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**N Pandey**
Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, Pauri Garhwal Uttarakhand, India

**S Ravi**
Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, Pauri Garhwal Uttarakhand, India

**Vivekanand**
School of Agricultural Science Career Point University Alaniya, Kota, Rajasthan, India

**M Gusain**
Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, Pauri Garhwal Uttarakhand, India

**Correspondence**
**N Pandey**
Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, Pauri Garhwal Uttarakhand, India

# *In vitro* efficacy of biocontrol agents and fungicides against anthracnose of french bean caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.)

**N Pandey, S Ravi, Vivekanand and M Gusain**

**Abstract**
Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) is an important seed borne disease throughout the world. This pathogen overwinters on bean seed hence the losses can be 100%. Three fungicides alone namely Hexaconazole, Captan, Carbendazim and three in combinations i.e. Hexaconazole + *Bacillus ceresus*, Captan + *Trichoderma harzianum* and Carbendazim + *Pseudomonas fluorescens* were used at different concentrations (100, 250, 500 and 750 ppm) by poisoned food technique. Alone Carbendazim and in combination, Carbendazim + *P. fluorescens* found complete inhibition of *C. lindemuthianum* at 100 ppm, while Captan at all concentration (52.48, 62.27, 68.05 and 69.75%) and in combination Hexaconazole + *B. ceresus* (53.30, 63.98, 80.89 and 81.77%) were found minimum inhibition. Three bio control agents viz. *B. ceresus*, *T. harzianum* and *P. fluorescens* were evaluated by dual culture technique, to check their antagonistic effect on the growth of *C. lindemuthianum*. Among bio agents *T. harzianum* observed (73.87%) maximum mycelium growth inhibition while *B. ceresus* minimum (31.33%).

**Keywords:** French bean, anthracnose, *Colletotrichum lindemuthianum*, fungicides and bioagents

## Introduction
French bean (*Phaseolus vulgaris* L.) is a leguminous vegetable belongs to the family Fabaceae. It is commonly known as Rajmash, haricot bean or common bean. French bean is considered to have originated in Mexico Maibam *et al.* (2015) [17]. Common bean (*Phaseolus vulgaris* L.) is a key grain legume crop and a vital source of nutrition worldwide. The FAO reports that half of the world's common bean production occurs in low income, food deficit countries where this staple crop contributes to food security. The other half is produced in countries like the U.S., where common bean is an important economic crop with 769 thousand ha of dry and snap beans planted in 2012, and with a farm gate value of $1.5 billion (Anonymous, 2013) [3]. Yesuf (2005) [25] emphasized that diseases are known to be the major factors which threaten the productivity of common beans in general and common bean in particular. Anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), angular leaf spot (*Phaeoisariopsis griseola*) and common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) are common diseases of bean in Ethiopia Habtu *et al.* (1996) [12]. The fungus *Colletotrichum lindemuthianum* is often present in or on the seed of the infected pods. Infected pods may show yellowish to brown sunken lesion (Agrios, 2005) [2]. Morphology of *C. lindemuthianum* causing anthracnose of bean and reported that, conidia were hyaline, single celled, dumbbell shaped born in acervuli bearing setae observed by Junaid *et al.* (2014) [13].
Plants at all stage are susceptible and susceptibility increase with age of the plant. Infection of a susceptible cultivar under favourable condition may results in 100% yield loss (Fernandez *et al.*, 2000) [9]. Heavy and frequent rains with moderate temperature (19-25ºC) and high humidity (<70%) favours the progress of anthracnose disease (Aggarwal *et al.*, 2017) [1]. In fungicides Carbendazim and *Trichoderma* sp. in biocontrol agents were reported effective to reduce disease (Rajesha *et al*., 2010) [21]. Ten fungicides at 0.05%, 0.1% and 0.15% against *C. gloeosporiodes* causes anthracnose of papaya and reported that Carbendazim inhibited (100%) per cent mycelium growth followed by Hexaconazole (93.50%) and Captan (84.09%) observed by Tasiwal *et al.* (2008) [22].

Keeping the above facts, the present investigation was carried out, some fungicides and bio agents against anthracnose of french bean caused by *Colletotrichum lindemuthianum.*

**Materials and Methods**

The present investigation was conducted in the laboratory of the Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, Pauri Garhwal, Uttarakhand during 2017-18.

