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In vitro Evaluation of Fungicides, Botanicals and Bio-agents against *Colletotrichum lindemuthianum* causing Anthracnose of Bean

Ashwini R. Bagade*, G. K. Giri, Archana W. Shingne and Punam N. Usendi

Department of Plant Pathology, Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola- 444104 (M.S.), India

**Corresponding author*

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A B S T R A C T

Common bean (*Phaseolus vulgaris* L.) also known as French bean, dry bean and field bean belongs to family leguminaceae. In the suitable condition bean is attacked by various diseases. Out of which, anthracnose caused by seed borne pathogen *Colletotrichum lindemuthianum* is an important fungal disease and major limiting factor for yield loss. Realizing the potentiality of the disease in causing economic losses, the different fungicides are tested against *Colletotrichum lindemuthianum* among the fungicides Carbendazim+ Mancozeb, Carboxin + Thiram showed 100 % mycelial inhibition followed by Pyraclostrobin, Coper oxychloride, Propineb and Azoxystrobin 91.48 %, 88.55%, 72.22%, 54.44% respectively. Among the botanicals *Lawsonia inermis* showed highest mycelial inhibition 88.55 % followed by *Zingiber officinale* 65.55%, *Pongamia pinnata* 56.00 %, *Azardirachta indica* 37.04%, *Eucalyptus globules* 32.22% and *Oscimum sanctum* 26.33 %. Among the bio-agents *Trichoderma reesei* inhibited 77.77% mycelial growth and found most superior bio-agents followed by *Bacillus subtilis* 59.44%, *Pseudomonas fluorescens* 56.77 % and *Trichoderma asperellum* 48.61%.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the important legumes crop belongs to family leguminaceae and occupies a premier place among grain legumes in the world including India (Jan *et al.*, 2014). It is an important constituent of people's diets especially in developing countries. Dry bean find a unique position in the culinary items because of their high nutritional value (Padder *et al.*, 2017). It is rich in calories, carbohydrates, protein, vitamins and minerals particularly calcium, phosphorus and iron, thus an excellent food for human consumption. Common bean suffers from many diseases caused by fungi, bacteria, viruses, nematode and also abiotic stresses.

Among the fungal diseases anthracnose are the most prevalent ones. The anthracnose caused by *Colletotrichum lindemuthianum* it is seed borne pathogen (Parthiban and Kavitha, 2014). Anthracnose is a wide spread problem limiting the profitable cultivation and seed production throughout the major common bean growing regions of India. The pathogen causes extensive damage to the

fruits since the lesions on the fruits considerably reduce the market value of the produce. In India, disease incidence has been reported to vary between 24.59 to 51.72 % Sharma and Sugha (1995). As a disease of minor important but during the last few year bean anthracnose has appeared as a potential threat to the (Sacc and Magnus) Briosi and Cavara is a major limiting factor in reduction of yield in subtropical and temperate regions. Anthracnose is mainly a seed-borne disease caused by a fungus which has a wide host range on many legume species (Goswami *et al.,* 2011).

Materials and Methods

Isolation and pathogenicity

The present investigation on *in vitro* efficacy of fungicides, botanicals and bio-agents against *Colletotrichum lindemuthianum* was conducted at the Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. PDKV), Akola. The culture of *C. lindemuthianum* used in this study was isolated from infected leaves of common bean plants collected from the fields of Chilli and Vegetable Research Unit, Dr. PDKV, Akola. In order to isolate pathogen infected leaf sample were cut along with healthy leaf and surface sterilized with 0.1% sodium hypochlorite solution for one minute and washing with three times by sterilized distilled water. The bits were placed in petriplates containing PDA medium. All the above operations were carried out in sterilized condition (under laminar air flow unit). Then plates were incubated at 27±2 ºC for 10 days. The fungal growth, which developed around each bit, was then transferred to PDA medium slant for sub culturing. The isolated fungi were identified as *C. lindemuthianum* on the basis of morphological characters and published literature. The inoculum was prepared and sprayed (1×10^{6}spore/ ml) on plants of common bean within 6-12 days typical anthracnose symptoms were observed. The pathogen was reisolated on the PDA medium from the inoculated plants for confirmation of Koch's postulates.

