

Morpho-taxonomic characters and cross pathogenicity of *Colletotrichum* spp., infecting the various beans

S Narasimha Rao¹, SL Bhattiprolu², V Prasanna Kumari³, V Sekhar⁴

¹ Department of Plant Pathology, College of Horticulture, Dr. YSRHU, VR Gudem, West Godvari, Andhra Pradesh, India

² Regional Agricultural Research Station, ANGRAU, Lam, Guntur, Andhra Pradesh, India

³ Department of Plant Pathology, Agricultural College, ANGRAU, Bapatla, Guntur, Andhra Pradesh, India

⁴ Department of Statistics, College of Horticulture, Dr. YSRHU, VR Gudem, West Godvari, Andhra Pradesh, India

Abstract

Anthrachnose incited by various species of *Colletotrichum* causes huge yield losses to beans. Cultural and morphological characteristics of *Colletotrichum* sp. from field bean, French bean, cowpea, mungbean, urdbean, soybean, horsegram, clusterbean, swordbean, wingedbean, yard longbean and pillipesara were studied *in vitro* on Potato Dextrose Agar. Variations were noticed with respect to colony colour and shape of conidia. Pathogenicity of these isolates was proved on their respective native hosts and cross pathogenicity varied from different test plant species. Among the test plant species, cowpea, horsegram and mungbean showed the positive reaction to the *C. lindemuthianum* and *C. truncatum* isolates, while clusterbean showed host specificity.

Keywords: beans, *Colletotrichum*, cross pathogenicity

1. Introduction

Vegetarian population in India consumes large amounts of legumes, particularly, vegetable beans, in their diet where they serve as a good source of protein. Nutritionally these crops have been named as “poor man’s meat and rich man’s vegetable, as these are rich in proteins, minerals (especially iron and zinc) and vitamins and used as green vegetables or green shelled seeds or dry seeds as pulse, based on the stage at which they are harvested [6].

Initially, there were a total of 900 *Colletotrichum* species and over 100 species of *Colletotrichum* species that cause anthracnose disease [8] and about nine species of *Colletotrichum* have been recorded on legume crops worldwide [19]. One hundred species of *Colletotrichum* have been recorded in India [30]. *C. gloeosporioides*, *C. lindemuthianum*, *C. capsici*, *C. falcatum*, *C. truncatum*, *C. sansevierie*, *C. acutatum* and *C. coccodes* are some important species reported to be associated with anthracnose disease in India during last ten years [14].

Colletotrichum is a hemibiotrophic pathogen exploiting biotrophic and necrotrophic strategies to infect various plant parts, at all stages of development, from seedlings to mature plants and seed, causing disease symptoms commonly known as anthracnose [2]. Apart from anthracnose, *Colletotrichum* species causes not only diseases such as damping-off, die back, blight, leaf spot, flower rot, fruit rot, stem end rot and root rots on infected plants [7,20] but also post-harvest diseases in many fruits such as apple, banana, chilli, coffee, grape, mango and strawberry [10]. It occurs worldwide regardless of differences in climate in all continents and causes significant economic damage to crops in tropical, subtropical and temperate regions [12]. Cereals, legumes, ornamentals, vegetables and fruit trees may be seriously affected by *Colletotrichum* spp., [2].

The genus *Colletotrichum* was ranked fourth most studied phyto-pathogenic fungus, being surpassed by *Fusarium*, *Phytophthora* and *Rhizoctonia* [17] and the eighth most important group of plant pathogenic fungi on a world scale based on economic-scientific perceptions [11]. *Colletotrichum* spp., can overwinter on alternative hosts such as other solanaceous or legume crops, plant debris and rotten fruits in the field [23]. Bean crops are infected number of pathogens, among them anthracnose caused by various species of *Colletotrichum* and causes considerable yield loss. Considering the ability of this pathogen to infect various host plants throughout the year, identification of pathogen responsible for anthracnose/ leaf spot disease in some natural hosts is essential by studying morpho-taxonomic characters and also to study the cross pathogenicity to find out the off season survival of pathogen, as the selected crops in the present study are cultivated throughout year continuously or intercropped or rotated with other crops.