**Collection and isolation of the pathogen:** The infected pods of bean showing typical symptoms of anthracnose were collected from Vegetable Research and Demonstration Block, Bharsar. Cut into small pieces of (1-2 mm) size along with some healthy portion. These are surface sterilized with 70% ethanol and 0.1% mercuric chloride ($HgCl_2$), for about 30 seconds followed by three washings in sterilized distilled water. Then the segments were blotted on the blotter papers and transferred into Petri plates containing 20 ml of molten and cooled potato dextrose agar (PDA) and incubated at 28±1 $^0$C for 7 days. The incubated plates were observed periodically for fungal growth (Baudoin, 2011) [5]. Isolated pathogen transferred into sterilized Petri plates containing PDA medium and incubated at 24± 1$^0$C for 7 days Junaid *et al.* (2014) [13].

**Purification, preservation and identification of pathogen:** Pathogenic isolates from host plant species were isolated from the moist chambers as well as on PDA Petri plates. All the isolates were cultured under aseptic conditions in a laminar air flow and incubated at 28±1 ºC for 7 days. Culture purified from single colony appearing on PDA after observing under microscope and then maintained on PDA slants at 4 ºC in a refrigerator for further use, sub culturing of the stored cultures was done periodically. The study was undertaken to confirm the identity of the isolated pathogen. Fungal growth, examined through the compound microscope, after making slides in lactophenol cotton blue stain. The shape and size of acervuli, conidia/spore; conidia hyaline, dumbbell, with obtuse ends and acervuli black in colour and saucer shaped. Microscopic observation was taken at (40 x) Junaid *et al.* (2014) [13].

***In vitro* study**

The evaluation of fungicides and bio-agents alone and in combination against *C. lindemuthianum* were tested by, poisoned food technique (Nene and Thapliyal, 1993) [18]. Three fungicides alone namely Hexaconazole, Captan, Carbendazim and three in combinations i.e. Hexaconazole + *Bacillus ceresus*, Captan + *Trichoderma harzianum* and Carbendazim + *Pseudomonas fluorescens* including control were used at different concentrations (100, 250, 500 and 750 ppm) with three replication and Petri plates were incubated at 28±1 ºC for 7 days.

Bio-agents by dual culture technique (Faheem *et al.,* 2010) [8] with five replications were maintained and incubated at 28±1 ºC, observed after 7 days. Three bio agents including control, namely *Bacillus ceresus*, *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated to check their antagonistic effect against *C. lindemuthianum*.

Per cent mycelium inhibition in each treatment over control was calculated with the following formula given by Vincent (1947) [23].

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

The data had obtained and analyzed by using simple complete randomized design (CRD) with the help of OPSTAT and STPR3.

**Results and Discussion**

Three fungicides and three bio-control agents alone and in combination with control were evaluated (Table -1). In alone Carbendazim (100%) inhibition was found highly effective at all concentrations (100, 250, 500 and 750 ppm) followed by Hexaconazole showed the disease inhibition of (75.02, 77.29, 82.55 and 84.98%) respectively at all concentration. Captan at all concentration showed mycelia inhibition of (52.48, 62.27, 68.05 and 69.75%). Deshmukh *et al.* (2010) [7] also found that Carbendazim was found highly effective with maximum inhibition (100%) against anthracnose of bean. Patil *et al*. (2009)[20] tested among chemicals, Mancozeb + Carbendazim (0.2%) was found most effective in inhibiting 96.26 per cent growth of *C. gloeosporioides* followed by Carbendazim (0.1%) 68.34 per cent, Mancozeb and Copper oxychloride. Rajesha *et al*. (2010) [21] found among different systemic fungicides tested, Carbendazim inhibited cent per cent (100%) mycelial growth of *C. lindemuthianum* followed by propiconazole (100%) and difenoconazole (84.87%) at a concentration of 400 ppm. Gawade and Suryawanshi (2009)[11] evaluated five fungicides *viz.*, Carbendazim, Chlorothalonil, Difenoconazole, Hexaconazole and Propiconazole (each @ 100, 150 and 250 ppm.) against *C. truncatum* causing soybean anthracnose and reported that Carbendazim as a most effective with maximum mycelium inhibition (90.59%), followed by Propiconazole, Hexaconazole, Difenoconazole and Chlorothalonil. Madhusudan (2002) [15] observed ten fungicides against *C. truncatum*, causing anthracnose of soybean. They reported that, Benomyl and Carbendazim inhibited per cent mycelium growth of the fungus at all the three concentrations tested (0.025, 0.05 and 0.1%), followed by the Combi fungicides Carbendazim 12% + Mancozeb 63% (SAAF 75 WP) with mycelial growth inhibition of (99.22%) and (85.92%) at 0.25 and 0.2% concentration, respectively.