In vitro evaluation of fungicides by poisoned food technique

Poisoned food technique was used to evaluate the efficiency of six fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved 1.05kg/cm2 for 15 min. Then before solidification of media different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks shaken to facilitate uniform mixture of fungicides thoroughly and poured in Petri plate's 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with seven days old pathogens separately. Five mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface of medium.

The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using following formula (Vincent, 1927).

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

Where,
PI = Per cent Inhibition

C = Growth of fungi in control (mm)
T = Growth of fungi in treatment (mm)

In vitro evaluation of botanicals by poisoned food technique

Aqueous leaf extracts of the test botanicals were obtained by grinding the washed rhizome and leaves (100 g) in mortal and pestle with equal volume (100 ml) of sterilized distilled water. The macerate obtained was filtered through the folds of muslin cloth and the filtrate obtained formed 100% phytoextracts, which were evaluated by poisoned food method. Twenty ml of poisoned medium was poured into each stetrile petriplates. Five mm diameter mycelial disc selected from periphery of actively growing culture were cut out by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface of medium. of each agar plate. Control were also maintained by growing the pathogen on PDA plates.

The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using above formula.

In vitro evaluation of bio-agents by dual culture method

The lawn culture of test fungi and bio-agents *viz.*, *Trichoderma asperellum* and *Trichoderma reesei* were prepared. Autoclaved, melted potato dextrose agar was poured in petri plates and allowed to solidify for obtaining levelled surface. The plates were inoculated with the culture of test fungi and bio-agents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bio-agents, *Bacillus subtilis* and *Pseudomonas fluorescens* were prepared by inoculating a loopful culture in sterilized conical flask containing 100 ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bio-agent lawn culture was cut with the help of sterilized cork borer lifted and transferred in petri plates, containing autoclaved solidified PDA medium. In each petri plates, four discs of bio-agents were inoculated at four peripheral points of the plates and the test fungi was placed in the center of petri plates. In case of *Pseudomonas fluorescens* and *Bacillus subtilis*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on potato dextrose agar without bio-agents served as control. All these plates were incubated at room temperature for seven days. After an expiry of seven days incubation period the mycelial inhibition was calculated as per formula mentioned in the poisoned food method.

Results and Discussion

In vitro evaluation of fungicides against *Colletotrichum lindemuthianum*

Fungi toxic activities of different fungicides was assayed against *Colletotrichum lindemuthianum* and observed in (Table 1 and Fig. 1) indicated that, Carbendazim+ Mancozeb @ 0.25% and Carboxin+Thiram @ 0.3% were the most effective for arresting 100% mycelial growth followed by Pyraclostrobin (91.48%), Copper oxychloride (88.55%) and Propineb (72.22%). Least mycelial growth inhibition observed in Azoxystrobin (54.44%). Similar results were observed by Chaudhari and Gohel (2016) who reported that Carbendazim+Mancozeb at 1000, 2000, and 2500 ppm conc. inhibited 100% mycelial growth of *Colletotrichum gloeosporioides*. Madhusudan (2002) reported that Carbendazim + Mancozeb at 0.25@ and

0.20@ inhibited mycelial growth by 99.22% and 85.92% respectively against the *Colletotrichum truncatum*. Ingle *et al.*, (2014) observed 93.15% mycelial growth inhibition of *Colletotrichum dematium* in Carbendazim+ Mancozeb @ 0.25%.

Table.1 Following fungicides were evaluated for their efficacy against *Colletotrichum lindemuthianum in vitro*

Sr. No.	Fungicides	Conc. (%)	Mean radial mycelial growth (mm) *	Mycelial inhibition (%)
1.	Carbendazim 12%+ Mancozeb 63% WP	0.25	0.00	100.0
2.	Coper oxychloride 50% WP	0.25	10.33	88.55
3.	Azoxystrobin 23% EC	0.1	41.00	54.44
4.	Pyraclostrobin 20% WG	0.1	7.66	91.48
5.	Carboxin 37.5% + Thiram 37.5% DS	0.3	0.00	100.0
6.	Propineb 70% WP	0.3	25.00	72.22
7.	Control	-	90.00	-
	F' test	-	Sig.	-
	SE(m)±	-	0.64	-
	CD(p=0.01)	-	2.70	-

