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Cultural and morphological variability in *Colletotrichum lindemuthianum* (Sacc. and Magn.) causing anthracnose of field bean

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Abstract
Colletotrichum lindemuthianum was isolated from 95 samples of anthracnose infected field bean, collected from nine major field bean growing districts in five Agro Climatic Zones of Andhra Pradesh. Thirty six isolates from stem bits collected from nine locations, eight isolates from pods and leaves of four different locations were screened to study the cultural and morphological variability. Cultural variations with respect to colony morphology, radial growth, colony colour, colony reverse and mycelial growth pattern and growth rate were examined by growing them on potato dextrose agar. The isolates varied in their cultural behaviour ranging from cottony to fluffy, mostly suppressed with regular to irregular margin and dark black, white and olive brown colony colour. Significant differences were noticed among the isolates with respect to mycelial growth ranging from 36.10 to 88.70mm. It was noticed that olive brown colour isolates showed slow growth rate compared to the other isolates. Morphological variations in spore dimensions (both in length and width, ratio of L:W), number of oil globules, spore shape and sporulation were recorded. The acervuli were embedded, isolates under study mostly produced pinkish acervuli on PDA except CI-2 and CI-4, which had pink to orange colour. Conidia were hyaline, single celled and most of the isolates possessed the cylindrical shape with one or both ends are pointed (12 isolates). Conidia of *C lindemuthianum* isolates contained zero to three oil globules per conidia.

Keywords: Anthracnose, *Colletotrichum lindemuthianum*, cultural variability, field bean, morphological variability

Introduction
Anthracnose was first described from plant specimens obtained from Germany in 1875 and derived from a Greek word meaning "coal like" as a common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 1992) [19] and pathogen *Colletotrichum* associated with the disease of grape in which blackening of tissues as first used by Scribner, 1888 [37].
Colletotrichum Corda is one of the economically important polyphagous, hemi-biotrophic genera of fungi, being responsible for anthracnose and other diseases of a wide range of plant species and recently designated as the world's fourth most studied pyhto-pathogenic fungus (Hyde *et al*. 2009) [18] and the eighth most important group of plant pathogenic fungi on a world scale based on economic-scientific perceptions (Dean *et al*. 2012) [10]. Infections and disease occurred at any stage of plant growth, either in the field or in the greenhouse and storage room and causes economic damage to crops in tropical, subtropical and temperate regions. Different disease symptoms may appear simultaneously in different plant parts e.g. leaf, stem, pods and roots, following the infection of *Colletotrichum* species (Lenne, 1992) [22].
Conventionally, *Colletotrichum* spp., are identified by a variety of cultural and morphological characteristics like colony colour, growth rate, colony texture, size of conidia, presence or absence of setae, *etc.,* (Barnett and Hunter 1972) [5]. Thus, the objective of our study was to identity and investigate the variability in *Colletotrichum* species populations infecting field bean anthracnose in Andhra Pradesh, India by using cultural and morphological characters, as four species of *Colletotrichum* have been reported so far in field bean *viz.*, *C. lindemuthianum* (Rahman *et al*. 1999 [31]; Babu *et al*. 2004 [3]; Pandravada *et al*. 2012 [28]; Rajesha and Mantur, 2014 [33]; Ewansiha *et al.* 2016) [15], *C. dematium* (Singh and Srivastava 1985 [39]; Rahman *et al.* 1994 [32]; Azad *et al.* 2005), [2[*C. truncatum* (Singh *et al.* 1993) [40] and *C. gloeosporioides* (Deshmukh *et al*. 2012) [12]. Rao *et al*. (1998) [34] and Guerber *et al*. (2003) [16] reported several

Colletotrichum species within which having diverse morphological, pathogenic and genetic diversities. Information on pathogen diversity and geographic distribution of the pathogen populations is therefore a prerequisite for designing effective disease management practices including accurate assessment of suitable resistant germplasm in breeding programs. Therefore, the present study, it was rather obligatory or to confirm the identity of the pathogen species as well as variability in it. Anthracnose is caused by the anamorphic form of the fungus *C. lindemuthianum*, which presents wide variability in its populations (Balardin *et al*. 1997; Pinto *et al*. 2012) [4, 30].

