungicides, Propiconazole (87.95%), Hexaconazole (86.15%), Difenconazole (84.81%) and Chlorothalonil (70.23%). Among five botanicals tested, Neem recorded highest mean inhibition (72.56%) of mycelial growth of the test pathogen, followed by the Parthenium (61.31%), Mehandi (46.03%) and Bougainnveilia (28.98%). Bioagents, *T. viride* and *V. lecanii* recorded mean mycelial growth inhibition of 41.79 and 23.75 per cent, respectively.

Key words : Colletotrichum truncatum, Tungicides, Botanicals, Bioagents

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Soybean [*Glycine max* (L.) Merill.] is known as golden/ miracle bean crop of the planet. It has dual qualities *i.e.* 40 per cent protein and 20 per cent oil without cholesterol. Among the conventional oil seed crops of India, soybean ranks first in its importance, next only to groundnut and rapseed-mustard. Soybean is able to leave residual nitrogen effect for succeeding crop equivalent to 35 to 40 kg / N_2 / ha.

In India, area, production and productivity of soybean during 2005-2006 were 76.720 lakh ha., 61.28 lakh metric tonnes and 799 kg/ha., respectively. In Maharashtra, the area, production and productivity of soybean during 2005-2006 were 23.89 lakh ha., 19.63 lakh metric tonnes and 822 kg/ha, respectively (Anonymous, 2006). 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).

More than 100 plant pathogens have been reported to affect soybean, but among them, very few are economically important causing yield losses to the tune of 12-20 per cent. The most important diseases reported to cause economic losses to the soybean are: Anthracnose incited by *Colletotrichum truncutum* (Schw.) Andrus and Moore causing yield losses of 16-100 per cent, frog eye spot (*Cercospora sojina*) causing 15 per cent losses, rust (*Phakospora pachyrhizi*) causing 10-90 per cent losses, downy mildew (*Peronospora monshurica*) causing 8 per cent losses, powdery mildew (*Microsphaera diffusa)* causing 10-35 per cent losses and soybean mosaic causing 25-50 per cent losses (Sinclair,1992). *Colletotrichum truncatum*, is the most common species recorded on soybean (Lenne, 1992) and the crop soybean is susceptible to *C. truncatum* at all the stages of development particularly from bloom to pod fill. The disease causes considerable damage by reducing plant stand, seed quality, seed germination and yield (Vyas *et al.*, 1997).

MATERIALS AND METHODS

The experiment was conducted during *kharif*, 2006 at Department of Plant Pathology, College of Agriculture, Latur, during the present investigations on anthracnose (*C. truncutum*) of soybean [*Glycine max* (L.) Merill.]. These diseased specimens (leaves, pods) were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half healthy and half

diseased portion intact. These pieces were surfaces sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. The surface sterilized diseased pieces were the inoculated on the solidified and cooled PDA (Potato dextrose agar) medium in Petri plates under aseptic conditions of Laminar-air-flow cabinet. Inoculated plates were then incubated in BOD incubator at 24 $\pm$ 2^0C temperature. After three to four days of incubation, the well developed mycelial growth free from any contaminant was obtained. A total of five fungicides (@ 100, 150, 200 ppm each) *viz*. Carbendazim (50 WP), Chlorothalonil (75 WP), Difenconazole (25 EC), Hexaconazole 5 EC) and Propiconazole (25 EC); five plant leaf extracts (@ 10 and 15%) *viz*., Neem, Mehandi, Nilgiri, Bougainnveilia, Parthenium and ready-made commercial formulations of two bioagents *Trichoderma viride* (Tricho -Action, 100% w/w) and *Verticillium lecanii* (Viro -Action, 100% w/w) @ 10 and 15% each were evaluated *in vitro* against *C. truncatum* applying Poisoned food technique (Nene and Tapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture media. The requisite quantity of each fungicide on the basis of active ingredient (a. i.) was calculated and thoroughly mixed with autoclaved and cooled (40-45^{0}C) PDA in conical flasks to obtain desired concentrations of 100, 150 and 200 ppm.

Healthy and disease free fresh leaf sample of selected plant species were brought to the laboratory and washed with sterile distilled water and then chopped into small bits with sterilized sharp knife. Each leaf sample was then separately grinded 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 No. 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 each leaf extract was incorporated separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentrations (10 and 15%) of each extract and autoclaved at 15 Lbs pressure for 15 to 20 minutes. Commercial formulations of the bioagents, *T. viride* (Tricho– Action, 100% w/w) and *V. lecanii* (Viro – Action, 100% w/w) were also evaluated *in vitro* applying positioned food technique. Stock solution of 10 and 15 ml of each bioagent was mixed separately with 90 and 85 ml of sterilized and molten PDA medium, respectively to get 10 and 15 per cent concentrations.