2. Materials and Methods

2.1 Collection of diseased samples

The diseased samples of leaves, stems and pods of field bean, French bean, cowpea, mungbean, urdbean, soybean, horsegram, clusterbean, swordbean, wingedbean, yard longbean and pillipesara showing typical symptoms of anthracnose/ leaf spot infection were collected from instructional farm of Agriculture College, Bapatla and College of Horticulture, Venkataramannagudem and investigated at Department of Plant Pathology, College of Horticulture, Venkataramannagudem. The details of different beans and varieties/cultivars used for cross pathogenicity studies were presented in the Table 1.

2.2 Isolation of fungi

Isolation of fungal pathogen was carried out directly or surface sterilization techniques depending on fungal sporulation on the diseased sample. For direct isolation, conidia were picked directly from sporulating samples and were transferred to PDA medium and incubated at $25\pm 1^\circ\text{C}$ until growth of mycelium was visible [1]. Alternatively, isolation of pathogen was performed from the infected plant parts showing typical symptoms of anthracnose by surface sterilization technique using tissue segment method on Potato Dextrose Agar (PDA) medium. Small bits measuring about 2.0 mm size were cut off from the infected parts showing lesion in such a way that each bit contains both infected as well as healthy portions. Such bits were surface disinfested in 1.0% sodium hypochlorite for 2 min and rinsed once in sterilised distilled water and dried on two folds of sterilized blotting papers to remove excess moisture under aseptic conditions. After drying these bits were transferred aseptically to Petri plates containing PDA, supplemented with rose bengal (30 mg l^{-1}) to show fungal growth and streptomycin sulfate (50 mg l^{-1}) to suppress bacterial growth, and incubated at $25\pm 1^\circ\text{C}$ in an incubator and observed periodically for mycelial growth. The actively growing tips of fungal hyphae were then transferred to new Petri plates containing fresh PDA medium. The PDA plates were sealed with paraffin film and incubated in an incubator at 12 h light and 12 h dark periods at $25\pm 1^\circ\text{C}$ and observed at regular intervals for the growth of pathogen. The culture thus obtained was further purified by single spore method and maintained on PDA slant for further use.

2.3 Purification and identification of pathogen

The isolated fungi was purified by the single spore method as per the procedure described [9]. Identification of pathogen from different test plants were carried out by studying the cultural and micro-morpho taxonomic characters on PDA medium and then compared with the available standard literature for establishing their identity. Micro morphological characters such as size and shape of conidia and existence of setae, cultural characters such as colony morphology was recorded right from the initiation of mycelial growth till the period of complete covering of mycelial growth in PDA Petri plates.

Conidia were harvested after 15 days of incubation for the presence of conidia as well as fruiting body (acervulus). Size of conidia was measured at 40X magnification compared with those as described [3,4,18]. After purification and identification, the isolates of *Colletotrichum* species were designated and shown in Table 1.

2.4 Cultural and morphological characters

Mono-conidial culture of each isolate was used to study cultural and morphological characters. Five mm mycelial disc of each isolate was collected from actively growing areas near the edges of seven days old cultures grown on PDA medium, transferred separately on to the centre of sterilized Petri plates containing autoclaved PDA medium and incubated at $25\pm 1^\circ\text{C}$ under 12 h lightness and 12 h darkness for about 10 days for sporulation.

2.5 Preparation of host for pathogenicity and cross pathogenicity studies in pot culture

Pathogenicity and cross pathogenicity of different *Colletotrichum* spp., from field bean and other plant species

was tested on seedlings by spray inoculation method. Ten seeds of each test plant species were sown into each of three 30 cm earthen pots containing 2:1:1 (v/v/v) mixture of soil, sand and peat moss. Seedlings were thinned to five per pot and kept in greenhouse. Two pots were selected for cross inoculation, while one pot was meant for control by inoculating with sterilised distilled water. Ten days old healthy seedlings were inoculated with freshly prepared spore suspension of *Colletotrichum* spp., and each plant species was inoculated with respective isolate and *vice versa*.

A concentration of 5×10^6 conidia ml^{-1} inoculum load was used as standard inoculum for carrying out pathogenicity and cross pathogenicity studies [22]. Tween 20 (poly oxy ethylene sorbitan monolaurate), a surfactant and dispersing agent, was added to the spore suspension @ 0.1 per cent to enable uniform spread of inoculum on the leaves as well as on stem.