*Average of three replications

Table.2 Following botaniclas were evaluated for their efficacy against *Colletotrichum lindemuthianum in vitro*

Sr. No.	Botanicals	Conc. (%)	Mean radial Mycelial growth (mm)*	Mycelial inhibition (%)
1.	*Zingiber officinale* (Ginger rhizome)	10.00	31.00	65.55
2.	*Azardirachta indica* (Neem leaves)	10.00	56.66	37.04
3.	*Lawsonia inermis* (Heena leaves)	10.00	10.33	88.55
4.	*Oscimum sanctum* (Tulsi leaves)	10.00	66.33	26.33
5.	*Eucalyptus globules* (Nilgiri leaves)	10.00	61.00	32.22
6.	*Pongamia pinnata* (Karanj leaves)	10.00	39.33	56.00
7.	Control	-	90.00	-
	F' test	-	Sig.	-
	SE(m)±	-	0.84	-
	CD(P=0.01)	-	3.59	-

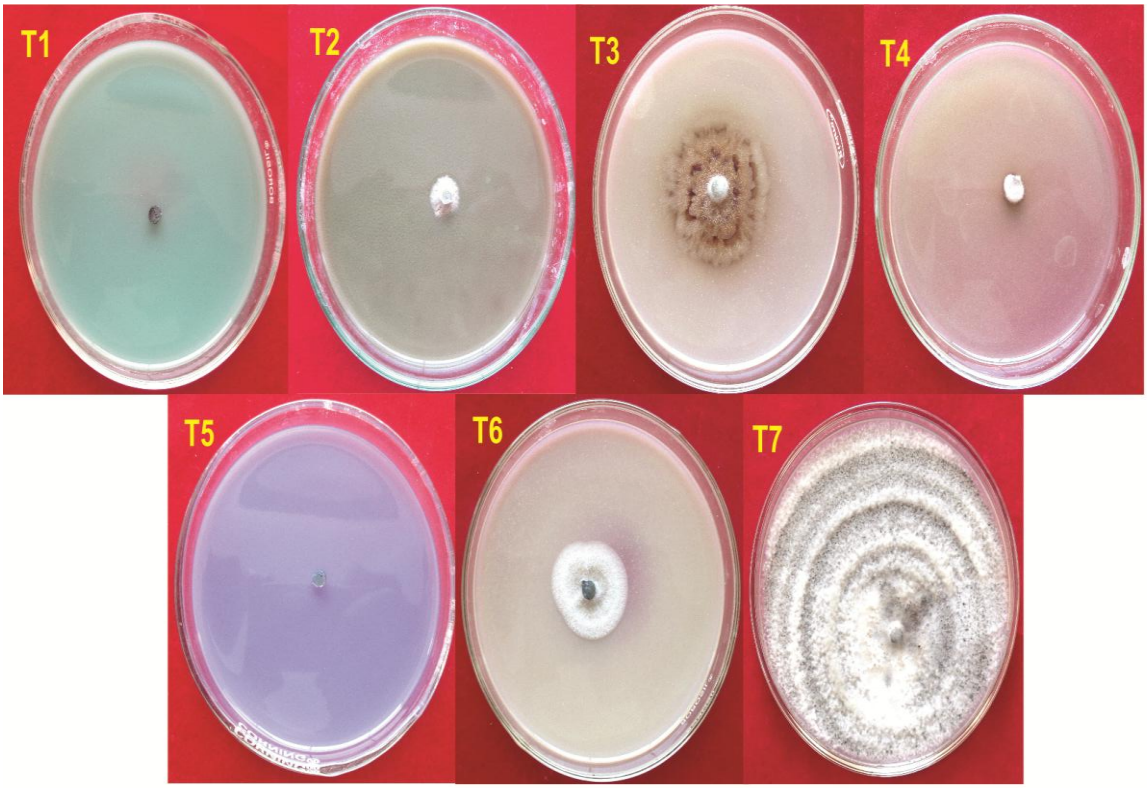
*Average of three replications

Table.3 Following bio-agents were evaluated for their efficacy against *Colletotrichum lindemuthianum in vitro*

