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Evaluation of antagonistic potential of bio-agents against anthracnose of French bean *Colletotricum lindemuthianum*

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Abstract
The French bean (*Phaseolus vulgaris*) is one of the most widely grown grain legume crop around the world covering an area of about 28 million hectares with an annual production of 20 million tones (FAO 2016). French bean suffers from many diseases caused by fungi, bacteria, viruses, nematodes and abiotic stresses. Among the fungal diseases anthracnose, are the most prevalent ones. *Colletotrichum lindemuthianum* attack on the bean leaves, causes dark brown necrotic lesions and decrease leaf photosynthesis activity. Yield loss is due to early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed that has lesions on its coat. Such beans have a repulsive appearance and are not preferred by consumers. The disease is characterized by serious leaf spotting ultimately resulting in 'shot hole' symptoms and finally defoliation which affects the yield greatly. In the present investigation five bio-agents *viz., Trichoderma viridae, Trichoderma harzianum, Chaetomium globosum, Pseudomonas fluorescence* and *Bacillius subtilis* were evaluated to see the efficacy against *C. lindemuthianum* through dual culture technique under *in-vitro* conditions. The present inhibition of mycelial growth of pathogen by bio-agents was recorded after 48, 96, and 168 hrs. At 96 hours of inoculation, maximum % inhibition of *C. lindemuthianum* was recorded by *Trichoderma viridae* (49.25%) which were significantly superior from all the tested bio-agents, followed by *P. fluorescence* (46.95%) while at 168 hours of inoculation, maximum % inhibition of *C. lindemuthianum* was recorded by *Chaetomium globosum* (59.50%) followed by *P. fluorescence* (58.14%) and *Trichoderma viridae* (57.04%).

Keywords: Evaluation, antagonistic, potential, French, *Colletotricum lindemuthianum*

1. Introduction
The French bean (*Phaseolus vulgaris*) is one of the most widely grown grain legume crop around the world covering an area of about 28 million hectares with an annual production of 20 million tones (FAO 2016). Asia is the largest continent producing common beans and exporting to other countries in the world. About 46% of common beans are produced in Asia. In India, it is grown in all most all part of India like as Himachal Pradesh, Uttarakhand, Jammu and Kashmir, Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. In India, it is cultivated both as dry and snap bean in an area of about 0.15 million hectares with an annual production of approximately 0.42 million tones (FAO 2016). In Uttar Pradesh it is grown in an area 9.8 mha and total production of bean 147.38 mt (NHB 2016). It is a nutritive vegetable, rich in protein (1.70 mg), calcium (1.32 mg), thiamine (0.08 mg) and vitamin C (2.4 mg) per 100 g of edible pods. Dry leaves, threshed pods and stalks are nutritious feed to the animals. It has anti-diabetic property and is good for natural cure of bladder burns, cardiac problems and diarrhea.

French bean suffers from many diseases caused by fungi, bacteria, viruses, nematodes and abiotic stresses. Among the fungal diseases, powdery mildew, anthracnose, *Cercospora* leaf spot, web blight and dry root rot are the most prevalent ones. In recent years, Green gram anthracnose caused by *Colletotrichum lindemuthianum.* Andrus and Moore has been reported that Green gram anthracnose (*Colletotrichum lindemuthianum)* become one of the major diseases which is known to occur in many countries *viz.,* India, Nigeria, Thailand, Philippines,

Upper Volta, Zambia, Palmira, Columbia, *etc.* (Agarwal, 1991) [3]. It occurs in all the parts of the world, wherever French bean is cultivated.

In India, the French bean anthracnose was first reported from Jorhat of Assam state in 1951 (Majid, 1953) [1]. The disease has been reported from all major French bean growing regions of India in mild to severe form and in tropical and subtropical areas it causes considerable damage by reducing seed quality and yield (Sharma *et al.*, 1971) [2].

The disease causes huge losses in temperate and subtropical zones. Plant at all growth stage is susceptible and susceptibility increase age in infection of a susceptible cultivar under favorable condition leading to an epidemic may result in 100% yield loss (Fernandez *et al.*, 2000). It produces symptoms as circular radish brown, sunken spots with dark centre and bright red orange margins on leaves and pods. The disease also produces cankers on petioles and on stems that cause severe defoliation and rotting of fruits and roots. Infected fruit has small, water soaked, sunken circular spots that may increase in size up to 1.2 cm in diameter.

Anthracnose, *Colletotrichum lindemuthianum* (Sacc. and Magn.). It is the most dangerous disease in common bean. Field losses in these regions, due to seedling, leaf, stem and pod infections, are up to 90% under favourable climatic condition. *Colletotrichum lindemuthianum* attack on the bean leaves, causes dark brown necrotic lesions and decrease leaf photosynthesis activity. Yield loss is due to early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed that has lesions on its coat. Such beans have a repulsive appearance and are not preferred by consumers.The disease is characterized by serious leaf spotting ultimately resulting in 'shot hole' symptoms and finally defoliation which affects the yield greatly. Infection of pods directly damages the seeds and reduces its germinability. Pod infection may result in complete loss in yield. The pathogen survives on seed and plant debris in soil. Disease spreads in the fields through air borne conidia. The disease is more severe in cool and wet regions.

