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Infection of *Colletotrichum lindemuthianum* through seed and soil inoculation

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Abstract
Anthracnose caused by seed borne pathogen *Colletotrichum lindemuthianum* is an important fungal disease. Diseased samples were collected, isolation was done, *C. lindemuthianum* was associated in collected diseased samples of bean. Isolation frequency of *C. lindemuthianum* was observed in the range of 12-16 per cent. *C. lindemuthianum* was found pathogenic to bean causing anthracnose disease. Inoculated plant showed symptoms within six to twelve days after inoculation. Reddish and brownish lesions were observed on the leaf surface. In seed and soil inoculation method, seed rot (60% and 40%) and seedling blight (20% and 10%) was observed respectively.

Keywords: Anthracnose, seed, soil, inoculation, *Colletotrichum lindemuthianum*

Introduction
Common bean (*Phaseolus vulgaris* L.) also called as french bean, kidney bean, dry bean and field bean belongs to family leguminaceae and occupies a premier place among grain legumes in the world including India. It is an important constituent of peoples diets especially in developing countries. It is rich in calories, carbohydrates, protein, vitamins and minerals particularly calcium, phosphorus and iron, thus an excellent food for human consumption. The crop is distributed worldwide and can grow under diverse agro climatic condition ranging from tropical, subtropical to temperate region. Major producing states in India are Maharashtra, Jammu and Kashmir, Himachal Pradesh, Tamil Nadu, Kerala, Karnataka and West Bengal. The first scientific report of anthracnose was in Italy and Germany (Sherf and Macnab). In India the disease was first reported by Butler from North and Nilgiri hills of South India. In India, disease incidence has been reported to vary between 24.59 to 51.72 % (Sharma and Sugha 1995) [10] as a disease of minor important but during the last few year bean anthracnose has appeared as a potential threat to the bean production. Anthracnose disease is caused by the fungus *C. lindemuthianum* (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. This disease can cause serious losses in bean crops in temperate and subtropical zones infecting leaves, stems and pods of bean plants. The anthracnose caused by *C. lindemuthianum* it is seed borne pathogen. 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.

Materials and Methods
Collection of diseased samples
Diseased samples showing typical symptoms of anthracnose were collected from the Vegetable Research Unit Dr. P.D.K.V, Akola.

Collection of seeds
Seeds were collected from Pulse Research Unit Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Isolation of fungal pathogen by tissue isolation method

Infected leaf samples were cut into small pieces with sterilized blade and disinfected with sodium hypochlorite solution for two minute. Pieces were then washed with three changes of sterilized distilled water and bits after dried on sterilized filter paper and around flame of spirit lamp were placed on sterilized solidified PDA medium in plate. Each plate contained five bits. The plates were incubated at room temperature (28 ± 2 °C). All these operations were carried out aseptically. The plates were examined regularly. The fungus colonies growing around the each bit were examined and sub cultured. Based on morphological characters and published literature the fungi were identified as *C. lindemuthianum*. The pure culture was transferred on PDA slants and maintained for further studies.

Purification and maintenance of fungal culture

The fungal culture was purified by following hyphal tip method and culture so obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

Pathogenicity test by spray inoculation method

Pathogenicity of *C. lindemuthianum* was tested by spray inoculation method. The seeds of host plant common bean were disinfected with sodium hypochloride solution for two minutes, followed by three subsequent washing with the sterilized distilled water. Seeds were sown in separate pots containing 1/3 rd of sterilized sand + soil mixture for raising of seedlings. For inoculation studies, ten day old seedlings (1-2 trifoliate leaf stage) were sprayed with standard inoculums. Inoculated pots were kept under the cover moist transparent polythene bags for 48 hrs. Inoculated plants were observed for the appearance of disease symptoms after 10-15 days. Healthy uninoculated plants were served as control. After two weeks of incubation period, typical symptoms of anthracnose on foliage were observed. The pathogen was reisolated on the PDA medium from the inoculated plants for confirmation of Koch's postulates.

The mode of infection through seed inoculation

The common bean seeds were mixed with seven day old culture of pathogen. The inoculated seed (25 seeds /pot) were sown in the pot containing sterilized soil mixture. The pot contains seeds without culture inoculation served as control. All these pots were then watered lightly and kept in a greenhouse for further recording of observations on seed germination, seedling mortality, diseased symptoms etc. The pots were kept under observations for 15 days from the date of sowing.

