

Growth Behavior and Morphological Characters of *Colletotrichum lindemuthianum* Causal Agent of Anthracnose of Bean

Shubham Singh*, Ramesh Singh, Anand Kumar Prajapati and Gaurav

ABSTRACT

Bean (*Phaseolus vulgaris* L.) is one of the most important legume crops grown throughout the world, used both as green vegetable and pulse. The crop succumbs to a number of biotic factors including anthracnose of beans that poses a serious threat to its production potential. The first symptom of the disease appears on all plant parts of the bean plant. The symptoms are most visible on leaves and mature pods. At first anthracnose generally appears on the cotyledons and primary leaves of the seedlings, it formed small circular reddish to brown coloured diseased spots. The highest radial growth of the pathogen was recorded on wheat meal medium (5.32 cm) followed by corn meal medium (4.40 cm) and Richard's Medium (4.04 cm), respectively. The poorest growth was observed in Sabouraud's Medium (3.19 cm). The mycelium of *Colletotrichum lindemuthianum* were consist of intracellular, septate, hyaline to pale brown, branched and developed as brown to black lesions mass of hyphae. The conidiophore arise from the culture these were hyaline, septate cylindrical, straight, flag at the base. The conidia are unicellular, hyaline, sickle shaped and non-septate.

Keywords: Media, Sporulation, Colony, Symptoms

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an important legume as well as vegetable crop its cultivation worldwide. It is most important legume crop in India belong to the family Fabaceae or Leguminosae. The bean crop is subjected to various disease caused by fungi, bacteria, viruses, nematode and physiological disorder at different stage of development of the plant growth. These are mainly Anthracnose (*Colletotrichum lindemuthianum*), Bean root rot (*Rhizoctonia solani*), Wilt (*Fusarium solani*), and two widespread bacterial blight that effect most type of beans, common blight (*Xanthomonas campestris* pv. *phaseoli*) and halo blight (*Pseudomonas syringae* pv. *phaseolicola*), Mosaic virus in which the leaf show sharply defined patches of unusually coloration may occur in bean (Padder and Sharma, 2010).

The anthracnose symptoms caused by *Colletotrichum lindemuthianum* appear as dark, sunken, lenticular necrotic lesion by a slightly raised black ring of acervuli of the bean fruit. The initial symptoms of disease appear as a dark lesion along the veins on underside of leaves and susceptible genotypes may exhibit symptom on all aerial parts of the plant. Identification and characterization of *Colletotrichum* species is based on morphological character such as shape and size of conidia and appressoria. Existents of setae or presents of a Teleomorph and cultural character such as colony color growth rate and texture (Mohammed, 2013).

MATERIALS AND METHODS

Effect of different media on the growth of pathogen

The isolates of the pathogen were grown on different culture media to study their cultural and morphological characters. A total of eight synthetic, semi synthetic and natural media were use Viz., Potato Dextrose Agar Medium, Corn Meal Agar Medium, Oat Meal Agar Medium, Leaf Extract Agar Medium, Wheat Agar Medium, Richard's Ager Medium, Asthana and Hawker's Media and Sabouraud's Medium

The culture media used in various experiments were prepared with the standard composition and standard formula given by (Ainsworth, 1961) and (Tute, 1969). Required amount of peeled potato was cut into fine pieces. It was boiled in 500 ml of distilled water for 30 minutes and filtered through muslin cloth. Thereafter, 20 gm of dextrose and 20 gm of Agar- Agar were dissolved in 500 ml boiling water. Potato extract was added in boiling mixture and mixed thoroughly by stirring with glass rod. The final volume 1000 ml maintained with added of distilled water, it was transferred to, about 200 ml in and, 500 ml capacity flask and plugged with non -absorbent cotton. The culture media used in various experiments were.

Inoculum

One week old culture of the pathogen, under study, was grown on 2 per cent sterilized Potato Dextrose Agar Medium and used as inoculum for all the experiments. Petri dishes containing equal quantities of Potato Dextrose Agar Medium

were inoculated with 5 mm. Culture discs, cut with a sterilized cork borer from the actively growing region of the pathogen. Flasks containing liquid media were also inoculated aseptically with 5 mm culture discs of the pathogen.

Measurement of fungal growth

In case of plate culture, surface growth was measured as the diameter of growing colony. The linear growth of mycelium was measured in two directions at right angle to each other along the diameter of developing colony in centimeter (cm) in case of circular and regular colony (Lilly and Barnett, 1951). The measurement of each colony was taken separately and an average was worked out to denote the radial growth of the colony. As regards irregularly developed colonies in plates, the measurements were taken along the largest and shortest diameter, and then average was worked out as the measurement of the growing colony.

