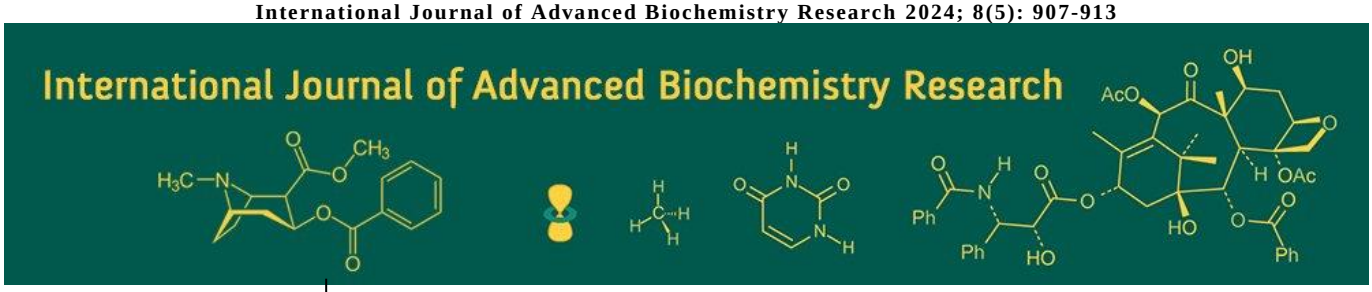

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(5): 907-913
www.biochemjournal.com
Received: 03-02-2024
Accepted: 10-04-2024

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Morphological characterisation of *Colletotrichum lindemuthianum* and *In vitro* efficacy of Bioagents: Insights from dual culture method

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DOI: https://doi.org/10.33545/26174693.2024.v8.i5k.1217

Abstract
The current study dealing with morphological characterization of *Colletotrichum* lindemuthianum and *in vitro* efficacy of various bioagents against the said pathogen by adopting the standard procedure of dual culture method was carried out. Morphological characterization revealed that the mycelium is hyaline to pale brown, branched with intercellular and intracellular septations. The conidia measured 9.87-11.30 × 3.43-4.19 µm (av. 9.98 × 4.11 µm) on host tissue and 11.76-21.44 × 3.88-5.94 µm (av. 16.73 × 4.32 µm) on potato dextrose agar media. The shape of conidia recorded was cylindrical with obtuse ends. Shape of acervulus observed under microscope was saucer like with dimensions ranging from 137-315 µm (av. 198.25 µm). The colour of conidia, acervuli and setae recorded was hyaline, black and brown to dark brown, respectively. No septation was observed in conidia or acervuli but setae had 0-3 septum. Among bio-agents tested *in vitro Trichoderma viride* proved to be the best with mean mycelial growth inhibition percentage of 69.44, followed by *Trichoderma harzianum* (63.88%), *Psuedomonas flourescens* (46.60%) and *Bacillus subtilis* (54.01%).

Keywords: Conidia, inhibition percentage, mycelium, pathogen, septation

Introduction
Common bean (*Phaseolus vulgaris* L.) is called by different names like French and kidney bean. Based upon its form of consumption it is called snap bean (green form) or pulse (dried form). Common bean (*Phaseolus vulgaris* L.) is a pulse crop grown for dry seeds as well as tender pods and taxonomically finds its position in the family Fabaceae (Romero-Arenas *et al*., 2013) [16]. Anthracnose of common bean was first reported in India in Nilgiri Hills in 1915 by Hutchinson and Ayer. Common bean is taken as a *Kharif* crop in general, so the humid and cool environmental conditions predispose the pathogenic attack (Tu, 1998; Kumar *et al*., 1999) [17, 7]. Infection can occur at any phenological stage provided the interaction (disease) quadrangle is satisfied. Drastic yield reduction was reported in anthracnose susceptible cultivars ranging from 38 to 100 percent under varying situations (Mukunya and Keya, 1979; Shao and Teri, 1985; Makini and Danial, 1995; Sartorato *et al*., 1998) [10, 18, 8, 17]. The impact of disease on yield loss is more under temperate conditions followed by subtropical climatic conditions. Symptoms of rajmash anthracnose can appear on any plant part although initial symptoms may appear on cotyledonary leaves as small, dark brown to black lesions. The infected tissues manifest minute rust coloured specks, which gradually enlarge longitudinally and form sunken lesions or eye spots that reach the hypocotyl of the young seedling, causing it to rot off (Pastor-Corrales and Tu, 1989) [13]. Common bean anthracnose is primarily a seed borne disease (Tu, 1983; Kumar *et al*., 1999) [20, 7]. *Colletotrichum lindemuthianum* survives inside the seed coat as dormant mycelium and can endure the temperature of -15 to -20 C for a shorter span of time (Mordue, 1971) [9].
Taking into account the prevalence of the major disease of common beans (Anthracnose) that limits the yield both quantitatively and qualitatively, present investigation was undertaken to study the morphological characters of *Colletotrichum lindemuthianum* and evaluate the efficacy of various bio-agents against it under *in vitro* conditions.