Materials and Methods

Collection of diseased samples of field bean

Samples were collected to explore the possibility of existence of different species of *Colletotrichum* and/or variability of *Colletotrichum* spp. infecting the field bean in Andhra Pradesh. Both bush (*Lablab purpureus* var. *lignosus*) and pole types (*Lablab purpureus* var. *typicus*) of field bean plants showing the conspicuous/ typical symptoms of anthracnose were collected from farmers' fields at flowering and pod formation stage. The diseased samples were collected based on visual symptoms and variability in size, shape and colour of lesions. Each sample was labelled carefully to depict places from which samples were collected, site, villages, district and name of the region. A total of 95 samples including stem, leaf, pods were collected in major field growing areas at 36 locations in 9 districts representing five agro-ecological zones in Andhra Pradesh as anthracnose symptoms occur on leaves, stems, petioles, pods and seeds (Table-1).

Table 1: Anthracnose infected field bean samples collected and number of isolates screened for variability

Agro Climatic Zones	District*	Name of the villages and Plant part used for isolation
North Coastal Zone	Srikakulam (3/8)	Bhavajipet (P-2), Pedibheemavaram (L-2), Pareedpet (S-4)
	Viziyanagaram (4/12)	Arigathota (S-3), Mentada (S-2), Manapuram (P-4), Ramabhadrapuram(S-3)
	Visakhapatnam (2/4)	Anandhapuram (S-4)
Godavari zone	East Godavari (4/11)	Burugupudi (S-2), Rajanagaram (S-2), Pithapuram (S-3), Madiki (S-4)
	West Godavari (4/11)	VR Gudem,College (S-2), Telikacherla (P-3), Peravelli (S-3), Vellamilli (S-3)
Krishna Zone	Krishna (2/6)	Avanigatta (L-2), Kankipadu (S-4)
	Guntur (6/12)	HRS, Lam (S-2), Bapatla (L-3), Narakoduru (S-1), Maddirala (S-2), Gopalamvaripalem (S-2), Kottappakonda (S-2)
South coastal zone	Prakasam (11/29)	HRS, Darsi (S-3), Korisapadu (S-2), M.N. Padu (L-2), Kopperapadu (S-3), Kommalapadu (S-2), Ballikuruva (S-2), J Panguloru (S-3) Bobbepalli (S-2), P.Gudipadu (S-4), CS Puram (S-2), Muppavaram (P-4)
Rayalaseema zone	Kadapa (1/2)	A.R Pet (S-2)

L= Leaf, P= Pods, S=Stem *(screened isolates/ total isolates collected)

Isolation of pathogen

After microscopic confirmation of *Colletotrichum* spores in the specimens, isolation of the pathogen was performed from the infected plant parts showing typical symptoms of anthracnose disease by surface sterilization technique using tissue segment method on PDA medium (Dhingra and Sinclair, 2012) [13].

Purification and identification of *Colletotrichum* isolates

Colletotrichum isolates were purified by the single spore method and subsequently the pure culture thus obtained was maintained on PDA slants at 25±1 °C in an incubator. Identification of the pathogen causing anthracnose of field bean was carried out by studying the cultural and micro-morpho taxonomic characters 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 growth, growth rate (colony growth day^{-1}), colony morphology, colony colour both upper and reverse side, pigmentation, zonation and nature of growing margin were recorded right from the initiation of mycelial growth till the period of complete covering of mycelial growth on PDA medium in Petri plates.

Conidia were harvested after 15 days of incubation from PDA plates and a loop full of fungal culture was taken on a clean glass slide mounted with lacto phenol cotton blue for identification under the microscope for the presence of conidia as well as fruiting body (acervulus). Size of conidia was measured at 40X magnification with the aid of ocular and stage micrometer in compound microscope.

Studies on the variability

Mono-conidial culture of each isolate was used to study cultural and morphological variability. Cultural and morphological features of each isolates were recorded as per method described by Baxter and van der Westhuizen, 1984 [6].

Cultural variability

Five mm mycelial disc was cut with the help of cork borer of each isolates 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 PDA medium and incubated at 25±1 °C with intermittent of 12 h day^{-1} from near ultra-violet and day light type fluorescent tubes for about 7-10 days. All experiments were conducted in a completely randomized design with two replications for each isolate and average value of two replicates was worked out and the data were analyzed statistically.