Since the synthetic fungicides are being widely used by the farmers to eradicate pathogens but it results in environmental hazards and have harmful effects on human beings and animals. The chemical fungicides not only develop fungicidal resistant strains but also accumulate in food and ground water as residues. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention toward the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly bio-degradable, one such strategy is use of bio-control agents (BCAs) to control fungal plant diseases. Therefore, keeping in view the importance of diseases and the role of bio-control to overcome them, based on the consideration highlighted present investigation.

2. Materials and Methods

2.1 Effect of bio-agents on mycelial growth of *C. lindemuthianum*

The bio-control agent, were taken from bio control laboratory, Department of Plant Pathology, Chandra Shekhar Azad University of agriculture and Technology, Kanpur (Uttar Pradesh) and evaluated for their antagonistic effect under *in-vitro* conditions against *C. lindemuthianum* by dual culture technique.

2.2 Dual culture technique

The antagonistic activity of five antagonistic bio agents to tested efficacy of inhibit the growth of the pathogen to a maximum extent. Effect on the growth of *Colletotrichum lindemuthianum* studied using dual culture technique. In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petri plates and allowed to solidify. Fungal antagonists were evaluated by inoculating the pathogen at one side of Petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used. In case of evaluation of bacterial antagonist, two mycelia (discs of pathogen) were inoculated and bacterial antagonist was streaked in the centre of the plate. Each treatment was replicated three times. After required period of incubation i. e. after control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control as worked out according to formula given by (Vincent 1947).

$$I = \frac{C-T}{C} X100$$

Where,
I = Percent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Table 1: List of bio agents used in *in vitro* evaluation against *C. lindemuthianum*

S. No.	Treatments	Bio agents
1	T_1	*Trichoderma harzianumm*
2	T_2	*Trichoderma viride*
3	T_3	*Chaetomium globosum*
4	T_4	*Bacillus subtilis*
5	T_5	*Pseudomonas fluorescencs*
6	T6	Control

3. Results and Discussion

3.1 Effect of bio control agents on mycelial growth of pathogen

Five bio agents were evaluated for their efficacy against *C. lindemuthianum* through dual culture technique as explained in 'Materials and Methods'. The studies on inhibitory effect of *Trichoderma viridae, Trichoderma harzianum, Chaetomium globosum* and bacterial antagonist *Pseudomonas fluorescence* and *Bacillius subtilis* against pathogen *Colletotrichum lindemuthianum* by using dual culture technique on PDA medium showed significant differences in reduction growth of the pathogen under *in vitro* conditions. The inhibition of mycelial growth of pathogen by bio agents was recorded after 48, 96, and 168 hrs.

Table 2: *In-vitro* evaluation of effect bio agents on radial growth of *C. lindemuthianum*

Treatments	Bioagents	Inhibition after 48 hrs		Inhibition after 96 hrs		Inhibition after 168 hrs	
		Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition
T_1	*T. viridae*	6.17	34.70	22.33	49.25	38.66	57.04
T_2	*T. harzianum*	5.18	45.18	31.19	29.11	44.46	50.6
T_3	*C. globosum*	6.23	34.07	25	43.18	36.45	59.5
T_4	*P. fluorescence*	4.09	56.71	23.34	46.95	37.67	58.14

T_5	*B. subtilis*	5.65	40.21	29.67	32.56	43.33	51.85
T_6	Control	9.45		44.00		90	
CD @ 5%		1.555		0.308		0.696	
S.Em ±		0.487		0.097		0.218	

The data presented in Table-2 revealed that there was significant mycelial growth % inhibition of *C. lindemuthianum* by all the tested bio agents. At 96 hours of inoculation, maximum % inhibition of *C. lindemuthianum* was recorded by *Trichoderma viridae* (49.25%) which were significantly superior from all the tested bio-agents, followed by *P. fluorescence* (46.95%). Whereas, in case of *Chaetomium globosum* (43.18%) inhibition was observed followed by *B. subtilis* (32.56%) and *Trichoderma harzianum* (29.11%) at 96 hours of inoculation. Whereas in case of control plate 44 mm radial growth was observed after 96 hours of inoculation.

At 168 hours of inoculation, maximum % inhibition of *C. lindemuthianum* was recorded by *Chaetomium globosum* (59.50%) which were significantly superior from all the tested bio agents, followed by *P. fluorescence* (58.14%) and *Trichoderma viridae* (57.04%) at 168 hrs caused the % inhibition of the pathogen. Whereas, in case of *Trichoderma harzianum* (50.60%) and *B. subtilis* (51.85%) % inhibition was observed on PDA media by dual culture method at 168 hours of inoculation. Whereas in case of control plate 90 mm radial growth was observed after 168 hours of inoculation. Reported that *Gliocladium virens*, *T. harzianum* and *T. viride* significantly inhibited growth of *C. lindemuthianum in-vitro*. The present investigations are in agreement with Gupta *et al*. (2005) [4], who found effectiveness of *Trichoderma* spp. against *Colletotrichum lindemuthianum*, where, as Laxman (2006) [5] against *C. truncatum*. This could be obviously due to several possibilities of existence of microbial interactions such as stimulation, inhibition, mutual intermingling of growth of antagonistic isolate over test pathogen *etc.* have been enumerated by many workers.

4. Conclusion

Based on the results of the present investigation, it can be concluded that *Chaetomium globosum* was found most effective against of *C. lindemuthianum* which suppress the growth of the pathogen.

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