Materials and Methods

Isolation of the pathogen

After collecting infected samples from the field, isolation of pathogen was done by tissue bit transfer method (Sicard *et al*., 1997) [19]. Infected samples of leaf and pod were cut in to minute pieces followed by surface sterilization with 0.1% mercuric chloride for a time period of 30 seconds (Jhonston and Booth, 1983) [4]. After surface sterilization, tissue bits were washed three times in the sterile distilled water serially. To remove the moisture, tissue bits were placed on sterlised filter paper under laminar-air-flow cabinet. Dry sterilized tissue bits were transferred in Petri plates and test tubes containing solidified cold potato dextrose agar under aseptic conditions i.e, under laminar-air-flow cabinet. Inoculated plates and slants were kept in incubator to maintain temperature at around 25 ºC for the promotion of test fungus growth. Purification of fungal culture was done by employing single spore method (Jhonston and Booth, 1983) [4]. The pure culture of test fungus was maintained for subsequent use by sub-culturing at 30 days interval. Storage of culture was done in PDA slants stored at 4-5 ºC.

Pathogenicity test

Test for pathogenicity of isolated fungal pathogen was done on common bean cultivar KDB-17 which is susceptible to anthracnose. The plants of said cultivar were raised from healthy and infection free seeds in plastic pots containing inoculum free soil, sand and farmyard manure in proportion of 2:1:1 by weight. Inoculum free growing media for plants was made possible by autoclaving it. Fresh culture of test fungus was prepared on PDA in Petri plates mixed with 10 ml distilled water. Conidia production occurred while mixing culture mass of said fungal pathogen in water. The resulted suspension was subjected to filtration through cheese cloth having four fold thicknesses. The concentration of conidia in the suspension was adjusted to about 1×10^6 ml^{-1} by making use of hemocytometer. Inoculation with the spore suspension was done to foliage of 20 days old plant in the evening time with atomizer. Un-inoculated plants were also given the foliar spray of sterilized distilled water to mask the effect of humidity difference which arises due to foliar spray of spore suspension in inoculated plants. Un-inoculated plants served as control and both inoculated and un-inoculated plants were kept under humid chamber (glass house) for two days. The observations were recorded periodically concerning the disease development. After observation of diagnostic symptoms and confirmations from microscopic examinations, the pathogenicity of test fungus was confirmed as it qualified the postulates of Robert Koch.

Morphological characterization

Semi-permanent slides were made from the 10 days old culture of the isolated fungus. The slide was made under aseptic conditions. Cotton blue and lactophenol were used to stain the fungus. Slide was examined under different resolutions of microscope and the following morphological characters were recorded at 10x and 40x resolution:

Colony: colour, shape and mycelial growth
Mycelium: colour, breadth, septation and branching
Acervulus: colour, shape and size
Conidia: colour, shape, size and septation

In vitro evaluation of Bio-agents

For evaluating the bio efficacy of different bio-control agents against *Colletotrichum lindemuthianum* dual culture technique (Dennis and Webster, 1971) [2] was adopted as a procedure. Following bio-control agents were evaluated: *Trichoderma viride*, *Trichoderma harzianum*, *Psuedomonas flourescens* and *Bacillus subtilis*. Discs of 5 mm diameter from pathogen as well as fungal bio-agent were inoculated at the opposite margins (approximately 10mm from wall of plate) of PDA media contained in Petri plates (90 mm) under laminar air flow cabinet. Bacterial bio-agents were streaked on opposite side of the pathogen. Control plates were also run which contained pathogen only. Now comparison was made for the mycelial growth of *Colletotrichum lindemuthianum* as well as colony diameter of bio-control agent up to zone of inhibition. The experimental design followed for this study was completely randomized design with four replications for every treatment. Inhibition of mycelial growth of pathogen was calculated using Vincent's (1927) formula as given below:

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

Where
I= percent inhibition;
C= colony diameter in control plate;
T= colony diameter in treated.