The mode of infection through soil inoculation

The sorghum grains were washed thoroughly in tap water and boiled. After removing the excess water, grains were allowed to air dry and cooled at room temperature. About 300 g moistened grains were filled in each 1000 ml conical flask with 10 ml water and autoclave for 30 minute at 15 Ibs psi pressure. The bit of pure culture of *Colletotrichum lindemuthianum* were inoculated under aseptic condition in those flask containing grains and incubated at 28± 2 ^{0} C for 15 days. Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of the fungus. The grains turn blackish due to mycelial growth of the test fungus. After 15 days of incubation, the inoculum was taken out from flask and mixed thoroughly with sterilized sand plus soil mixture (1:1) at 100 g inoculums per kg soil. This potting mixture (sand +soil +inoculums) was filled in the earthen pots and watered lightly and incubated for four days. Then seeds were sown (25 seeds / pot) in the earthen pot. The pots with uninoculated soil served as control. All these pots were then watered lightly and kept in a greenhouse for further recording of observations on seed germination, seedling mortality, diseased symptoms etc. The pots were kept under observations for 15 days from the date of sowing.

Results and Discussion

Collection of diseased samples

Diseased samples showing the typical symptoms induced by anthracnose as oval or irregular brown or deep brownish spots of various sizes scattered all leaves surface were collected from Chilli and Vegetable Research Unit. Kulkarni *et al.* (2009) described the symptoms induced by anthracnose of green gram (*C. lindemuthianum*) as appearance of reddish brown lesions on lower surface, later they turned to deep dark brown and became chlorotic forming the lesions on both surfaces. Parthiban and Kavitha (2014) [7] also studied anthracnose of beans (*C. lindemuthianum*) and reported that, appearance of small reddish-brown, slightly sunken spots on the pods, which enlarge forming, dark-sunken lesions. The frequencies of isolation are given in (Table 1). It was observed that the infected bits yielded the fungus *C. lindemuthianum* in the range 12-16 per cent.

Isolation of the pathogen

The target anthracnose pathogen was isolated from collected bean leaves. Isolation were made on PDA medium by tissue isolation method. Fitson *et al.* and Jan *et al.* (2014) [2] used diseased pods and leaf tissue for the isolation of *Colletotrichum lindemuthianum* causing anthracnose of bean.

Purification and identification of fungus

The fungus was identified based on morphological characters and published literature as *C. lindemuthianum* and purified by hyphal tip method. The isolated fungus culture was maintained on PDA slants for further studies.

Table 1: Association frequency of *C. lindemuthianum* recorded in collected diseased samples

Location	Sample No.	Plant part used	No. of bits used for isolation	No. of bits yielded fungi	Fungi obtained (No. of bits)		Occurrence of fungi (%)	
					C. lindemuthianum	Other fungi	*C. lindemuthianum*	Other fungi
Chilli and Vegetable Research Unit Dr. PDKV, Akola	Sample 1	Leaves	25	8	3	5	12.00	20.00
	Sample 2	Leaves	25	10	4	6	16.00	24.00
	Sample 3	Leaves	25	8	3	5	12.00	20.00
	Sample 4	Leaves	25	7	3	4	12.00	16.00

Pathogenicity test

Pathogenicity test was carried out by using conidial suspension of *C. lindemuthianum* on bean plants by spray inoculation method. After two weeks incubation period, typical symptoms of anthracnose as reddish brown lesions on the leaf surface were observed (Plate 1). The pathogen was reisolated from artificially inoculated diseased plants on PDA medium and microscopic observations made were found similar to that of the pathogen isolated from naturally diseased bean plants. Thus, the test pathogen was confirmed as *C. lindemuthianum* which ascertained the Koch's postulates and pathogenicity of *C. lindemuthianum* was proved. The results of present pathogenicity test are similar to those of Kelly *et al.* (1994) [4] and Nkalubo *et al.* (2007) [6] who proved the pathogenicity of *C. lindemuthianum* on dry bean.

Plate 1: Pathogenicity test

Mode of infection of *C. lindemuthianum* through seed and soil inoculation

Seed inoculation

The pathogen *C. lindemuthianum* inoculated to the seed and sown in earthen pots containing sterilized soil. 100 seeds were used for seed inoculation and observation were recorded on seed rot and seedling blight.