Cultural variability among the isolates was studied on the basis of observations on texture of aerial mycelium, nature of colony edges and zonation recorded for each isolate as described by using the terminology of Baxter and van der Westhuizen 1984 [6]. The colour of colonies was assessed by matching with the Mycological Colour Standards and Colour Nomenclature (Rayner, 1970) [35].

a. **Type and colour of colony:** Colony colour and nature of colony margins were recorded 7 days after incubation.
b. **Colony growth:** Radial growth of the isolates was determined directly by measuring the diameter of

colonies from underside the culture plate against light in the perpendicular axis when the maximum growth was attained in any one of the isolate tested with the maximum of 15 days.

c. **Growth rate:** Average mycelial growth rate (AMGR) was evaluated by taking the average colony measurements (mm) in two perpendicular directions every 72 h up to 15 days of incubation due to slow growth. Growth rate (mm day^{-1}) of each isolate was calculated by dividing the colony diameter with number of days kept for incubation (Jahan *et al.* 2013) [20].

$$\text{Average mycelial growth rate} = \frac{C_8 - C_0}{C_8}$$

Where, C_8 is colony growth (diameter) after 8 days of incubation and C_0 initial colony growth of same isolate

Grouping of isolates based on mycelial growth

The isolated isolates were grouped based on colony growth as given under table 2

Table 2: Scale for grouping of isolates by measurement of radial growth.

S. No	Mycelial growth (mm)	Type of growth	Symbol
1	20 and less	Very poor	-
2	21 to 40	Poor	+
3	41 to 60	Average	++
4	61 to 80	Good	+++
5	Above 80	Excellent	++++

D. Mycelial dry weight: To study the mycelial dry weight of each isolate, 100 ml of PDB was poured into 250 ml conical flask, plugged and sterilized. Five mm mycelial discs were cut aseptically with a cork borer from the margin of seven days old culture of each isolate and transferred to conical flask containing sterilized PDB medium under aseptic conditions. After 15 days of incubation at 25±1°C, the mycelial mat was harvested on to previously weighed and oven dried Whitman's filter paper No. 42. The mycelial mat was removed by filtering through Whitman's No.42 filter paper discs of 12.50 mm diameter. The filter papers along with mycelial mats were dried in an electric oven at 60°C for 12 h and weighed immediately in electric analytical balance. Dry weight of the mycelium was recorded by deducting the weight of filter paper, weighed previously.

Morphological variability

Mono-conidial culture of each isolate was first grown on PDA medium and then semi-permanent slides were prepared from 15 days old culture, stained with lacto phenol cotton blue. The morpho-taxonomic characters such as spore dimensions (length and breadth), shape, colour of conidial mass, number of conidia ml^{-1}, number of oil globules per spore of the each isolate were recorded to know the variability.

a. **Morphology and measurements of conidia:** Micrometric technique was followed to measure length as well as breadth. Size was measured with the help of ocular measurements after calibrating the stage micrometer. The shape and size (length and width) of conidia produced by the each isolate were recorded from 10 randomly selected conidia, measured using the 40X objective of the microscope.

b. **Number of oil globules:** Number of oil globules present per conidia in each isolate was recorded using microscope under 40X magnification.

c. **Spore mass:** Fifteen days after incubation of culture plates, the spore mass appeared on the periphery of the colony. Colour of spore mass and appearance was recorded by visual observation.

d. **Assessment of sporulation:** Sporulation of individual isolates was assessed on PDA medium. At the end of incubation period, five mm discs of each isolate was cut with a sterile cork borer from the centre to the margin at three different equidistant places along the radius of the growth. Three discs were placed in a sterile test tube containing 15 ml of sterile distilled water and the conidia were brought into the suspension by crushing with a glass rod. The suspension was shaken in a vortex mixer to separate the conidia. From this spore suspension, 0.01 ml was transferred on to the counting chamber of the haemocytometer and the number of spores in a small square at the centre was counted to calculate for the total number of spores in 1 ml of the spore suspension. Three replications were maintained for each isolate and average number of spores per ml was determined. The sporulation of each isolate was categorized as poor, average, good and excellent based on the relative number of spores as given under table 3 (Pandey and Vishwakarma, 1998) [27].