Results and Discussion

Experimental findings of the investigation conducted at FoA SKUAST-K Wadura Sopore are presented under various headings:

Isolation of the pathogen

The incitant of anthracnose of common bean i.e., *Colletotrichum lindemuthianum* was isolated on potatao dextrose agar from the disease specimen following the standard phytopathological techniques. The test fungus formed greyish colony during juvenile phase and dark black colony with compact mycelial growth at maturity. The 90 mm Petriplates were fully occupied by the test fungus within 8 to 12 days of incubation period.

Purification and maintenance of the pathogen

Purification of fungal culture was done by employing single spore method. The pure culture of test fungus was maintained for subsequent use by sub-culturing at 30 days interval. Storage of culture was done in PDA slants kept in refrigerator having temperature of 4-5 ºC (plate 1).

Pathogenicity test

Test for pathogenicity of isolated fungal pathogen was done by inoculating it on healthy Common bean potted plants kept under controlled conditions. Post inoculation symptoms were recorded and these were found to be the replica of symptoms of Anthracnose of common bean. The characteristic anthracnose symptoms were recorded after 7-8 days of inoculation (plate 2). The fungus isolated from the symptoms of potted plants was identical to that of fungus isolated from diseased specimen collected from the field hence confirmation of pathogenicity of test fungus was observed by following Koch's postulates.

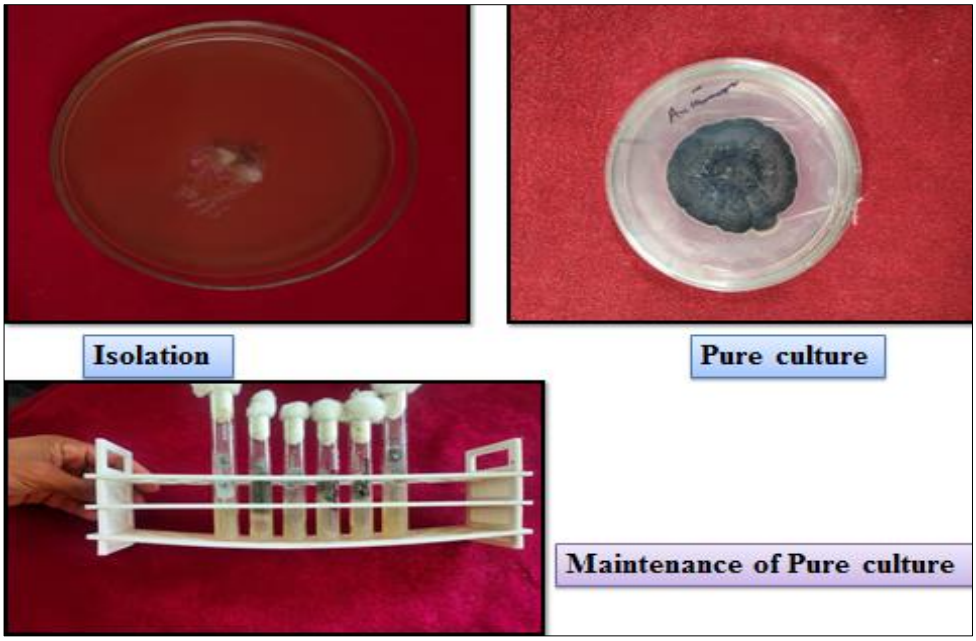

Plate 1: Isolation, Purification and Maintenance of the Pathogen

Plate 2: Pathogenicity test of *Colletotrichum lindemuthianum* on bean plant

Morphological characterization

The observations concerning morpho-cultural characteristics of said fungus were recorded from the culture growth on potato dextrose agar for 11-12 days which was incubated at 25 ± 1 ºC. Observations concerning morphology of structures like mycelium, acervulus, setae and conidia were recorded and are presented in Plate-3 and Table-1.

The pure culture of test pathogen was examined vigilantly for noting the colony characteristics and growth pattern. During the initial phase of incubation (3-4 days) of pure culture of test pathogen, the colony colour was greyish, loose and fluffy in appearance but with the maturity of the culture of test pathogen, the colony turned dark black compact mycelail growth. Acervulus began to develop within 8-10 days of incubation. Entire 90 mm Petri plate was occupied by the fungus in 10-12 days of incubation.

