Research Paper :

In Vitro evaluation of fungicides and biocontrol agents against *Colletotrichum lindemuthianum* causing anthracnose of dolichos bean

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SUMMARY

The experiment was conducted to know the efficacy of different fungicides and bioagents against inhibition of *Colletotrichum lindemuthianum* growth, causing anthracnose of Dolichos bean. Among different contact fungicides tested *in vitro,* mancozeb was found to be more effective and inhibited centper cent (100%) followed by propineb (48.32%) and chlorothalonil (37.39%) inhibited the mycelial growth of *C. lindemuthianum* at a concentration of 800 ppm. Among different systemic fungicides tested, carbendazim inhibited cent per cent (100%) mycelial growth followed by propiconazole (100%) and difenoconazole (84.87%) at a concentration of 400 ppm. Among the biocontrol agents, *Trichoderma harzianum* was found to be the best in inhibiting the mycelial growth of *C. lindemuthianum* to an extent of 73.54 per cent followed by *T. viride* (50.90%). Least mycelial growth inhibition was observed in *Bacillus megaterium* (39.46%).

Key words : Fungicides, Bioagents, *Colletotrichum lindemuthianum*, Anthracnose

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Dolichos bean (*Dolichos lablab* L.) is an important pulse-cum-vegetable crop. Dolichos bean is good source of the amino acid, lysine, and it contains 20-28% crude protein. The green pods are a good protein source as well as a valuable source of fibre. The dolichos bean is affected by many diseases and among which, anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scriber. is the most devasting disease in India (Sharma and Sugha, 1995) and disease occurs in tropical and subtropical regions but it causes greater losses in the temperature zones than it does in tropics. Anthracnose is caused by *C. lindemuthianum* (Sacc and Magn.) Scriber. affecting all plant parts *viz.*, stem, pods and seeds (Zaumeyer and Thomas, 1957). Keeping in view the importance of anthracnose disease, the present investigation was carried out to test the efficacy of different fungicides and biocontrol agents against *Colletotrichum lindemuthianum.*

MATERIALS AND METHODS

The experiment was conducted at Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, G.K.V.K., Bangalore, during 2008. Under *in vitro* condition, eight treatments of fungicides *viz.*, carbendazim, chlorothalonil, copper-oxy-chloride, difenconazole, hexaconazole, mancozeb, propiconazole and propineb were tested against *Colletotrichum lindemuthianum* by employing poisoned food technique. The desired concentrations were obtained by adding appropriate amount of stock solution of fungicides to Potato dextrose agar (PDA) in Petriplates and repeated thrice for each treatment. PDA without fungicides served as control. Each plate was inoculated with a 5 mm mycelial disc of the pathogen taken from 7 day old culture grown on PDA. The inoculated plates were incubated at 28±1^{0}C. after 7 days of incubation, colony diameter was recorded and per cent inhibition in each treatment over control was calculated by using the formula given by Vincent (1947):

$$I = \frac{C - T}{C} x100$$

where, I =Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

The different bioagents *viz., Trichoderma viride, T. harzinaum, Bacillus megaterium* and *Pseudomonas fluorescens* were evaluated against anthracnose pathogen, *C. lindemuthianum* by dual plate technique. Twenty ml of PDA medium was poured into sterile Petriplates and allowed for solidification. Seven day old, 5 mm culture disc of *C. lindemuthianum* was taken with the help of sterilized cork borer and placed in the one side

of Petriplate. The biocontrol agents were cut with a sterile cork borer and transferred aseptically to Petriplates on opposite sides, but in case of bacterial antagonists, streak was done in Petriplates. The disc of *C. lindemuthianum* was placed two days earlier than bio-control agents to compensate for the slow growth of pathogen. A Petriplate without antagonist was maintained as control. The inoculated plates were incubated in a BOD incubator at 28±1^{0}C and each treatment was replicated thrice.

Observation on growth of antagonist and *C. lindemuthianum* from centre of disc towards centre of plate was recorded 7 days after inoculation. The growth of inhibition of *C. lindemuthianum* over control was calculated as per Vincent (1947). The PDA medium was used for maintaining the fungal biocontrol agents but the Nutrient agar was used for the maintenance of bacterial biocontrol agents.

RESULTS AND DISCUSSION

An *in vitro* the evaluation of fungicides provides useful and preliminary information about efficacy against the pathogen within a shortest period of time. Fungitoxicants play an important role in checking the fungal growth. In the present investigation, *in vitro* evaluation of four contact and four systemic fungicides were undertaken against the pathogen by following poisoned food technique. Four contact fungicides *viz.*, chlorothalonil, copper-oxy-chloride, mancozeb, propineb and four different systemic fungicides *viz.*, carbendazim, difenconazole, hexaconazole and propiconazole were evaluated against *C. lindemuthianum*. The data on per cent (%) inhibition of radial growth of the fungus are presented in Table 1.