2.6 Inoculation for pathogenicity and cross pathogenicity

Ten days old seedlings (2-4 trifoliate leaf stage) were sprayed with standard spore suspension using hand atomizer after pricking the leaves with sterile needle on both dorsal as well as ventral side. Alcohol washed hand atomizer was used separately for spraying of spore suspension of each *Colletotrichum* spp. Such inoculated plants were kept in growth chambers at $25\pm 1^\circ\text{C}$ for 10-15 days and sufficient humidity was provided for three days by covering the inoculated plants with moist transparent polythene bags and then transferred to a greenhouse. Healthy un-inoculated plants served as control. Plants were observed at regular intervals for the appearance of disease symptoms starting from second day up to 25 days after inoculation. Time of appearance of typical anthracnose symptoms was recorded on each test plant.

To determine whether the observed symptoms were caused by respective *Colletotrichum* sp. or not, a small piece of infected plant part from each susceptible host was excised/removed, cleaned with sterilised distilled water and incubated in a humidity chamber for 2-3 days under fluorescent light and then observed under the light microscope for the presence of conidia and compared with that of original conidia isolated from the respective test plant species.

3. Results and Discussion

3.1 Collection of diseased samples

Typical anthracnose/leaf spot symptoms were observed on leaves, stems and pods of different hosts (Plate 1) and such specimens were brought to the laboratory for isolation on PDA medium in Petri dishes under aseptic conditions.

3.2 Isolation and identification of pathogen

Tissue isolation technique was followed to isolate the pathogen causing anthracnose/leaf spot symptoms from test plants and identified as *Colletotrichum* species. Based on cultural and morphological characters, *Colletotrichum* spp., infecting the different hosts were *C. lindemuthianum*, which infect the field bean, French bean, urdbean, cowpea and horsegram, *C. truncatum* on mungbean, pillipesara, soybean, yard longbean, wingedbean and swordbean and *C. capsici* f. sp. *cyamopsicola* on clusterbean through standard mycological keys [3,4,18].

3.3 Cultural and morphological characters of *Colletotrichum* isolates

Cultural and morphological characters of each *Colletotrichum* species in respective test plant species infected were recorded and characters were presented in the Table 2 and Plate 2.

3.3.1 Cultural characters of *Colletotrichum* isolates

Cultural characters of different *Colletotrichum* species with respect to mycelial growth and colour on potato dextrose agar were presented in Table 2. All the species produced branched septate mycelium and at initial stages colonies were fluffy white, thick and later they produced different shades of colour like blackish brown or grey or white or olive brown. The growth pattern of different *Colletotrichum* species was either circular with the mycelia showing a uniform growth pattern or radial ring like pattern (Plate 2).

3.3.2 Morphological characters of *Colletotrichum* isolates

Morphological characters with respect to conidia and setae were presented in Table 2. Conidia of all species were hyaline, unicellular with variable dimensions, formed in acervuli, usually produced on the top of dark brown to black stromata and all conidia having one or two fat globule at centre. Setae were black and multi septate. But the species showed differences in conidial shape (Plate 2).

3.4 Pathogenicity on native host plant species

The data presented in Tables 3 and 4 revealed that all the test host species were readily infected by respective *Colletotrichum* sp. by producing typical symptoms of anthracnose /leaf spot within 5-8 days after of inoculation (DAI), in which they were originally isolated, whereas uninoculated plants remained free from infection even after 25 days. Re-isolations were made from the artificially inoculated test plant species and compared with the original isolate. Re-isolations made from infected parts, invariably yielded cultures of respective species of *Colletotrichum*, which were found to be identical to the type cultures originally inoculated.

3.5 Cross pathogenicity

The cross-inoculation studies demonstrated variations in terms of pathogenicity on their original host and also variation in host preference of other test plants. Different species of *Colletotrichum* were proved as the pathogen and caused anthracnose/leaf spot symptoms after challenge inoculation with different species of *Colletotrichum*. This was verified by the fact that mock inoculation using sterile water had no effect on inoculated test plants.

3.5.1 Cross pathogenicity of *Colletotrichum lindemuthianum*

C. lindemuthianum was isolated from field bean, French bean, urdbean, cowpea and horsegram plants and inoculated to their respective host plants and other test plants to know the cross pathogenicity. Among five *C. lindemuthianum* isolates, isolate CI 4 from cowpea infect all the test plants except wingedbean, swordbean, clusterbean and soybean, this was followed by CI 5 isolate from horsegram, infect all test plants except pole type beans and clusterbean. Similarly, CI 3 from urdbean unable to infect the pole type beans, soybean and clusterbean, while CI 1 and CI 2 from field bean and French bean, respectively, showed similar

type of host preference (Table 4). CI 1 from field bean readily infected the field bean, French bean, urdbean and cowpea, while others were disease free. The cross pathogenicity of French bean isolate (CI 2) as same as field bean (CI 1) isolate but varied with incubation period.