Mycelium: Microscopic examination of isolated pathogen unveiled that the fungus contains hyaline to pale brown mycelium measuring 11.4-13.12 µm with inter and intracellular septations.

1. **Acervulus:** Shape of acervulus observed under microscope was saucer like with dimensions ranging from 137-315 µm (av. 198.25 µm) in culture media and 177-281 µm (266 µm) on host surface. Colour of acervulus recorded was black and septation was absent.
2. **Setae:** The size of pointed appendages associated recorded in culture media was 61-104 × 3-4 µm (av.79.54 × 3 µm). The colour of setae ranged from brown to dark brown and cylindrical in shape with pointed ends having 0-3 septa.
3. **Conidia:** Size of conidia varied significantly on host tissue and culture media. Conidia are hyaline and aseptate. The conidia measured 9.87-11.30 × 3.43-4.19 µm (av. 9.98 × 4.11 µm) on host tissue and 11.76-21.44 × 3.88-5.94 µm (av. 16.73 × 4.32 µm) on potato dextrose agar media, respectively. The shape of conidia recorded was cylindrical with obtuse ends.

Table 1: Morphological characteristics of *Colletotrichum lindemuthianum* causing anthracnose of Common bean

Fungal structure		Shape	Colour	Size (µm)	Septation
Mycelium on PDA		Branched	Hyaline to pale brown	11.4-13.12 µm (width)	Septate
Acervulus	On host	Saucer like	Black	177-281 µm (266 µm)	Aseptate
	In culture (PDA)	-do-	-do-	137-315 µm (av. 198.25 µm)	-do-
Setae		Cylindrical with pointed ends	Brown to dark brown	61-104 × 3-4 µm (av.79.54 × 3 µm)	0-3 septum
Conidia	On host	cylindrical with obtuse ends	Hyaline	9.87-11.30 × 3.43-4.19 µm (av. 9.98 × 4.11 µm)	Aseptate
	In culture (PDA)	-do-	-do-	11.76-21.44 × 3.88-5.94 µm (av. 16.73 × 4.32 µm)	-do-