Table 3: Scale for grouping isolates based on sporulation.

S. No	Average no. of conidia ml^{-1}	Grade	Score
1	$> 9.0 \times 10^4$	Abundant	++++
2	$4.0 \times 10^4 - 9.0 \times 10^4$	Good	+++
3	$1.0 \times 10^4 - 4.0 \times 10^4$	Average	++
4	$<1.0 \times 10^4$	Poor	+
5	-	No sporulation	-

Results and Discussion

Successful implementation of a disease management practice, in addition to other factors, depends on the understanding of pathogen population structure and mechanism by which variation arises within population. Variations in pathogen population can be detected with cultural, morphological and pathogenic characters. During the present work, thirty six isolates screened from thirty six locations showed variations in their cultural and morphological characteristics.

Collection of anthracnose infected field bean plant parts

The primary cultures of symptomatic segments yielded many mould species. The isolations from leaf, stem and pod samples of field bean revealed the association of *Colletotrichum* sp., with other fungi *viz.*, *Alternaria* sp. *Curvularia* sp. *Isariopsis* sp. and *Fusarium* sp. (Plate 1). *Colletotrichum* species were separated based on spore/ conidiomata characters and further purified by single spore technique. The infected samples on the PDA media gave slightly whitish pink to pale orange mycelium.

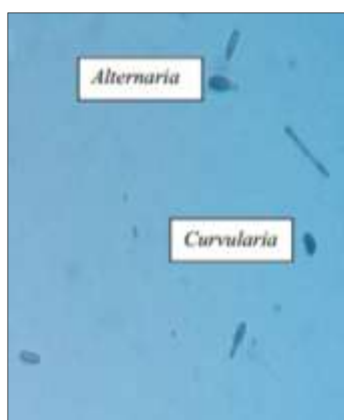


Plate 1: Association of *Alternaria* and *Curvularia* in infected field bean samples

A total of thirty six isolates were screened out of ninety five samples collected. *Colletotrichum* isolates (28 no.) were obtained from stem bits collected from nine locations. The remaining eight were isolated from pods (4 no.) and leaves (4 no.) collected at four different locations. In most of the cases single pathogen was observed and in ten cases mixed infection was noticed. *Colletotrichum* was the main anthracnose pathogen with 100 per cent recovery rate compared with 5.50 per cent for *Alternaria*, *Curvularia* and *Fusarium* and 11.11 per cent with *Isariopsis*. However, the recovery rate differed between tissues (Table 4). Infected leaves yielded pathogens namely *Alternaria*, *Curvularia* and *Isariopsis* whereas *Fusarium* was noticed along with *Colletotrichum* from stem samples. On the contrary, *Isariopsis* was also observed along with *Colletotrichum* on pods. These results are in accordance with Maria *et al*. (2011) [24], Abdulwehab *et al.* (2015) [1] who reported association of *Colletotrichum* sp, *Fusarium oxysporum* and *Curvularia lunata* in Sudanese legumes.

Distribution and isolation rate of *Colletotrichum* isolates

Table 4: Isolation frequency of different pathogens from diseased plant parts

S. No	Plant part used for isolation	No. of samples	Associated pathogens				
			Colletotrichum	*Curvularia*	*Alternaria*	*Isariopsis*	*Fusarium*
1	Stem	28	28	0	0	0	2
2	Leaves	4	4	2	2	2	0
3	Pods	4	4	0	0	2	0
	Total	36	36	2	2	4	2

Screening of isolates

After 10 days of incubation, plates were preliminarily examined for presence of conidia of *Colletotrichum* species. The screened isolates of *Colletotrichum* was identified as *C. lindemuthianum* based on morpho taxonomic characters as well as Koch postulates and compared with the findings of Baxter *et al*. 1983 [7], Wijesekara and Agarwal (2006) [44], Damasceno Silva *et al.* (2007) [9]. Out of 95 samples, 36 isolates were selected based on difference in symptom expression under filed condition and also representing one sample for

each location in order to study the variations among the isolates of *C. lindemuthianum.* Both cultural and morphological variation of *C. lindemuthianum* grown of PDA were noticed with respect to type of growth, colony diameter and colony colour, mycelial dry weight and results presented in Table 5. There were differences in mycelial growth and sporulation of different isolates in the same media.