C. lindemuthianum isolates like CI 1, CI 2, CI 3, CI 4 and CI 5 produced anthracnose symptoms within 5-6 days in original test plants, while on other test plants symptom appeared after 6 days of inoculation. CI 3 isolate from urdbean, anthracnose symptoms appeared on 6th day after inoculation on urdbean, while rest of test plants like field bean, French bean, cowpea, mungbean and horsegram the incubation period varied from 7-9 days (Table 4). These results are in conformity with the findings [24] who reported urdbean and horsegram were susceptible to all isolates derived from different hosts.

3.5.2 Cross pathogenicity of *Colletotrichum truncatum*

C. truncatum was obtained from pillipesara, soybean, mungbean, yard longbean, wingedbean and swordbean and designated as CT 1, CT 2, CT 3, CT 4, CT 5 and CT 6, respectively. Cross pathogenicity of *C. truncatum* isolates revealed that CT 3 isolate from mungbean showed positive reaction and produced anthracnose/leaf spot symptoms on field bean, French bean, urdbean, cowpea, horsegram, pillipesara and mungbean. All the remaining crops like soybean, pole type bean and clusterbean exhibited negative reaction. Bharadwaj and Singh [5] reported that isolates of *C. dematium* f. sp. *truncatum* from mungbean, horsegram, urdbean and soybean differed in pathogenic behaviour on six leguminous host species. *C. truncatum* isolates from three pole type beans viz., yard longbean, swordbean and winged bean cross infected each other, while isolate from yard longbean cross infected the cowpea in addition to the pole types (Table 4). Gopalakrishnan and Prakasam [15] reported cross pathological variations in isolates of *C. gloeosporioides* collected from different places of Coimbatore. Soybean isolate (CT2) preferred only horsegram in addition to the native host. The present results of pathogenic variation in isolates of *C. truncatum* is supported by the work [16] who reported the isolates of *C. truncatum* from 11 weeds and soybean varied significantly in pathogenicity on two soybean cultivars.

C. truncatum isolates viz., CT 1, CT 2, CT 3, CT 4, CT 5 and CT 6 produced anthracnose symptoms within 5-8 days in original test plants, while on other test plants symptom development varied from 6 -10 days after inoculation (Table 4).

3.5.3 Cross pathogenicity of *Colletotrichum capsici* f. sp. *cymopsicola*

Colletotrichum species isolated from clusterbean infect only native host and unable to infect other test plants, which means that the host factor is very influential to the initiation and development of disease. This indicated the host specificity of pathogen. Clusterbean produced the anthracnose symptoms within 5 DAI (Table 4). This outcome of host-pathogen interaction indicated host preference or host specialization [28].

The above the cross-pathogenicity study indicated different levels of host specificity and host preference. Among the tested plants, cowpea, horsegram, pillipesara, mungbean, yard longbean and soybean infected by *C. lindemuthianum* and *C. truncatum* these crops act as collateral hosts to the *C.*

lindemuthianum and *C. truncatum* during off season. In general, horsegram is naturally infected by several species of *Colletotrichum*, including, *C. dematium*, *C. lindemuthianum* and *C. truncatum*. In India, *C. lindemuthianum* [27], *C. capsici* [21], *C. dematium* f. sp. *truncatum* [5] and *C. dematium* [25] were identified as pathogens responsible for anthracnose of horsegram.

Similarly, cowpea is infected by *C. dematium*, *C. lindemuthianum*, *C. capsici* and *C. truncatum* [19]. Three different species of *Colletotrichum* viz., *C. truncatum*, *C. lindemuthianum* and *C. dematium* were reported to cause leaf spots or anthracnose disease of mungbean in India [26, 5, 29].