The fungicides, plant extracts and bioagents amended PDA was then poured (15 – 20 ml/plates) in sterilized Petriplates (90 mm dia.) under aseptic conditions. Four plates / treatments / concentrations were maintained and each treatment with respective concentration was replicated for four times. On solidification of PDA in Petriplats, all the 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. Petriplates containing plain PDA without any fungicide, plant extract and bioagent were inoculated with 5.0 mm disc of the test pathogen and maintained as suitable untreated control. All the treatment (inoculated) and control Petriplates were then incubated at 24 $\pm$ 2^0C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen.

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.

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

where,

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

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

RESULTS AND DISCUSSION

Effect of fungicides:

The results (Table 1 and Fig. 1) revealed that all the five fungicides tested *in vitro* against *C. truncatum* significantly inhibited the mycelial growth of the test pathogen over untreated control. Carbendazim found highly effective which recorded minimum mean colony diameter (8.45 mm) and maximum mean inhibition (90.59 %) of mycelial growth of the test pathogen over untreated control (mean col. dia. 90.00 mm and mean inhibition, 0.00%). This was followed by the fungicide, Propiconazole, (mean col. dia., 10.83 mm and mean inhibition, 87.95%), Hexaconazole (mean col. dia., 12.45 mm and mean inhibition, 86.15%) and Difenconazole (mean col. dia., 13.60 mm and mean inhibition, 84.81%). The maximum mean colony diameter (26.78 mm) and minimum mycelial growth inhibition (70.23%) of the test pathogen were recorded by the fungicide, Chlorothalonil. All the concentrations of the fungicides tested significantly inhibited the mycelial growth of the test pathogen. However, higher concentration (@ 200 ppm) recorded maximum inhibition (range, 75.94 to 91.94%) followed by 150 ppm (range, 69.19 to 90.69% inhibition) and 100

Table 1 : Effect of fungicides at different concentrations on radial growth of *C. truncatum*

Fungicides	Colony diameter* (mm)			Mean	% Inhibition			Mean
	100 ppm	150 ppm	200 ppm	(mm)	100 ppm	150 ppm	200 ppm	%
Carbendazim	9.75	8.37	7.25	8.45	89.16	90.69	91.94	90.59
					(70.77)	(72.23)	(73.50)	(72.16)
Chlorothalonil	30.97	27.72	21.65	26.78	65.58	69.19	75.94	70.23
					(54.08)	(56.28)	(60.63)	(56.99)
Difenconazole	15.37	13.87	11.75	13.66	82.91	84.58	86.94	84.81
					(65.58)	(66.87)	(68.81)	(67.08)
Hexaconazole	14.25	12.25	10.87	12.45	84.16	86.38	87.91	86.15
					(66.54)	(68.34)	(69.65)	(68.17)
Propiconazole	12.12	11.25	9.12	10.83	86.52	87.49	89.86	87.95
					(68.46)	(69.29)	(71.43)	(69.77)
Control	90.00	90.00	90.00	90.00	00.00	00.00	00.00	00.00
					(4.05)	(4.05)	(4.05)	(4.05)
S.E. ±	0.16	0.11	0.23	--	0.13	0.10	0.19	--
C.D. (P=0.05)	0.51	0.35	0.72	--	0.40	0.33	0.59	--

* Average of four replications
Figures in parenthesis are angular transformed values

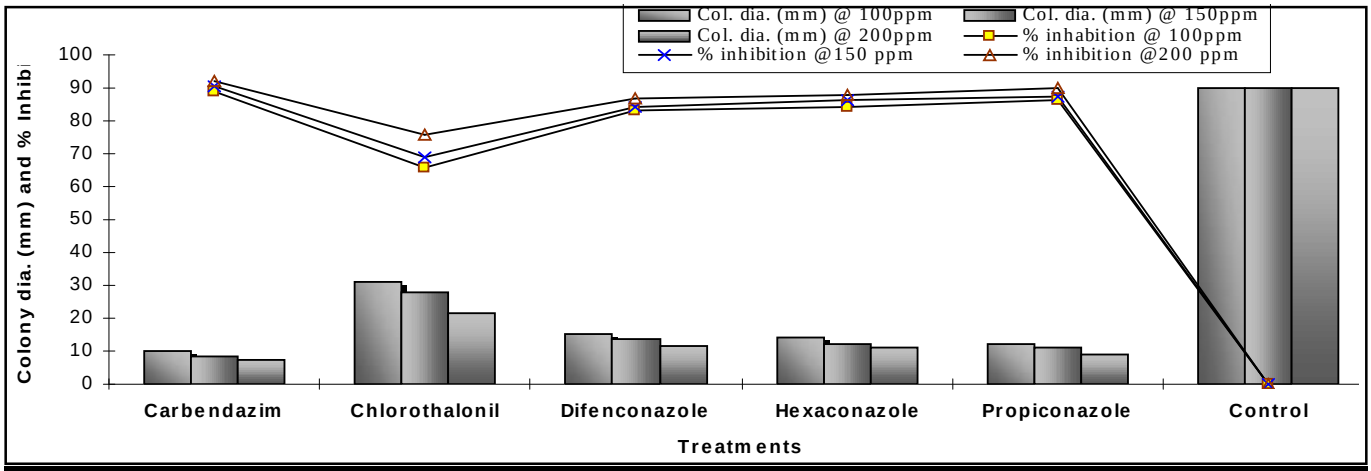

Fig. 1 : Effect of fungicides at different concentrations on radial growth of *C. truncatum*

ppm (range, 65.58 to 89.16% inhibition). Earlier workers reported the efficacy of Carbendazim against *C. lindemuthianum* causing anthracnose of French bean (Chakraborty and Shyam, 1988 and Gupta *et al*., 2005), and Swamy and Kulkarni (2003) reported that the fungicides, Hexaconazole, Propiconazole, Penconazole and Difenconazole were inhibitory to *C. truncatum, C. gleosporioides. C. capsici* and *C. lindemuthianum* and these findings are inconformity with the present study.