Morphological Characters

The morphological study of the fungus obtained from culture pertaining to mycelium, conidiophores and conidia was microscopically examined. The microscopic examination of morphological parts of test fungus was done by using the compound microscope.

Mycelium characters

1. Growth
2. Colour
3. Septation
4. Branching

Conidiophore characters:

1. Colour
2. Septation
3. Size

Conidia characters:

1. Colour
2. Shape
3. Size
4. Arrangement

RESULTS AND DISCUSSION

Symptomatology of the disease

The pods were also affected by the disease at different stages of the plant growth. The first symptom of the disease appears on all plant parts of the bean plant. The symptoms are most visible on leaves and mature pods. At first anthracnose generally appears on the cotyledons and primary leaves of the seedlings, it formed small circular reddish to brown coloured diseased spots. The lesions at the collar region showed enlarged reddish-brown discolouration sometimes, the lesions extended down to the roots just below the ground

level forming reddish brown lesion resulting in collapse of the seedlings. The older seedlings which did not collapse, exhibited yellow leaves with retarded growth on the leaves, circular spots were seen on leaf lamina, on the margin or near the tip of the leaves (Fig. 1). These spots were reddish turning dark with a deep brown margin, which were about 24- 82 mm in diameter. Acervuli of the pathogen were isolated from these spots particularly from the lower leaves. On the pods exhibited as small, circular, slightly depressed reddish-brown spots. As the lesions enlarge in size, the centres became black with purple borders (Fig. 2). The spots had a tendency to coalesce forming larger diseased areas. The central portion turned black with purple margin in the later stage, slimy pink conidial masses are found on these spots which after drying form a crust. The interior of the pod looked discoloured when cut open the seed was stained yellow to brown in colour. If the pathogen attacked immature pods, the pods dry out prematurely, ceased to grow leading to development of cracks on the pods. In such cases, the hyphae of the pathogen reached outer integument then placenta and finally to inner integument and embryo badly affected seeds were light, brownish in colour, poorly developed and fail to germinate (Fig. 3). It was also observed that the seeds which were not destroyed in the pods, were internally infected and carried the conidial mass of the fungus on the seed coat from such seeds, the seedlings showed the typical lesions on the cotyledons. The symptom which appear on the pod of bean plant are more or less similar to those as described by (Kulkarni, 2009) described similar symptoms on green gram crop and (Martinez-Pacheco *et al.*, 2009) on bean



Fig. 1: Symptom on leaves



Fig. 2: Symptom on Pods

Fig. 3: Symptom on seeds

Effect of different solid media on the growth of the Pathogen

The pathogen was grown on 8 solid different media viz. natural, semi synthetic and synthetic. Observation on radial growth, cultural and synthetic linear growth, sporulation morphological characters was recorded after 7 days of interval incubation at room temperature (28°C). Fungi can be grown on different solid media comprising of known and unknown constituents but *In- Vitro*, vegetative as well as reproductive, they require some specific media for their best vegetative as well as reproductive growth. The results are presented in Table 1.

Table 1: Radial growth and sporulation of *Colletotrichum lindemuthianum* on different solid media after 7 days of incubation at 28±10C

S.N.	Solid Media	Avg. Diameter of colonies (cm)	Sporulation
1.	Wheat Extract Medium	5.32 (13.31)	Excellent
2.	Corn Meal Agar Medium	4.40 (12.11)	Good
3.	Rechard's Medium	4.04 (11.54)	Good
4.	Oat Meal.	3.96 (11.39)	Fair
5.	P D A	3.88 (11.24)	Fair
6.	Leaf Extract Medium	3.59 (10.78)	Fair
7.	Asthana and Hawker's Medium	3.33 (10.47)	Poor
8.	Sabouraud's Medium	3.19 (10.14)	Poor
	C.D. @ 5%	2.562	

The result are obtained from the (Table 1) and Fig. 4 and its corresponding histogram (Fig. 5) indicate that the highest radial growth of the pathogen was recorded on Wheat Meal Medium (5.32) followed by Corn Meal Medium (4.40 cm) and Richard's Medium (4.04 cm) respectively. These were statistically at par with each other. The next order of superiority Oat Meal Medium (3.96 cm) and PDA Medium (3.88 cm) support good fungal growth respectively. These were statistically at par with each other. The rest of the media in descending order of superiority was Leaf Extract Media (3.59 cm) and Asthana and Hawker's Medium (3.33 cm) average colony growth. The poorest growth (3.19 cm) was observed in Sabouraud's Medium. All the media test with close agreement with the findings of (Sharma and Kaushal, 1999). Studied the cultural characters of *C. truncatum* on urd bean in seven different media. The study revealed that PDA and Oat Meal Agar supported maximum mycelial growth and Chopped French bean pod media supported maximum sporulation. Singh (2006) conducted cultural studies of *C. lindemuthianum* on bean. They used eight different solid media for the growth of *C. lindemuthianum*. The maximum mycelial growth was seen in PDA followed by Richard's Agar of 82.34 and 75.33mm respectively. Also PDA and Richard's Agar recorded excellent sporulation of *C. lindemuthianum*.