Data presented in Table 2, (Plate 2) indicated that the inoculated seeds caused seed rot and seedling blight 60% and 20% respectively. Thus, studies revealed that the presence of seed borne inoculums plays an important role resulting into seed rot and seedling blight. Prasanna (1985) [8] reported severe infection and seed borne nature of *Colletotrichum lindemuthianum* in cowpea. Quandah and Memony (2003) [9] also reported that the infected seeds as a primary source of infection, causing seed rot and seedling mortality, in bean due to *C. lindemuthianum.* Seed borne nature of *C. lindemuthianum* was also studied by Goswami *et al.* (2011) [3] in bean.

Table 2: Mode of infection of *C. lindemuthianum* through seed inoculation

Sr. No	Fungi	No of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling blight	Seed rot	Seedling blight
1.	*C. lindemuthianum*	100	40	60	20	*C. lindemuthianum*	*C. lindemuthianum*

Soil inoculation

The pathogen *C. lindemuthianum* inoculated in earthen pots containing sterilized soil. 100 seeds were used for soil inoculation and observation were recorded on seed rot and seedling blight. Data presented in Table 3, (Plate 2) indicated that the pathogen inoculated soil caused seed rot and seedling blight 40% and 10% respectively. A result of present investigation indicates that the soil borne inoculums of the pathogen may cause the disease in the form of seed rot and seedling blight. Parthiban and Kavitha (2014) [7] reported that anthracnose of bean caused by *C. lindemuthianum* is a seed borne pathogen and the infected seed is the primary source of inoculums and young seedlings often suffer from damping - off. Anthracnose of green gram caused by *C. lindemuthianum* is soil as well as seed borne pathogen (Agrios, 2006) [1].

Table 3: Mode of infection of *C. lindemuthianum* through soil inoculation

Sr. No.	Fungi	No of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling blight	Seed rot	Seedling blight
1	*C. lindemuthianum*	100	60	40	10	*C. lindemuthianum*	*C. lindemuthianum*

Plate 2: seed and soil inoculation of *C. lindemuthianum*

Conclusion

Colletotrichum lindemuthianum was associated in collected diseased samples of bean.

Colletotrichum lindemuthianum was pathogenic to bean causing anthracnose disease.

In seed and soil inoculation method, seed rot (60% and 40%) and seedling blight (20% and 10%) was observed respectively.

References

1. Agrios GN. Plant pathology 4th ed. Academic Press. London, 2006.
2. Fitson S, Mohammed Amin, Thangavel S, Adungna A. *In vitro* evaluation of some fungicides and bioagents against common bean anthracnose (*C. lindemuthianum* Sacc. & Magnus) Briosi & Cavara. African J. Microbiol. Res. 2014; 8(20):2000-2005.
3. Goswami RS, Del Rio-Mendoza LE, Lamppa RS, Prischmann J. *Colletotrichum lindemuthianum* races prevalent on dry beans in North Dakota and potential sources of resistance. Plant diseases. 2011; 95:408-412.
4. Kelly JD, Afanador L, Cameron LS. New races of *Colletotrichum lindemuthianum* in michigan and implications in dry bean resistance breeding. Plant Dis. 1994; 78:892-894.
5. Kulkarni S, Benagi VI, Patil PV, Hegde Y, Konda CR, Deshpande VK. Sources of resistance to anthracnose in green gram and biochemical parameters for resistance. Karnataka J Agric. Sci. 2009; 22(5):1123-1125.
6. Nkalubo S, Melis R, Laing MD, Opio F. Yield loss associated with anthracnoe disease on Ugandan market-class dry bean cultivars. African Crop Science Conference Proceedings 2007; 8:869-874.
7. Parthiban VK, Kavitha R. *In vitro* screening of effective biocontrol agents against bean anthracnose pathogen, *Colletotrichum lindemuthianum.* Internat. J Pharma. Screening Methods 2014; 4:32-35.
8. Prasanna KP. Seed health testing of cowpea with special reference anthracnose caused by *Colletotrichum lindemuthianum*. Seed Sci. and Technol. 1985; 13(3):821-827.
9. Quandah ISA. A1- Memony The occurrence and seed transmission of bean anthracnose caused by *Colletotrichum lindemuthianum* in Jordan. Dirasat Agricultural Sciences. 2003; 30(2):205-210.
10. Sharma PN, Sugha SK. Management of bean anthracnose through chemicals. Indian Phytopathology. 1995; 48:304-307.