Effect of botanicals and bioagents:

The results (Table 2 and Fig. 2) revealed that all the botanicals / plant leaf extracts and bioagents tested *in vitro* were found significantly effective in reducing the percentage mycelial growth of *C. truncatum* over untreated control. Neem plant leaf extract (@ 10 and 15%) was found highly effective which recorded lowest mean colony diameter (24.68 mm) and highest mean mycelial growth inhibition (72.56%) of the test pathogen over untreated control. This was followed by the botanicals, parthenium (mean col. dia, 34.81 mm and mean inhibition, 61.31%), Mehandi (mean col. dia. 48.56 mm and mean inhibition 46.03%) and Bougainnveilia (mean col. dia, 64.81 mm and mean inhibition, 28.98%). The plant leaf extract of Eucalyptus was found least effective and reported maximum mean colony diameter (81.00 mm) and lowest inhibition (9.99%) of the test pathogen. Earlier workers reported the fungistatic action of leaf extract against several *Colletotrichum* species causing anthracnose, blights and leaf spots in many crops (George *et al*., 2003 and Rao and Narayana, 2005).

In between the two bioagents, *T. viride* was found

Table 2 : Effect of different plant extracts and bioagents on radial growth of *C. truncatum*

Treatments	Colony Diameter* (mm) at Conc.		Mean (mm)	% inhibition* at Conc.		Mean %
	10%	15%		10%	15%	
Neem	28.12	21.25	24.68	68.74 (56.03)	76.38 (60.93)	72.56 (58.48)
Mehandi	52.50	44.62	48.56	41.66 (40.19)	50.41 (45.23)	46.03 (42.71)
Eucalyptus	85.50	76.50	81.00	4.99 (12.81)	14.99 (22.77)	9.99 (17.76)
Bougainnveilia	68.12	61.50	64.81	24.30 (29.50)	33.66 (35.44)	28.98 (32.47)
Parthenium	39.87	29.75	34.81	55.69 (43.86)	66.94 (54.90)	61.31 (49.38)
T. viride	58.75	46.00	52.37	34.71 (36.09)	48.88 (44.36)	41.79 (40.22)
V. lecanii	74.00	63.00	68.50	17.77 (24.90)	29.74 (33.04)	23.75 (28.97)
Control	90.00	90.00	90.00	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
S.E. ±	0.99	0.56	--	1.65	4.46	--
C.D.(P=0.05)	2.93	1.65	--	4.85	1.36	--

* Average of four replications
Figures in parenthesis are angular transformed values

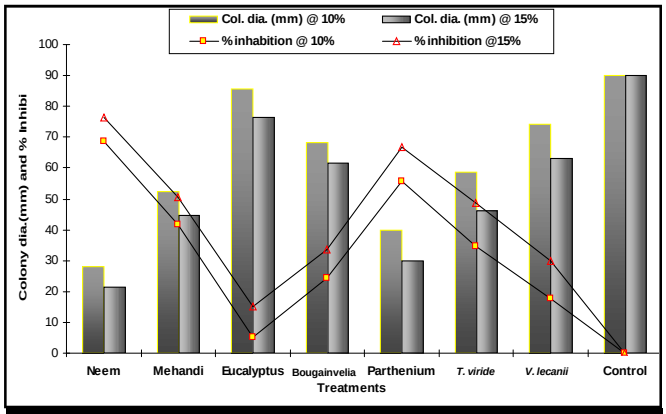

Fig. 2 : Effect of different plant extracts and bioagents on radial growth of *C. truncatum*

most effective which recorded 52.37 mm mean colony diameter and 41.79 per cent inhibition of the test pathogen followed by *V. lecanii* (mean col. dia, 68.50 mm and mean inhibition, 23.75%). Both concentrations (@ 10 and 15%) of the plant extracts and bioagents tested were found effective in the inhibition of the test pathogen. However, higher concentration (@ 15%) caused maximum (range, 14.99 to 76.38%) inhibition of mycelial growth compared to lower concentration (@ 10%) which recorded comparatively minimum inhibition of mycelial growth in the range of 4.99 to 68.74 per cent. The bioagents, *T. viride, T. harzianum, T. virens* were reported as effective antagonists against *Colletotrichum* species by several workers (Barros *et al.*, 1995 and Kaur *et al.*, 2006).

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