Sr. No.	Bio-agents	Mean radial mycelial growth (mm)*	Mycelial inhibition (%)
1.	*Trichoderma asperellum*	46.25	48.61
2.	*Trichoderma reesei*	20.00	77.77
3.	*Bacillus subtilis*	36.50	59.44
4.	*Pseudomonas fluorescens*	39.00	56.77
5.	Control	90.00	-
	F' test	Sig.	-
	SE(m)±	0.55	-
	CD(p=0.01)	2.31	-

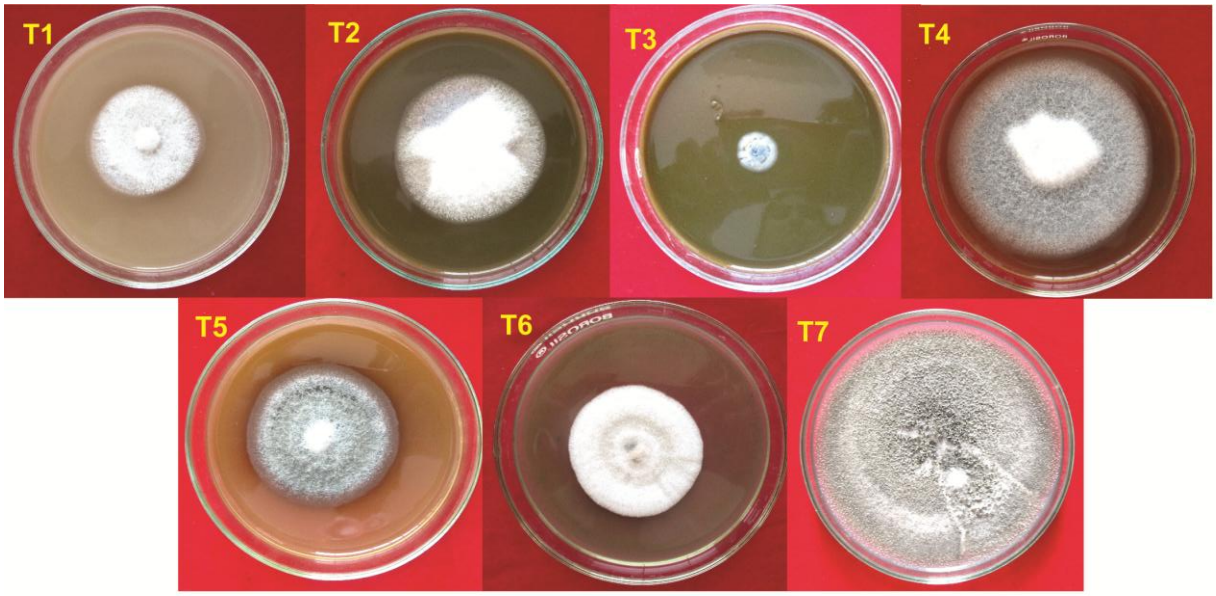
*Average of four replication

Fig.1 *In vitro* efficacy of fungicides against *Colletotrichum lindemuthianum*



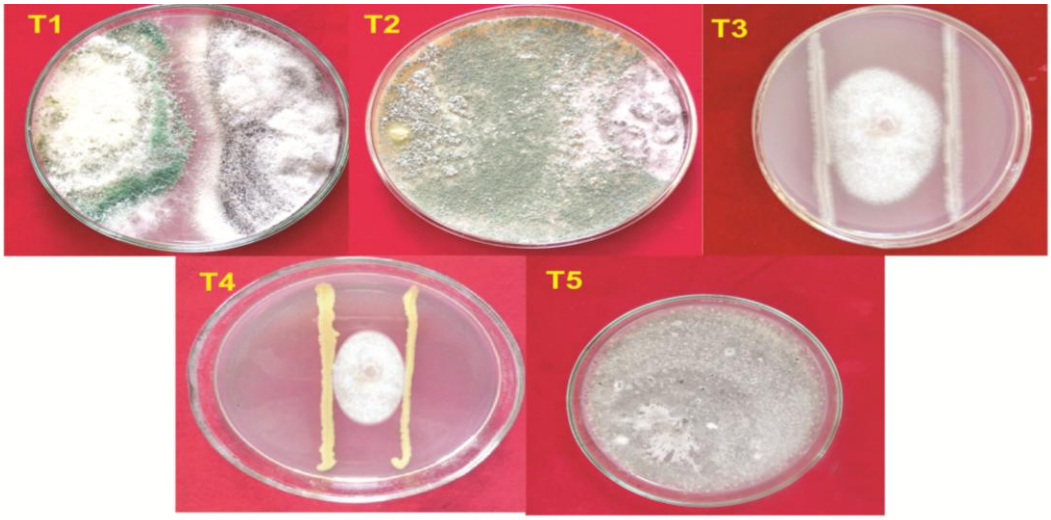
T1- Carbendazim + Mancozeb (0.25%) T2- Copper oxychloride (0.25%)
T3- Azoxystrobin (0.1%) T4- Pyraclostrobin (0.1%)
T5- Carboxin + Thiram (0.3%) T6- Propineb (0.3%)
T7- Control

Fig.2 *In vitro* efficacy of botanicals against *Colletotrichum lindemuthianum*



T1- *Zingiber officinale* T2- *Azardirachta indica* T3- *Lawsonia inermis*
T4- *Oscimum sanctum* T5- *Eucalyptus globules* T6- *Pongamia pinnata*
T7- Control

Fig.3 *In vitro* efficacy of bio-agents against *Colletotrichum lindemuthianum*



T1-*Trichoderma asperellum* T2-*Trichoderma reesei*
T3-*Bacillus subtilis* T4- *Pseudomonas fluorescens*
T5- Control

In vitro evaluation of botanicals against *Colletotrichum lindemuthianum*

In the present investigation of six aqueous extract of botanicals were evaluated under *in vitro* condition against *C. lindemuthianum.* Among the six extract (Table 2 and Fig. 2) showed that highest mycelial growth inhibition was observed in Heena (88.55%) followed by Ginger (65.55%) and Karanj (56.00%). Lowest mycelial growth inhibition was observed in Neem (37.04%) followed by Nilgiri (32.22%) and Tulsi (26.33%). The present results of botanicals are in agreement with Khan and Nasreen (2010) who reported maximum mycelial growth inhibition by *Lawsonia inermis* (81.81%) against *C. lindemuthianum.