A. Cultural variation of *C. lindemuthianum* isolates

All the 36 isolates of *C. lindemuthianum* were analysed for traits like colony morphology, growth pattern, mycelial growth, growth rate and mycelial dry weight to know the cultural variability. Mycelium of *C. lindemuthianum* isolates consisted of hyaline, branched, septate hyphae and it was 4 to 5µm thick.

1. Colony colour: At initial stages most of isolates were white in colour later turned to different shades like whitish, light grey, grey to dark grey, olive brown and orange pink (Plate-2). The initial growth of fungus on PDA was slow and abundance sporulation was noticed after 12 DAI. Lardner *et al*. (1999) [21] and Baxter *et al*. (1983) [7] observed differences in colony colours of *Colletotrichum* populations.

2. Colony morphology: Aerial mycelium was even and felted or in tufts, whereas on the reverse it was uneven. The colony margins varied from regular to irregular.

3. Growth pattern: All isolates exhibited cottony growth or fluffy growth and some isolates showed the sparse aerial mycelium. Among the fluffy type of colonies, isolate CI-4 showed raised appearance at centre. The present investigation revealed differences in colony colour and also growth pattern of isolates, some showed appressed mycelial growth whereas others with slightly fluffy to aerial mycelial growth on the medium.

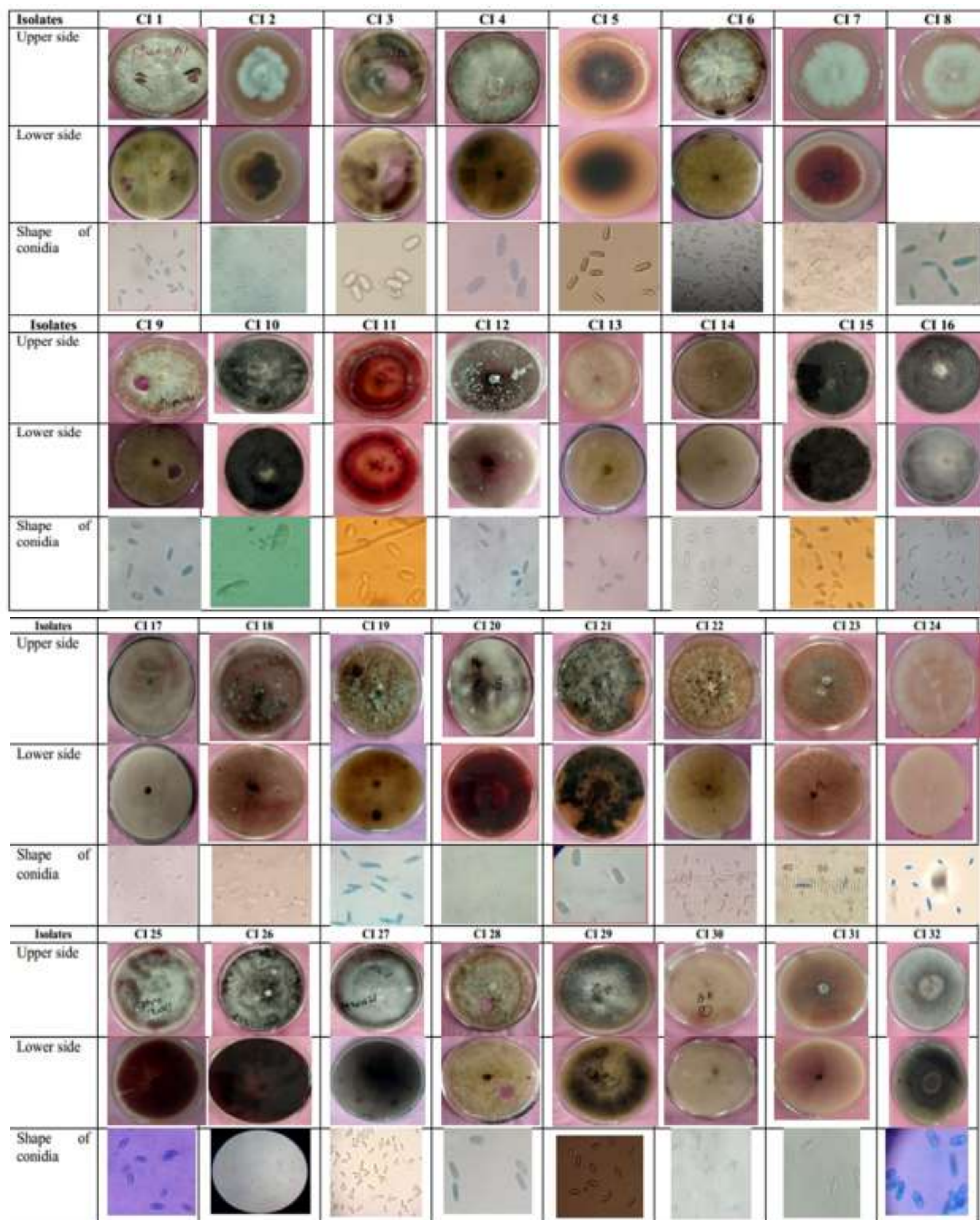
4. Radial mycelial growth: Significant differences were observed among the isolates. Maximum radial growth was recorded in CI-33 isolate (88.70 mm), statistically at par with that of isolates CI-32 and CI-34 (87.75 mm), CI-35 (86.00 mm) and CI-25 (85.50 mm) but differed significantly from all other isolates. Least growth was noticed in CI-28 (36.70 mm), CI-5 (36.80 mm) and CI-9 (37.10 mm) (Plate-3). The results of the present study revealed considerable variation among the isolates from large samples of *C. lindemuthianum* collected from different plant parts of field bean and different geographical locations and the results in agreement with Pandey *et al.* 2012[26] who reported the cultural, morphological variability among the isolates of *Colletotrichum* spp.