Among the contact fungicides evaluated, mancozeb inhibited cent per cent (100%) mycelial growth at 400 and 800 ppm, followed by 49.58 and 30.67 per cent inhibition at 200 and 100 ppm, respectively. Propineb inhibited 48.32 per cent at 800 ppm followed by 33.60, 30.66 and 17.22 per cent inhibition at 400, 200 and 100 ppm, respectively, while least per cent inhibition was observed in case of copper-oxy-chloride (06.72%) at 100 ppm followed by 11.34, 8.80 and 9.20 per cent inhibition at 200, 400 and 800 ppm, respectively. Mancozeb was found to be superior among the contact fungicides in inhibiting the growth of the pathogen. Among the systemic fungicides evaluated, carbendazim inhibited cent per cent (100%) mycelial growth in all the concentration tested. Propiconazole inhibited cent per cent mycelial growth at 400 ppm followed by 91.18, 86.13 and 84.87 per cent inhibition of mycelial growth at 200, 100 and 50 ppm, respectively. The least inhibition of 28.56, 37.82 and 42.01 per cent was observed in case of hexaconazole at 50, 100 and 200 ppm, respectively. Carbendazim was superior in inhibiting the growth of the pathogen.

Table 1 : *In vitro* evaluation of different fungicides against *Colletotrichum lindemuthianum*

Contact fungicides					
Sr. No.	Fungicides	Per cent inhibition of mycelial growth			
		Concentration (ppm)			
		100 ppm	200 ppm	400 ppm	800 ppm
1.	Chlorothalonil	22.26	23.52	35.29	37.39
2.	Copper oxy chloride	6.72	11.34	8.80	9.20
3.	Propineb	17.22	30.66	33.60	48.32
4.	Mancozeb	30.67	49.58	100	100
	Fungicides (F)	Concentrations (C)		F×C	
S.E.±	0.41	0.36		0.8	
C.D. (P=0.01)	1.41	1.26		2.80	
Systemic fungicides					
Sr. No.	Fungicides	Per cent inhibition of mycelial growth			
		Concentration (ppm)			
		50 ppm	100 ppm	200 ppm	400 ppm
5.	Carbendazim	100	100	100	100
6.	Difenoconazole	77.31	79.83	84.87	84.87
7.	Hexaconazole	28.56	37.82	42.01	49.58
8.	Propiconazole	84.87	86.13	91.18	100
9.	Control	0.00	0.00	0.00	0.00
	Fungicides (F)	Concentrations (C)		F×C	
S.E.±	0.43	0.38		0.9	
C.D. (P=0.01)	1.48	1.32		2.9	

Oliveira *et al.* (1992) and Suresh Ekbote (2005) also observed carbendazim as most effective in inhibiting the growth of *C. lindemuthianum*. Mancozeb was also reported best in inhibiting the growth of *C. lindemuthianum* by Joshi and Tripathi (2002), which is in conformity to the present studies as these workers have not used carbendazim in their studies. The present study showed that, carbendazim was superior in inhibiting the growth of the *C. lindemuthianum*. The effects of microbial biocontrol agents on the growth of *C. lindemuthianum* were evaluated under laboratory condition by dual culture technique and the data are presented in Table 2.

The results of the study (Table 2) indicated that among biocontrol agents, the maximum mycelial growth inhibition

Table 2 : *In vitro* evaluation of biocontrol agents against *Colletotrichum lindemuthianum*

Sr. No.	Biocontrol Agents	Per cent inhibition over control
1.	*Trichoderma viride*	50.90
2.	*Trichoderma harzianum*	73.54
3.	*Bacillus megaterium*	39.46
4.	*Pseudomonas fluorescens*	46.50
5.	Control	0.00
S.E.±		1.78
C.D. (P=0.01)		0.53
CV (%)		0.74

of 73.54% was observed in *Trichoderma harzianum* followed by *T. viride* (50.90%) and *Pseudomonas fluorescence* (46.50%). Least per cent inhibition of 39.46% was observed in *Bacillus megaterium*. Similar result were observed by Barros *et al.* (1995) who showed that *T. harzianum* and *T. viride* produced a noticeable decrease in colony diameter of *C. lindemuthianum* and noted that growth of *T. harzianum* was vigorous in dual culture and effectively hyper parasitized the pathogen by penetrating and coiling its hyphae around the hyphae of *C. lindemuthianum*. Rajathilagam and Kannabiran (2001) also studied the antifungal effect of the biocontrol agents; *Trichoderma harzianum* and *T. viride* which were reported to be effective in controlling the anthracnose fungus.

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