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In vitro evaluation of fungicides and bioagents against Anthracnose disease of Dolichos bean caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scri

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Dolichos bean (*Lablab purpureus* L.), a multipurpose crop, is affected by several fungal diseases among which anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scri is an important disease of economic significance. Investigations though *in vitro* evaluation of the effectiveness of different fungicides and biocontrol agents was carried out to manage this disease. Out of six systemic fungicides tested *in vitro* against *C. lindemuthianum*, carbendazim and carbendazim + mancozeb were found best in inhibiting cent per cent mycelial growth of the fungus at all four concentrations tested (100, 250, 500 & 750 ppm) and the next best was propiconazole (100%) at 750 ppm concentration. Mancozeb was found superior among the various non-systemic fungicides evaluated by inhibiting 100 per cent mycelial growth at 2000 ppm concentration. Among the bioagents, *Trichoderma viride* (92.22%) inhibited the mycelial growth of fungus to the maximum extent followed by *Bacillus subtilis* (89.44%).

Key words: Anthracnose, Bioagents, *Colletotrichum lindemuthianum*, Dolichos bean, fungicides

INTRODUCTION

Dolichos bean, *Lablab purpureus* L. (Sweet) is an ancient legume crop widely grown throughout the world as a vegetable or pulse crop for human consumption, animal forage or feed. It is cultivated either as a pure crop or mixed with other crops such as finger millet, groundnut, pigeonpea, castor, corn, bajra or sorghum in the tropical regions. India is the leading producer of dolichos bean in the world where it is grown in an area of 0.83 lakh ha with a production of 0.70 lakh tonnes and a productivity of 961 kg ha^{-1} (Anon., 2011). Karnataka contributes a major share, accounting for nearly 90 per cent in terms of both area and production of dolichos bean in India. Despite the fact that the area under dolichos bean is increasing in Karnataka, its production and productivity is considerably low (Rekha and Mallapur, 2007). This may be attributed to various biotic stresses (pests and diseases). The crop is affected by many fungal diseases, among which anthracnose is an important disease occurring throughout the world (Pastor-Corrales *et al.*, 1995). The incidence of dolichos bean anthracnose disease has been reported to range from 0.5 to 88.0 per cent in different locations. However, not much systematic research work has been carried out on management aspects of this disease. Considering the magnitude of loss caused by anthracnose disease of dolichos bean, the present investigation was undertaken with an aim to screen the most effective fungicides and bioagents against *Colletotrichum lindemuthianum*.

MATERIALS AND METHODS

The experiments were conducted following Completely Randomized Design (CRD) with three replications. Data was analysed statistically.

In vitro evaluation of systemic and non systemic fungicides against C. lindemuthianum

Six systemic (Carbendazim, Propiconazole, Hexaconazole, Thiophanate methyl, Difenconazole and Carbendazim+ Mancozeb) and five non-systemic (Mancozeb, Propineb, Copper oxychloride, Chlorothalonil and Captan) fungicides were evaluated against the fungus *C. lindemuthianum* under *in vitro* conditions. All systemic fungicides were tried at 100, 250, 500 and 750 ppm concentrations, while nonsystemic fungicides were evaluated at 250, 500, 1000 and 2000 ppm concentrations. The poisoned food technique (Shravelle, 1961) was followed to evaluate the efficacy of fungicides in inhibiting the mycelial growth of *C. lindemuthianum*. Fungicidal suspensions of different concentrations were prepared by dissolving requisite quantities of each fungicide in warm Richard's broth. The fungicides were thoroughly mixed with the medium by shaking with hands before autoclaving. About 20 ml of sterilized medium was poured in each 9 cm sterilized petriplates. After solidification, the plates were inoculated by placing 5 mm discs of 12 days old cultures of *C. lindemuthianum*. Three replicated plates were used for each concentration of every fungicide. The contents of the flasks were autoclaved at 121 °C at 15 *psi* for 20 minutes. The flasks were placed inside a clean bench for cooling at ambient temperature. Three replicated Richard's agar plates received no fungicides to serve as control. The inoculated plates were incubated at 27±1 °C and data on the radial colony diameter was recorded after 12 days of incubation when the growth of the control plates completely covered the plate. At the end of incubation, the cultures in all flasks were filtered separately through pre-weighted filter paper. Dry weight of mycelium was obtained by subtracting weight of filter paper from weight of filter paper and mycelium. Per cent inhibition of mycelial dry weight was determined by comparing the growth in control flasks using the formula of Vincent (1947).