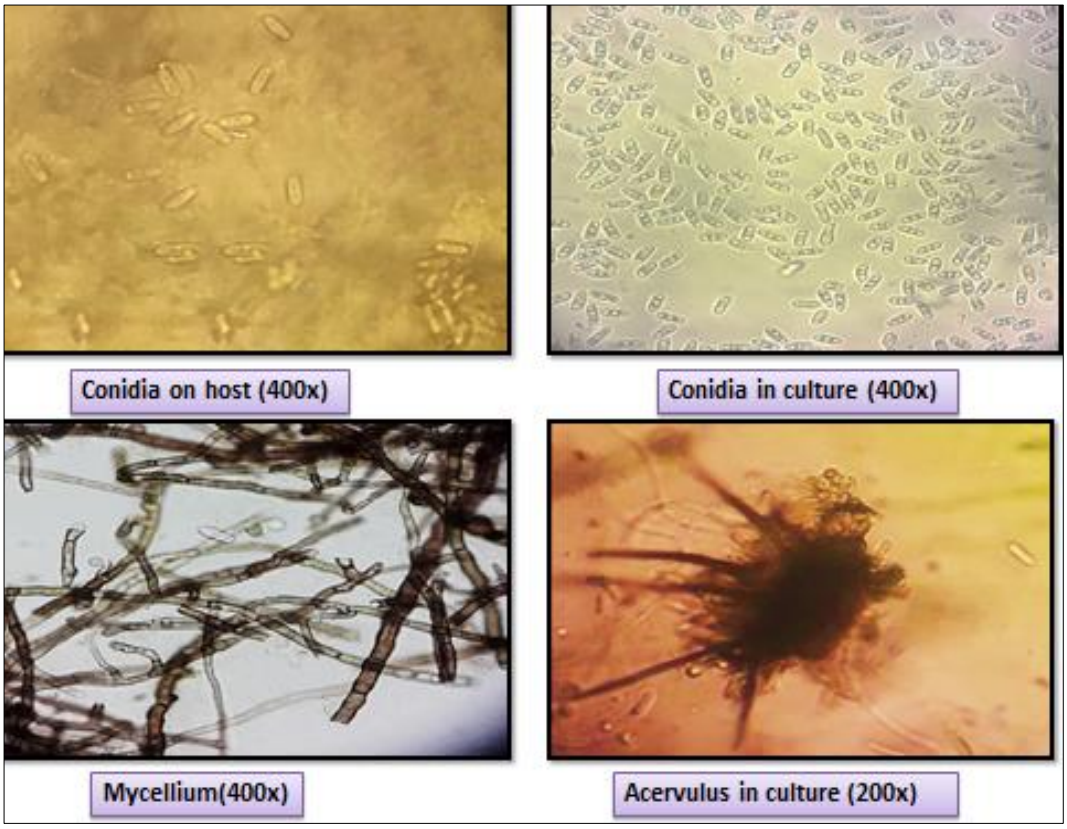

Plate 3: Morphological characteristics of *Colletotrichum lindemuthian*

Bio-agents: Four bio control agents were evaluated under *in vitro* conditions against *Colletotrichum lindemuthianum* through dual culture technique. The bio control agents namely *Trichoderma viride*, *Trichoderma harzianum*, *Psuedomonas flourescens* and *Bacillus subtilis* recorded 69.44, 63.88, 46.60 and 54.01 percent mycelial growth inhibition of test fungus. The best inhibitory response was shown by *Trichoderma viride* and least effective bio control agent screened was *Psuedomonas flourescens* as depicted in Table-2, Plate-4 and Fig.-1.

Table 2: *In vitro* efficacy of various Bio-agents in inhibiting mycelial growth of *Colletotrichum lindemuthianum*

S. No.	Bio-agent	Mycelial growth inhibition (%)
01	*Trichoderma viride*	69.44
02	*Trichoderma harzianum*	63.88
03	*Bacillus subtilis*	54.01
04	*Psuedomonas flourescens*	46.60
C.D. ($p \leq 0.05$)		5.58

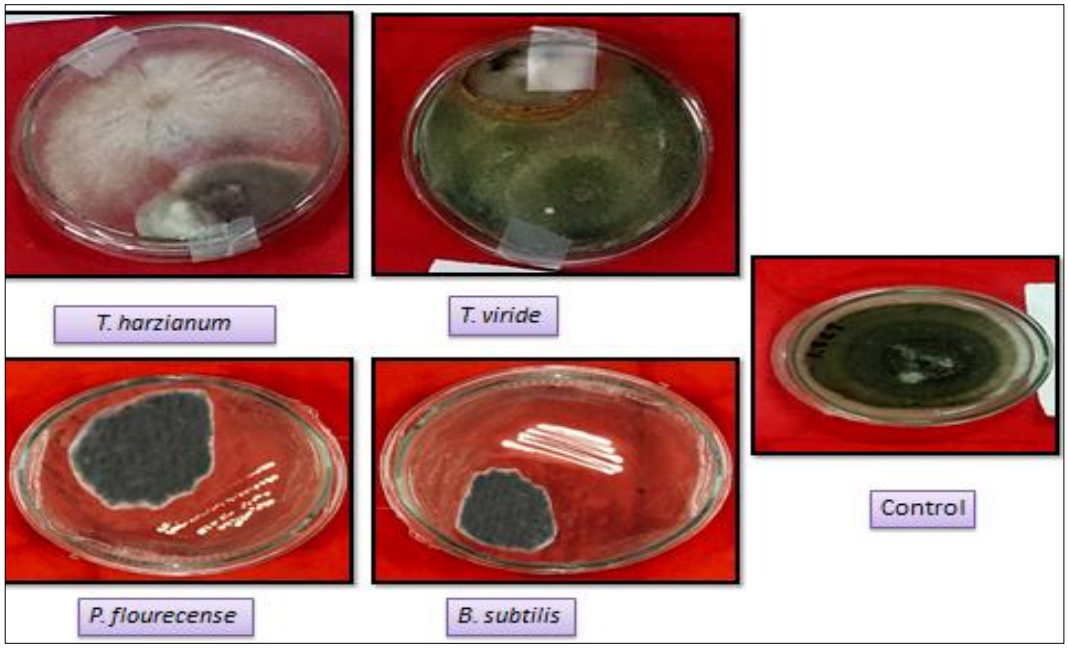

Plate 4: *In vitro* efficacy of the bio-agents against the mycelial inhibition of *Colletotrichum lindemuthianum*