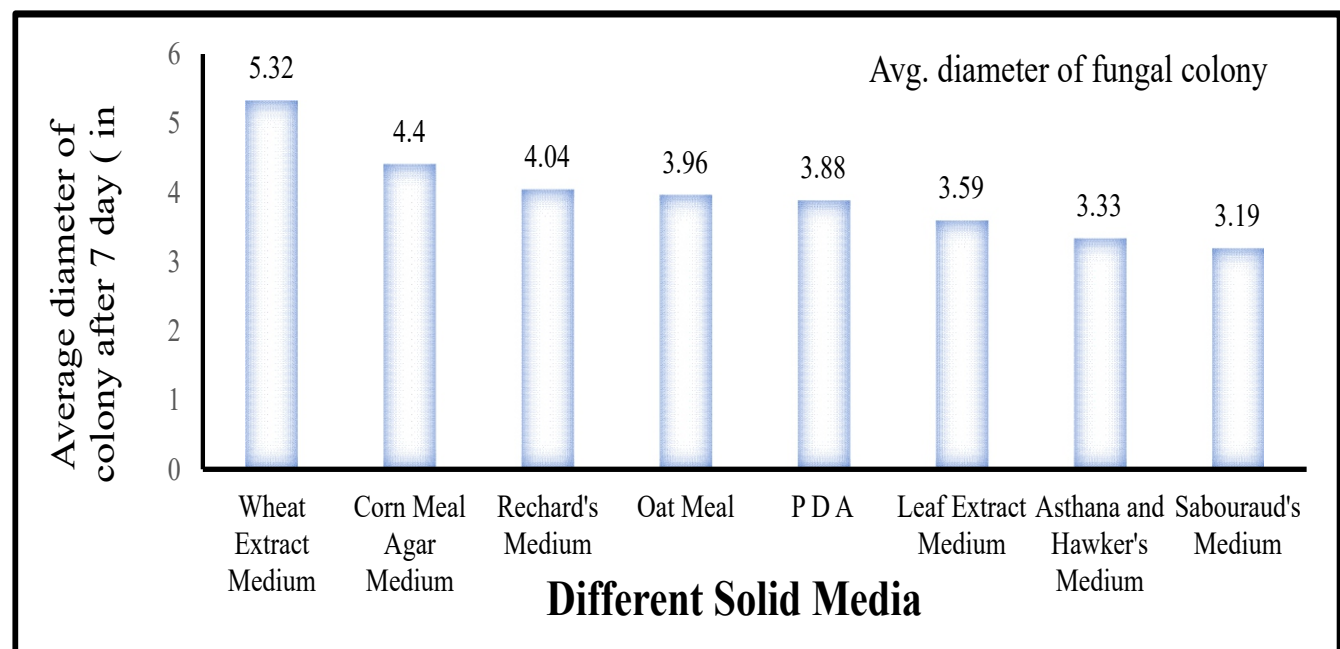


Fig. 4: Fungal diameter and Sporulation of *Colletotrichum lindemuthianum* on different solid media after 7 days of incubation at 28±10C