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

Where, I: Per cent inhibition ; C: Mycelial growth in control; T: Mycelial growth in treatment

In vitro evaluation of bioagents

The fungal and bacterial bioagents were evaluated *in vitro* for their antagonistic effect against *Colletotrichum lindemuthianum* by dual culture method (Dennis and Webster, 1971) on Richard's agar medium. Twenty ml of sterilized and cooled Richard's agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agent, mycelial discs of test fungus were inoculated at one end of petriplate and antagonistic fungus was placed on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked at ends of the Petriplates and mycelial discs of the fungus was placed at the centre. Three replications were maintained for each treatment. The plates were incubated at 27 ± 1ºC and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

RESULTS AND DISCUSSION

In vitro evaluation of fungicides provides useful and preliminary information regarding efficacy of fungicides against pathogen within a short time,

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

Fungicides	Per cent inhibition of mycelial growth Concentration (ppm)				Mean
	100	250	500	750	
Carbendazim	100	100	100	100	100
Propiconazole	70.92	89.81	97.04	100	89.44
Hexaconazole	72.63	79.57	81.87	86.53	84.76
Thiophanate methyl	59.62	66.29	83.33	96.67	76.47
Difenoconazole	84.00	93.00	94.33	98.00	92.33
Carbendazim 12% + Mancozeb 63%	100	100	100	100	100

Fungicides		Concentration	FxC
SEm±	1.47	1.47	2.95
CD at 1%	5.72	5.72	11.44

thereby, serving as a guide for field testing of such fungicides. Among the six systemic fungicides tested, Carbendazim and Carbendazim 12% + Mancozeb 64% were the best in inhibiting (100%) the growth of *C. lindemuthianum* at all the four concentrations (100, 250, 500 and 750 ppm) and the next best was propiconazole (100%) at 750 ppm concentration. The least inhibition of mycelial growth among the systemic fungicides was observed in thiophanate methyl (59.62%) at 100 ppm concentration (Table 1). Efficacy of these fungicides was previously reported by Sunil Kulkarni (2009), Madhusudhan (2002), Laxman (2006) and Rajesha *et al.* (2010).

Table 2: *In vitro* evaluation of non systemic fungicides against *Colletotrichum lindemuthianum*

Fungicides	Per cent inhibition of mycelial growth Concentration (ppm)				Mean
	250	500	1000	2000	
Mancozeb	47.03	68.15	83.82	100	74.75
Propineb	19.43	39.26	53.15	71.41	45.83
Copper oxy chloride	0.00	12.00	45.04	58.48	28.88
Chlorothalonil	45.41	53.52	68.99	78.11	61.51
Captan	50.03	67.50	71.48	80.00	67.25
Fungicides		Concentration		FxC	
SEm±	0.96	0.86		1.93	
CD at 1%	2.76	2.47		5.53	

Table 3. *In vitro* evaluation of bioagents against *C. lindemuthianum*

Bio agents	Name of the isolates	Colony diameter (mm)	Per cent inhibition over control
Trichoderma viride	Native isolate (GKVK)	7.00	92.22
Trichoderma viride	Tv-23-PDBC	20.00	77.78
Trichoderma harzianum	ThB9-PDBC	26.16	70.93
Bacillus subtilis	Bs-GKVK	9.50	89.44
Pseudomonas fluorescens	Pf-2-GKVK	24.83	72.41
Pseudomonas aeruginosa	Pa-PDBC	48.83	45.74
Control		90.00	0.00
S.Em ±		0.42	0.49
C.D at 1 %		1.78	1.96

Among the five non-systemic fungicides tested, manocozeb was found effective in inhibiting the growth of mycelium upto 100 per cent (2000 ppm). The least inhibition of mycelial growth was observed in copper oxychloride (0.00%) at 250 ppm concentration (Table 2). These results are in accordance with the reports of Rajesha *et al.* (2010), Hegde (1967) and Madhusudhan (2002).

Biological control through the use of antagonistic microorganisms is a potential non chemical means of controlling plant disease by reducing inoculum levels of pathogens. In the present investigation, the antagonistic effect of different bioagents against *C. lindemuthianum* was assessed by dual culture technique. Among the different bioagents evaluated, *Trichoderma viride* gave the highest growth inhibition (92.22%) followed by *Bacillus subtilis* (89.44%), *T. viride* isolate Tv PDBC (77.78 %), *Pseudomonas fluorescens* isolate Pf-2 GKVK (72.41%), *Trichoderma harzianum* ThB9-PDBC (70.93%). The least growth inhibition of the fungus was observed in *Pseudomonas aeruginosa* Pa-PDBC (45.74%) (Table 3). Gupta *et al.* (1991) reported that *Gliocladium virens*, *T. harzianum* and *T. viride* significantly inhibited growth of *C. lindemuthianum in vitro*. The present investigations are in agreement with the results reported by Varaprasad (2000), who found effectiveness of *Trichoderma* sp. against *Colletotrichum dematium* and Laxman (2006) against *C. truncatum*. This could be due to several possibilities of existence of microbial interactions such as stimulation, inhibition, mutual intermingling of growth of antagonistic isolate over test pathogen *etc.* as enumerated by many workers (Porter, 1924, Ghaffar, 1969 and Naik and Sen, 1995).

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