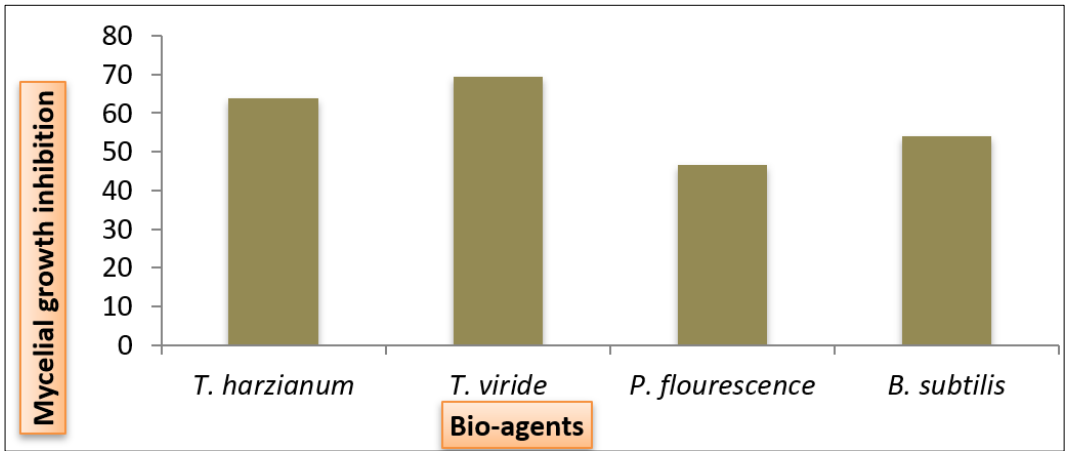

Fig 1: *In vitro* efficacy of various Bio-ag ents in inhibiting mycelial growth of *Colletotrichum lindemuthianum*

Discussion

The infected specimen were collected from common bean fields and microscopy was done for validating that the symptoms were incited by the target pathogen i.e, *Colletotrichum lindemuthianum*. After microscopic confirmation, the said pathogen was isolated from the infected specimen by employing standard tissue bit transfer method (Sicard *et al*., 1997) [19]. Isolation of *Colletotrichum lindemuthianum* from infected leaves, stem and pods was done on potato dextrose agar by tissue bit isolation method (Rio *et al*., 2002) [15]. Morphological characterization revealed that the size of conidia varied significantly on host tissue and culture media. Mycelium is hyaline to pale brown, branched with intercellular and intracellular septations. The conidia measured 9.87-11.30 × 3.43-4.19 µm (av. 9.98 × 4.11 µm) on host tissue and 11.76-21.44 × 3.88-5.94 µm (av. 16.73 × 4.32 µm) on potato dextrose agar media. The shape of conidia recorded was cylindrical with obtuse ends. Shape of acervulus observed under microscope was saucer like with dimensions ranging from 137-315 µm (av. 198.25 µm) in culture media and 177-281 µm (266 µm) on host surface. The size of pointed appendages associated with acervulus recorded in culture media was 61-104 × 3-4 µm (av.79.54 × 3 µm). The colour of conidia, acervuli and setae recorded was hyaline, black and brown to dark brown respectively. No septation was observed in conidia or acervuli but setae had 0-3 septum. The morphological observations recorded were in consonance with the findings of Junaid *et al*., (2014) [5], Khan *et al*., (2009) [6] and Wijesekara and Agarwal (2006) [23]. The test fungus was isolated on potato dextrose agar with colour of colony as grey for young colony to dark black with compact mycelail growth for completely developed colony. Acervuli were seen to be developed within 8 to 10 days of incubation Junaid *et al*., (2014) [5]. The 90 mm Petriplates were fully occupied by the test fungus within 8 to 12 days of incubation period. The culture plates were purified by employing the standard procedure of single spore method. After successfully isolating the pathogen as pure culture, pathogenicity test was done. For pathogenicity test spore cum mycelial inoculation was done on potted bean plants which were treated with carborundum powder. Within a period of 7-8 days characteristic anthracnose symptoms were produced on inoculated potted plants. Validation of the symptoms being caused by the test fungus was done by microscopy.