5. Growth rate analysis

Significant differences among the isolates were noticed with average growth rate of 2.45 mm day^{-1} to 5.92 mm day^{-1} on PDA. Fastest growth rate was observed in CI-33 (5.92 mm day^{-1}) and it was at par with CI-32 (5.85 mm day^{-1}), CI-34 (5.85 mm day^{-1}), CI-35 (5.73 mm day^{-1}) and CI-25 (5.70 mm day^{-1}), while slow growth rate was observed in CI-28 (2.45

mm day^{-1}), CI-5 (2.46 mm day^{-1}) and CI-9 (2.47 mm day^{-1}) (Table 5). It was noticed that olive brown colour isolates showed slow growth rate compared to the other isolates.

The colony diameter was evaluated separately in each isolate of *C. lindemuthianum* and groped into five groups based on mycelial growth**.** Out of 36 isolates screened, 22 isolates (61.11%) recorded good mycelial growth (61 to 80 mm) followed by six isolates (16.66%) with average mycelial growth (41 to 60 mm). Five isolates (13.88%) recorded excellent mycelial growth above 80.00 mm (Table 7). Significant differences were observed in relation to AMGR and colony diameter, this variability has been previously reported (Souza *et al*. 2007 [41]; Pinto *et al*. 2012 [30] and Liu *et al*. 2013) [23].