* Choudhary *et al.,* (2017) also reported mycelial growth inhibition of *C. lindemuthianum* by mehandi (64%). Gawade *et al.,* (2009) and Jagtab *et al.,* (2014) reported, (46.30%) and (40.36%) mycelial growth inhibition by used mehandi against *Colletotrichum truncatum.*

In vitro evaluation of bio-agents against *Colletotrichum lindemuthianum*

In the present investigation (Table 3 and Fig. 3) showed that two fungal and two bacterial bio-agents were tested against *C. lindemuthianum.* The results of dual culture technique on *C. lindemuthianum* reported that maximum growth inhibition was recorded with *Trichoderma reesei* (77.77%) followed by *Bacillus subtilis* (59.44%), *Pseudomonas fluorescens* (56.77%), and *Trichoderma asperellum* (48.61%). The present result in respect of antagonistic activity of bio-agents are in agreement with Fitson *et al.,* (2014) who reported highest inhibition of the mycelial growth by *Trichoderma viride* (80.39%) followed by *Trichoderma harzianum* (75.49%) against *Colletotrichum lindemuthianum.* Rajesh *et al.,* (2010) also reported that *Trichoderma harzianum* was most effective inhibiting the mycelial growth of *Colletotrichum lindemuthianum* to an extent of (73.54%) followed by *Trichoderma viride* (50.90%). The effective results of *T. harzianum* and *T. viride* against *C. dematium* was also recorded by Shovan *et al.,* (2008) and Kothikar and Koche (2017) respectively.

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