Bio-control agents have become the talk of town and have proven to be better alternatives to fungicides (Fokkema,

1993) [3]. Biological control involves the exploitation of microbes which show antagonism with the pathogens infecting crops without affecting the ecological balance (Chakraborty *et al*., 2007) [1]. Four bio control agents were evaluated under *in vitro* conditions against *C. lindemuthianum* through dual culture technique. The bio control agents namely *Trichoderma viride*, *Trichoderma harzianum*, *Psuedomonas flourescens* and *Bacillus subtilis* recorded 69.44, 63.88, 46.60 and 54.01 percent mycelial growth inhibition of test fungus. The best inhibitory response was shown by *T. viride* and least effective bio control agent screened was *P. flourescens.* The antagonistic activity may be attributed to different modes of action like antibiosis, competition and mycoparasitism of bio control agents over the test fungus. Pandey *et al*., (2019) [12] laid an *in vitro* trial for evaluating the inhibitory effect of different bio control agent's namely *B. ceresus*, *T. harzianum* and *P. fluorescens* by dual culture technique. The recorded antagonistic effect of these bio control agents on the mycelial growth of *C. lindemuthianum* was maximum with *T. harzianum* (73.87%) while as least effective records corresponded to *B. ceresus* (31.33%). Rajesha *et al*., (2010) [14] conducted an *in vitro* study concerning the evaluation of efficacy of different bio control agents namely *T. viride*, *T. harzianum* and *B. megaterium* against *C. lindemuthianum*. The mycelial growth inhibition recorded was 73.54, 50.90 and 39.46 percent by *T. harzianum*, *T. viride* and *B. megaterium*, respectively. Results of Padder and Sharma (2011) [11] were also in consonance with the findings of present investigation. They inferred that maximum inhibition of mycelial growth of *C. lindemuthianum* was recorded with *T. viride* in dual culture technique (59.48%) and inverted plate (55.98%).

Conclusion

The experimental findings concerning the above investigation are summarized as under: Isolation of fungus was done from the infected specimen that was collected from the common bean field and by employing the spore suspension technique. Pathogenicity of the test fungus was confirmed by following Koch's postulates. Disease symptoms on inoculated potted plants were seen with 10- 12 days of inoculation. Pathogen was cultured on potato dextrose agar medium and was subsequently incubated at 25±1 ºC. The isolated fungus having pathogenic tendency on common bean was identified as *Colletotrichum lindemuthianum* during microscopic investigations and symptomatological analysis.

The test fungus isolated on potato dextrose agar had grey colony colour initially and dark black colour with compact mycelail growth for completely developed colony. Acervuli were seen to be developed within 8 to 10 days of incubation. The 90 mm Petriplates were fully occupied by the test fungus within 8 to 12 days of incubation period. The culture plates were purified by employing the standard procedure of single spore method. Morphological characterization revealed that the size of conidia varied significantly on host tissue and culture media. Mycelium is hyaline to pale brown, branched with intercellular and intracellular septations. The conidia measured 9.87-11.30 × 3.43-4.19 µm (av. 9.98 × 4.11 µm) on host tissue and 11.76-21.44 × 3.88-5.94 µm (av. 16.73 × 4.32 µm) on potato dextrose agar media. The shape of conidia recorded was cylindrical with obtuse ends. Shape of acervulus observed under microscope was saucer like with dimensions ranging from 137-315 µm (av. 198.25 µm). The colour of conidia, acervuli and setae recorded was hyaline, black and brown to dark brown respectively. No septation was observed in conidia or acervuli but setae had 0-3 septum.

From the list of selected bio-agents evaluated under *in vitro* conditions against *C. lindemuthianum*, *T. viride* showed most promising results in limiting the growth of test fungus. The decreasing efficacy order of other bio-agents tested against the target fungus is as *T. harzianum* > *B. subtilis* > *P. flourescens.*

Declarations

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. The raw data as well as processed data is stored securely and will be made accessible to ensure transparency and reproducibility of the research findings.

The data can be obtained by contacting the corresponding author at sahilquyoom103@gmail.com;suhailquyoom@skuastkashmir.ac.in

Ethical statement

The research conducted in this study involving Suhail Quyoom Wani has been approved by the Shere Kashmir University of Agricultural Science and Technology, Kashmir, 190025.

Author contributions

All authors of this paper have contributed significantly to the research and preparation of the manuscript. Authorship has been determined in accordance with the criteria outlined in the journal of Indian Phytopathology guidelines.

Conflict of interest

The authors declare that they have no conflicts of interest relevant to this research.

Consent to Publish

The authors affirm that human research participants provided informed consent for publication of manuscript in the concerned journal.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Chakraborty B, Biswas R, Sharma M. Induction of resistance in tea plants against Alternaria alternata by foliar application of leaf extracts. J Mycol Plant Pathol. 2007;37:60-64.
2. Dennis C, Webster J. Antagonistic properties of species groups of Trichoderma III. Production of volatile antibiotics. Trans Br Mycol Soc. 1971;51:363-369.