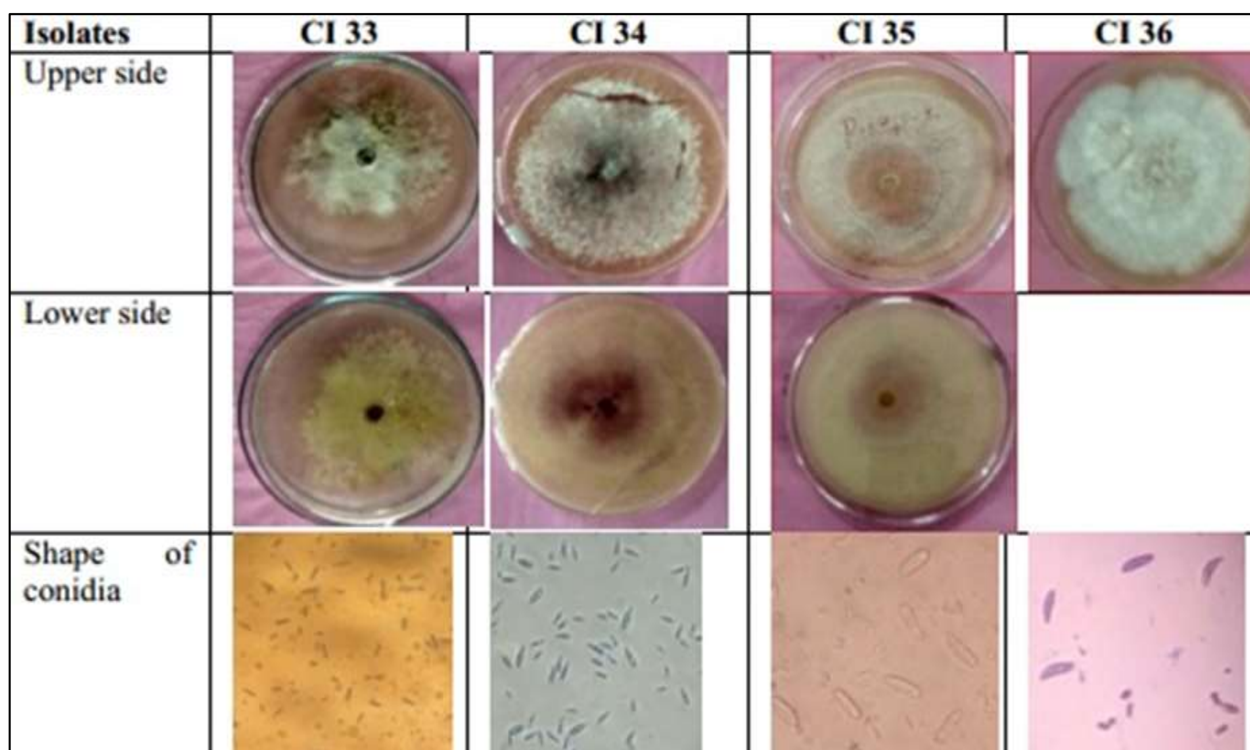


Plate 2: Cultural and morphological characters of different isolates of *C. lindemuthianum*

Plate 3: Colour of spore mass on PDA after 25 day of incubation

6. Mycelial dry weight

The growth responses of different isolates of *C. lindemuthianum* were also studied on PDB and results are summarized in Table 5. Significant difference was noticed in dry mycelial weight and it ranged from 235.00 to 295.50 mg. Among the different isolates, highest dry mycelial weight was noticed in CI-32 isolate (295.50 mg), which was statistically superior over the other isolates. The next isolate was CI-22 but differed significantly from all other isolates. Lowest mycelial dry weight was recorded in isolate CI-5 (235.00 mg). These results were in accordance with Sharma and Kaushal, 2001 [38] who reported varied cultural and morphological characteristics in eleven mono-conidial isolates of *C. truncatum*.

Table 5: Cultural variability of different isolates of *Colletotrichum lindemuthianum*

Isolate code	Colony characters				Colony colour		Mycelial dry weight
	Growth pattern	Mycelial growth (mm)^{a}	Growth rate (mm day^{-1})	Margin	Upper surface	Lower surface	(mg)
CI-1	Cottony growth	74.35	4.96	Regular	white	Olive greenish grey	276.00
CI-2	Fluffy growth	63.85	4.26	Irregular	white	Light brown	251.00
CI-3	Fluffy growth	74.45	4.97	Regular	Greyish white	Light grey	254.35
CI-4	Cottony growth	73.90	4.13	Regular	Whitish grey	Mouse grey	280.70
CI-5	Fluffy growth	36.80	2.46	Regular	Chocolate brown	Chocolate brown	235.00
CI-6	Cottony growth	74.00	4.93	Irregular	Olive brown	Olive brown	280.50
CI-7	Cottony growth	75.10	5.01	Regular	White	Purple	270.50
CI-8	Cottony growth	65.60	4.38	Irregular	White	-	280.70
CI-9	Fluffy growth	37.10	2.47	Irregular	Whitish brown	Olive brown	250.50
CI-10	Fluffy growth	67.65	4.51	Regular	Whitish black	Black	252.25
CI-11	Sticky growth	57.70	3.85	Irregular	Pink	Pink	260.50
CI-12	Fluffy growth	74.15	4.94	Regular	Whitish grey	Whitish grey	267.85
CI-13	Sparse growth	69.35	4.63	Regular	Olive brown	Olive brown	278.00
CI-14	Cottony growth	68.15	4.54	Irregular	Light brown	Creamy white	270.65
CI-15	Compact growth	73.50	4.90	Irregular	Black	Black	248.00
CI-16	Fluffy growth	76.00	5.07	Regular	Whitish black	Milky wite	260.50
CI-17	Fluffy growth	73.50	4.90	Regular	Creamy white	Creamy white	240.00
CI-18	Sparse growth	71.00	4.74	Irregular	Greyish white with purple tinge	Greyish white with purple tinge	265.10
CI-19	Fluffy growth	47.00	3.14	Regular and aerial mycelium	Brownish white	Olive brown	270.40