In combinations Carbendazim + *Pseudomonas fluorescens* was found most effective with complete inhibition at 100 ppm. Followed by Captan + *Trichoderma harzianum* and Hexaconazole + *Bacillus ceresus*. In other way control was observed least effective with (0.00%) inhibition.Vivekanand *et al.* (2018)[24] also observed that combi, *Pseudomonas fluorescens* + Carbendazim were tested against chilli anthracnose caused by *C. capsici* at 50, 100, 200, 400 and 600 ppm, they have found mycelia inhibition i.e.(60.86, 66.20, 69.59, 71.17 and76.57%). Basamma and Kulkarni (2017) [4]; Khan and Shahzad (2007) [14] also found the compatibility between these treatments combination.

**Table 1:** Effect of fungicides and bioagents at different concentration on per cent mycelia growth inhibition of *C. lindemuthianum*

| Treatments | Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 ppm | | 250ppm | | 500ppm | | 750ppm | |
| | G | I | G | I | G | I | G | I |
| Control | 38.10 | 0.00 | 38.10 | 0.00 | 38.10 | 0.00 | 38.10 | 0.00 |
| Hexaconazole | 9.52 | 75.02 | 8.63 | 77.29 | 6.66 | 82.55 | 5.70 | 84.98 |
| Captan | 18.09 | 52.48 | 14.33 | 62.27 | 12.18 | 68.05 | 11.50 | 69.75 |
| Carbendazim | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 |
| Hexaconazole + *Bacillus ceresus* | 17.77 | 53.30 | 13.66 | 63.98 | 7.26 | 80.89 | 6.92 | 81.77 |
| Captan + *Trichoderma harzianum* | 11.01 | 70.58 | 8.70 | 77.22 | 7.83 | 79.39 | 6.48 | 82.92 |
| Carbendazim +*Pseudomonas fluorescens* | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 |
| CD $_{(p=0.05)}$ | 1.52 | - | 2.38 | - | 1.63 | - | 1.47 | - |

G=Average mycelia growth in (mm); I= Average mycelia growth inhibition in (%).

Antagonistic effect of three bio-agents with control were observed for mycelia growth inhibition of *C. lindemuthianum* (Table-2). *Trichoderma harzianum* (73.87%) was found highly effective against test fungus, followed by *Pseudomonas fluorescens* (52.93%) and minimum in *Bacillus ceresus* (31.33%) mycelia growth inhibition. In other way control was observed least effective with (0.00%) inhibition. Padder *et al.* (2010) [19] and Rajesha *et al.* (2010) [21] also reported the similar result against *C. lindemuthianum.* Fitsum *et al.* (2014) [10] have found the highest percentage of inhibition of the mycelia germination against C. *lindemuthianum*, *T.viride* was obtained (80.39%) followed by *T. harzianum* and *P. fluorescens*. Begum *et al.* (2015) [6] among bioagents *T. harzianum* -2 isolate found most effective (77.78%) inhibition of mycelial growth and minimum in *P. fluorescens* was least effective against *Colletotrichum capsici.* (Mahadev, 2018) [16] also found that *Trichoderma harzianum* was effective against bean anthracnose. Vivekanand *et al.* (2018) [24] also reported that among bioagents *T. harzianum* was found effective and minimum in *P. fluorescens* against chilli anthracnose, *C. capsici.*

**Table 2:** Effect of biocontrol agents against *C. lindemuthianum*

| Treatments | Mycelia growth in (mm) | Mycelia growth inhibition in (%) |
|---|---|---|
| Control | 38.10 | 0.00 |
| *Bacillus ceresus* | 26.09 | 31.33 |
| *Trichoderma harzianum* | 9.92 | 73.87 |
| *Pseudomonas fluorescens* | 17.89 | 52.93 |
| CD $_{(p=0.05)}$ | 1.77 | - |

## Conclusion

Three fungicides alone and three in combinations were used at different concentrations. It has found that alone Carbendazim and in combination (Carbendazim + *P. fluorescens*) complete inhibition of *C. lindemuthianum* at 100 ppm, while Captan and (Hexaconazole + *B. ceresus*) had observed minimum growth inhibition. Three bio control agents among these *T. harzianum* found maximum mycelium growth inhibition while minimum in *B. ceresus.*

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