3. Fokkema NJ. Opportunities and problems of control of foliar pathogens with micro-organisms. Pest Manag Sci. 1993;37:411-416.
4. Johnston A, Booth C. Plant Pathological Pocket Book. Kew, Surrey, England: Commonwealth Mycological Institute; 1983. p. 439.
5. Junaid JM, Shah TA, Bhat AH, Bhat NA, Dar NA, Ambardar VK. Morphology and status of occurrence of anthracnose of bean (*Phaseolus vulgaris* L.) caused by Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib. in Kashmir valley. The Bioscan. 2014;9(Suppl 1):235-241.
6. Khan NA, Sagar V, Khursheed A, Ahmad M, Beigh MA. Occurrence of bean anthracnose in Kashmir valley - A preliminary study. SKUAST J Res. 2009;1:164-166.
7. Kumar A, Sharma PN, Sharma OP, Tyagi PD. Epidemiology of bean anthracnose under sub-humid mid-hills zone of Himachal Pradesh. Indian Phytopathol. 1999;52:393-397.
8. Makini FW, Danial DL. Bean production and constraints in Kenya with emphasis on diseases, breeding for disease resistance with emphasis on durability. In: Proceedings of a Regional Workshop for Eastern, Central and South Africa, Njoro, Kenya; 1995. p. 104-109.
9. Mordue JEM. *Glomerella cingulata*. CM Descriptions of Pathogenic Fungi & Bacteria. Wallingford, UK: Centre for Agriculture and Bioscience International; c1971. p. 315.
10. Mukunya DM, Keya SO. The influence of seed borne anthracnose and halo blight inocula on yield and disease development in a Canadian Wonder bean selection at Kabete. In: Symposium on Grain Legume Improvement in East Africa, University of Nairobi, Kenya; c1979. p. 20-4.
11. Padder BA, Sharma PN. *In-vitro* and *in-vivo* antagonism of biocontrol agents against Colletotrichum lindemuthianum causing bean anthracnose. Arch Phytopathol Plant Prot. 2011;44:961-969.
12. Pandey N, Ravi S, Vivekanand, Gusain M. *In vitro* efficacy of biocontrol agents and fungicides against anthracnose of French bean caused by Colletotrichum lindemuthianum (Sacc. and Magn.). Int J Chem Stud. 2019;7:3266-9.
13. Pastor-Corrales MA, Tu JC. Anthracnose in bean production problems in the tropics. In: Schwartz HF, Pastor-Corrales MA, editors. International Centre for Tropical Agriculture, California, Colombia; c1989. p. 77-104.
14. Rajesha G, Mantur SG, Shankar MR, Boranayaka MB, Shadakshari TV. *In vitro* evaluation of fungicides and biocontrol agents against Colletotrichum lindemuthianum causing anthracnose of dolichos bean. Int J Plant Prot. 2010;3:114-116.
15. Rio LE, Del-Lamppa RS, Gross PL. First report of the bean anthracnose (*Colletotrichum lindemuthianum*) race 73 in North Dakota. Plant Dis. 2002;86:562.
16. Romero-Arenas O, Huato DMA, Tapia RJA, Simon BA, Lara HM, Huerta CE. The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding rural communities in Puebla-Mexico. Int Res J Biol Sci. 2013;2:59-65.
17. Sartorato A, Zimmermann MJ, Rocha M, Yamada T. Anthracnose cultura-de-feijoeiro. Fatores-que-afetam-a-produtividade; 1998. p. 457-77.
18. Shao FM, Teri JM. Yield losses in Phaseolus beans induced by anthracnose in Tanzania. Trop Pest Manag. 1985;31:60-62.
19. Sicard D, Michalakis Y, Dron M, Neema C. Genetic diversity and pathogenic variation of Colletotrichum lindemuthianum in three centres of diversity of its host, Phaseolus vulgaris. Phytopathology. 1997;87:807-813.
20. Tu JC. Epidemiology of anthracnose caused by Colletotrichum lindemuthianum on white bean (*Phaseolus vulgaris*) in southern Ontario: Survival of the pathogen. Plant Dis. 1983;67:402-404.
21. Tu JC. Control of bean anthracnose caused by Colletotrichum lindemuthianum in Canada. Plant Dis. 1988;72:5-8.
22. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1927;159:850.
23. Wijesekara RHT, Agarwal DK. Taxonomic studies on five species of the genus Colletotrichum. Indian Phytopathol. 2006;59:203-209.