Table 1: List of different hosts used for cross inoculation studies

S. No	Crop	Scientific name	Variety	Place of collection	Common Name(s)
1	Field bean	<i>Lablab purpureus</i>	Arka Amogh	COH, V.R. Gudem	Hyacinth bean, lablab-bean, bonavist bean/ pea, dolichos bean, sem bean, lablab bean, Egyptian kidney bean, Indian bean and Australian pea
2	French bean	<i>Phaseolus vulgaris</i>	Arka Komal	HRS, Lam	Common bean, Green bean, Kidney bean, Rajmash, Sticky bean, Haricot bean
3	Urdbean	<i>Vigna mungo</i>	PU-31	Ag. College Bapatla, Farm	Blackgram or black maple bean
4	Mungbean	<i>Vigna radiata</i>	T-9	Ag. College Bapatla, Farm	Moong bean, Monggo, Greengram, Golden gram
5	Cowpea	<i>Vigna unguiculata ssp. cylindrica</i>	PKM-1	COH, V.R.Gudem	Chowli, Lobiya, Southern pea or Black eye peas
6	Yard longbean	<i>Vigna unguiculata ssp. sesquipedalis</i>	Arka Mangala	COH, V.R.Gudem	Asparagus bean, Snake bean, Chinese long bean, bodi, long-podded cowpea, pea bean
7	Clusterbean	<i>Cyamopsis tetragonoloba</i>	Pusa Navbahar	COH, V.R.Gudem	Guar, Gavar, Guwar, or Guwar bean
8	Pillipesara	<i>Vigna trilobata</i>	Local	M.N. Padu	African gram, Three-lobe-leaf cowpea, Jungle mat bean, Jangli, Mugun, Mungan, Mukni
8	Soybean	<i>Glycine max</i>	JS-335	Ag. College Bapatla, Farm	Soya bean, soya, soy, haba soya, soja bean, miracle bean
10	Horsegram	<i>Macrotyloma uniflorum Dolichos biflorus</i>	Local	Tallur	Kulthi bean, Hurali, Madras gram
11	Wingedbean	<i>Psophocarpus tetragonolobus</i>	Local	COH, V.R. Gudem	Rekkachikkudu
12	Swordbean	<i>Canavalia gladiata</i>	Red local	COH, V.R. Gudem	Thammakai

Table 2: Cultural and morphological characters of *Colletotrichum* spp., isolated from different host test plant beans

S. No	Isolate from	Cultural characters	Morphological characters					Isolate ID
			Conidia (µm)			Setae (µm)		
			Length	Width	Shape	Length	Width	
1	Field bean	Fluffy light pinkish homogeneous growth, mycelium first white, became olive brown in later stage	10.5 - 22.5	4.5 - 4.9	Oblong, rod shaped	82.2-89.9	3.6-4.2	CI 1
2	French bean	The mycelium was colourless at initial stages and turn to pure white	11.5-13.9	3.8-4.2	Oblong with one end slightly pointed end	79.2-82.9	3.5-3.9	CI 2
3	Urdbean	Hyaline at first but becoming light brown with age.	9.5 - 19.5	3.5 - 5.5	Oblong	80.2-88.9	3.1-3.9	CI 3
4	Cowpea	Colony was first white, became dull white in later stage	10.5 - 15.5	3.5 - 4.5	Rod shaped	79.9-92.2	2.9-3.2	CI 4
5	Horsegram	Colonies were brownish white and later turned dark grey in colour	10.7 -12.8	3.9- 5.1	Rod shaped	70.5-83.8	4.7- 4.8	CI 5
6	Pillipesara	Colonies were whitish at initial stages and turn to light brown	12.6- 15.5	3.6- 4.9	Rod shaped	78.9-89.9	4.1-5.1	CT 1
7	Soybean	Appeared white with smooth margin, compact mycelial growth and black in colour	20.4-23.7	3.8- 4.1	Spindle shaped	80.5 - 119.9	4.8-7.8	CT 2
8	Mungbean	Colonies were dark brown to black	14.5- 21.5	3.0-4.5	Fusiform/spindle	80.1-89.9	3.9-4.2	CT 3
9	Yard longbean	Appeared white with irregular margin, thick and compact growth	21.9- 26.8	3.4- 4.1	Fusiform	88.9-101.5	4.8-5.2	CT 4
10	Winged bean	Appeared white with smooth margin with greyish black	19.9- 22.7	3.9-4.2	Fusiform	91.5-98.9	5.1-5.5	CT 5
11	Sword bean	Appeared white with smooth margin with dull white	22.7-24.8	3.5-4.8	Fusiform	92.9-101.8	5.2-6.5	CT 6
12	Clusterbean	The fungal colony was fluffy white, later becoming blackish brown	18.9-25.5	4.5-5.5	Curved with both ends and oval to elliptical shaped	120.3-159.2	5.6-6.2	CC 1