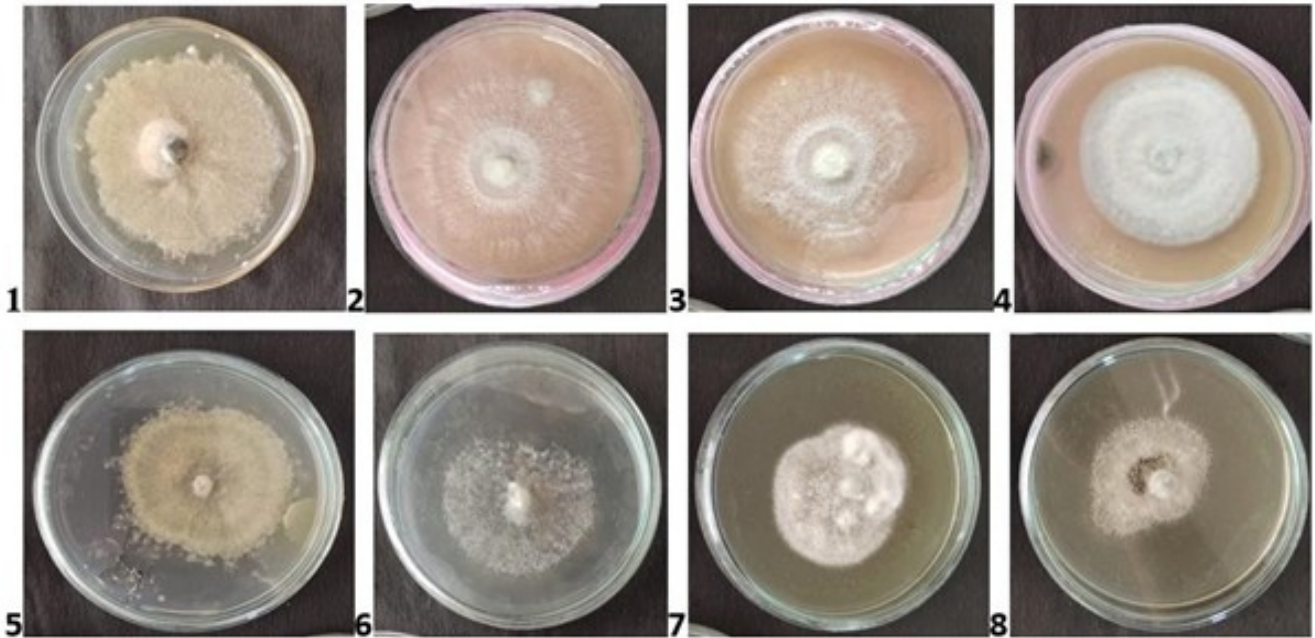


Fig. 5: dial growth of the pathogen on different solid media after 7 days of incubation at 28 ± 1°C

Sporulation

Excellent sporulation of the fungus was recorded on Wheat Meal Medium were as the sporulation was good obtained on Corn Meal Media and Rechar'd's Media and fair sporulation was showed Oat Meal, P D A and Leaf Extract Media. The Hawker's Medium and Sabouraud's Medium show poor sporulation of the pathogen.

Morphological character of the pathogen

Mycelium

The mycelium of *Colletotrichum lindemuthianum* were consist of intracellular, septate, hyaline to pale brown, branched and developed as brown to black lesions mass of

hyphae on the host part and in the culture. After initial growth the individual hyphae close to each other (Fig. 6).

Conidiophore: The conidiophore arise from the culture these were hyaline, septate cylindrical, straight, flag at the base and rounded at apex bearing many cylindrical conidia at the tip (Fig. 7).

Conidia: The conidia are unicellular, hyaline, Sickle shaped Non-Septet. The setae from culture were septate, long, pointed, gradually tapering towards the tip. The conidia are represented of sickles shaped like structure (Fig. 8).



Fig. 6: Mycelium

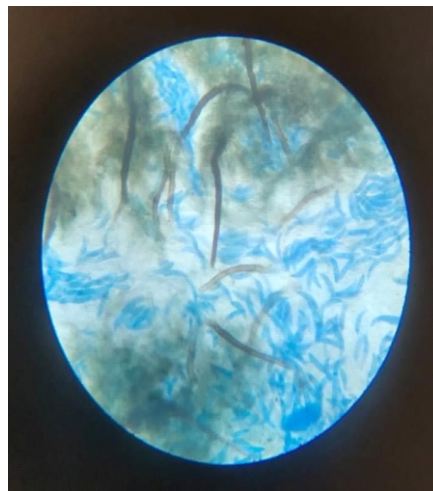


Fig. 7: Conidiophore



Fig. 8: Conidia

The morphological characters of the fungus studies on Potato Dextrose Agar Medium (in culture) and host (in nature) for the identification of the fungus revealed that the colonies in cultural were white, compact, circular and fast growing. The morphological characters of the pathogen described above agree very closely with those described by (Marak et al., 2019) and (Singh, 2006) for *Colletotrichum lindemuthianum* and thus, the fungus was identified as such.

CONCLUSION

From the finding of the cultural studies, it was ascertained that Wheat Extract Agar Medium and Corn Meal Agar Medium were the best suited media of maximum mycelial growth of pathogen. The Sabouraud's Medium showed minimum (3.19cm) radial growth of the pathogen. The views, various characters of the pathogen under investigation were studied on Potato Dextrose Agar Medium. Mycelium were consist of intracellular, septate, hyaline to pale brown, branched and developed as brown to black lesions mass of hyphae on the host plant part and in the culture. Conidiophore arise from the culture hence they were hyaline, septate cylindrical, straight, flag at the base. Conidia were arising from the unicellular, hyaline, Sickle shaped Non-septate. The setae from culture were septate, long, pointed, gradually tapering towards the tip.

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