Table 3: Cross pathogenicity of *Colletotrichum* isolates in different beans

S. No	Test plant	Reaction of the <i>Colletotrichum</i> isolates											
		CI 1	CI 2	CI 3	CI 4	CI 5	CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	CC 1
1	Field bean	+	+	+	+	+	-	-	+	-	-	-	-
2	French bean	+	+	+	+	+	-	-	+	-	-	-	-
3	Urdbean	+	+	+	+	+	-	-	+	-	-	-	-
4	Cowpea	+	+	+	+	+	-	-	+	+	-	-	-
5	Horsegram	-	-	+	+	+	+	+	+	-	-	-	-
6	Pillipesara	-	-	+	+	+	+	-	+	-	-	-	-

7	Soybean	-	-	-	-	+	-	+	-	-	-	-	-
8	Mungbean	-	-	+	+	+	+	-	+	-	-	-	-
9	Yard longbean	-	-	-	+	-	-	-	-	+	+	+	-
10	Winged bean	-	-	-	-	-	-	-	-	+	+	+	-
11	Sword bean	-	-	-	-	-	-	-	-	+	+	+	-
12	Clusterbean	-	-	-	-	-	-	-	-	-	-	-	+

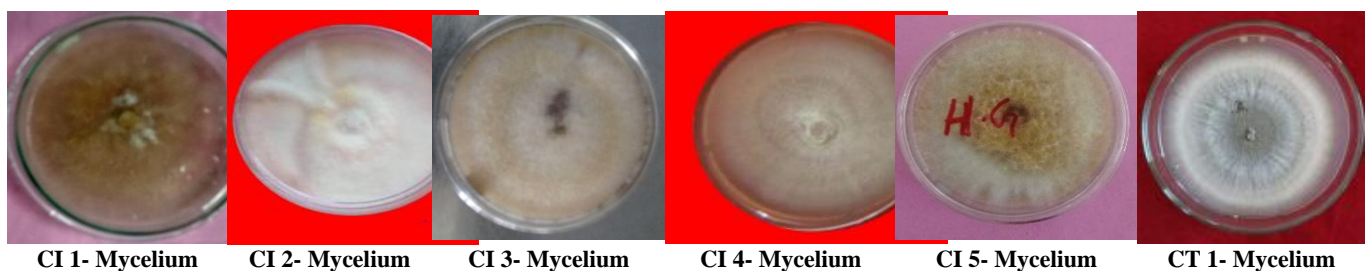
‘+’= Positive ‘-’ = Negative

Table 4: Cross pathogenicity of *Colletotrichum* isolates in different beans with respect to incubation period (days)

S. No	Test plant	CI 1	CI 2	CI 3	CI 4	CI 5	CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	CCI
1	Field bean	5	8	7	8	8	0	0	9	0	0	0	0
2	French bean	6	5	7	8	7	0	0	6	0	0	0	0
3	Urdbean	7	8	6	8	8	0	0	9	0	0	0	0
4	Cowpea	8	6	8	6	7	0	0	7	7	0	0	0
5	Horsegram	0	0	8	9	6	7	8	6	0	0	0	0
6	Pillipesara	0	0	9	7	9	5	0	7	0	0	0	0
7	Soybean	0	0	0	0	7	0	8	0	0	0	0	0
8	Mungbean	0	0	8	7	8	7	0	8	0	0	0	0
9	Yard longbean	0	0	0	7	0	0	0	0	5	6	9	0
10	Winged bean	0	0	0	0	0	0	0	0	7	8	9	0
11	Sword bean	0	0	0	0	0	0	0	0	10	10	7	0
12	Clusterbean	0	0	0	0	0	0	0	0	0	0	0	5



Plate 1: Anthracnose symptoms on different beans under field conditions.



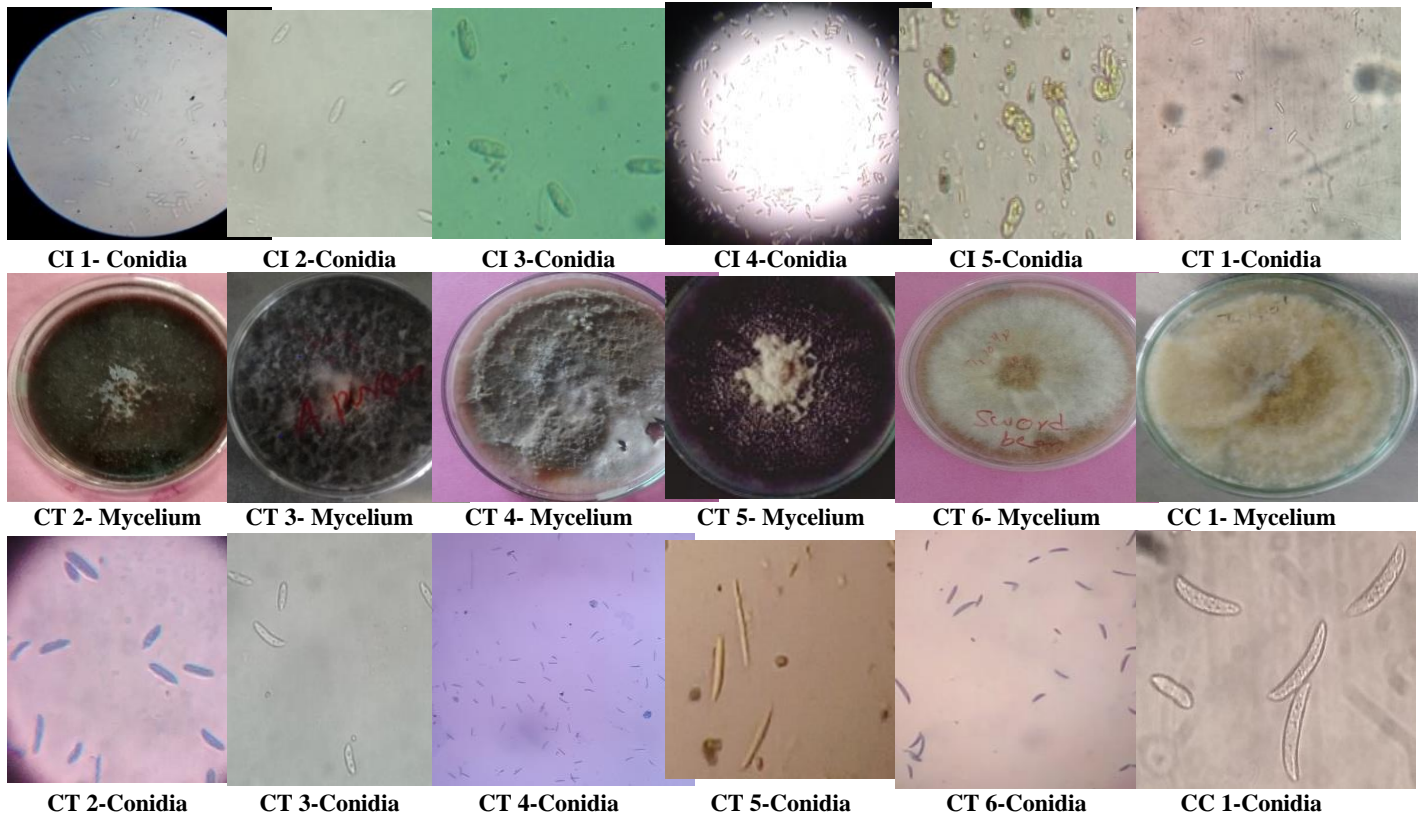


Plate 2: Cultural and micro morpho taxonomic characters of *Colletotrichum* spp., isolated from different host beans

4. Conclusion

Beans considered as both pulses and vegetables. Nutritionally these crops have been named as “poor man’s meat and rich man’s vegetable. From the present study, three species of *Colletotrichum* viz., *C. lindemuthianum*, *C. truncatum* and *C. capsici* f. sp. *cyamopsicola* were identified based on morpho-taxonomic characters on potato dextrose agar. Among the tested bean crops, clusterbean shows the host specificity, whereas cowpea, horsegram and mungbean showed the host preference to various species of *Colletotrichum*. In the absence of native host bean crop, these identified *Colletotrichum* species can survive on other bean crops, which helps continuity of disease cycle of pathogen.

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