CI-20	Cottony growth	63.15	4.21	Regular	White	Pink	279.00
CI-21	Fluffy growth	44.50	2.97	Regular	Blackish white	Black	235.50
CI-22	Aerial growth	56.65	3.78	Irregular	Olive brown	Creamy white	290.50
CI-23	Sparse growth	61.50	4.10	Irregular	Light greyish white	Mouse grey	259.00
CI-24	Cottony growth	64.00	4.27	Regular	White	Creamy white	250.50
CI-25	Cottony growth	85.50	5.70	Regular	White	Pink	240.50
CI-26	Fluffy growth	58.00	3.87	Regular	Blackish white	Black	275.50
CI-27	Sparse growth	71.45	4.76	Irregular	White with light grey	Light grey	258.00
CI-28	Cottony growth	36.70	2.45	Regular	Olive brown	Creamy white	255.50
CI-29	Cottony growth	63.50	4.24	Regular	Greyish white	Mouse gery	260.50
CI-30	Fluffy growth	71.50	4.77	Irregular	Creamy white	Creamy white	270.50
CI-31	Sparse growth	72.00	4.80	Irregular	Light greyish white	Mouse grey	280.50
CI-32	Cottony growth	87.75	5.85	Regular	Greyish white	Black	295.50
CI-33	Fluffy growth	88.70	5.92	Irregular	Creamy white	Creamy white	285.50
CI-34	Cottony growth	87.75	5.85	Irregular	White with grey centre	Pink with light grey border	285.50
CI-35	Cottony growth	86.00	5.73	Regular	White	Creamy white	280.50
CI-36	Cottony growth	51.00	3.40	Irregular	white	-	235.50
CD		4.78	0.329				3.13
SE(m)±		1.66	0.11				9.39
CV		3.52	3.52				18.77

B Morphological variability of *C. lindemuthianum* isolates

Analysis of morphological traits is useful in the characterization of different strains of a species, and can assist in genetic analyses when contrasting strains are identified for each trait, and also for the differentiation of a genus (Souza *et al*. 2007) [41]. All the 36 isolates of *C. lindemuthianum* were analysed for traits like length and width of the conidia (µm), shape, oil globules and sporulation capacity.

1. Acervuli: Acervuli were observed in some culture plates as well as on diseased plant parts, especially on stem. The acervuli were embedded with very light pinkish coloured mucilaginous mass containing numerous conidia. Isolates under study mostly produced pinkish acervuli on PDA except CI-2 and CI-4, which had pink to orange colour. Acervuli were produced 25 days after incubation. Conidial mass was whitish to brick red in colour (Plate-3). Similarly Mota *et al*. (2016) [25] reported the colonies *Colletotrichum* spp., produced orange masses of conidia on the surface.

2. Setae: Acervuli produced by different isolates also possessed dark blackish to greyish setae had pointed tips with variable septation. Setae were longer than conidiophores and it were erect, hair like, broader at base, tapering at apex and arising through the mucilaginous mass of conidia.

3. Conidia analysis: Average measurements of spore dimensions (length, width and ratio of length: width) showed significant differences among the isolates (Table-6). The length of conidia of all isolates was significantly different from each other. Isolate CI-11 had longest conidia (14.85 µm) followed by isolate CI-9 (14.50 µm), CI-14 (14.05 µm), CI-10 (14.00 µm) and CI-30 (13.95 µm) all were at par with each other. Whereas the lowest conidial length was observed in CI-22, this was at par with the isolates *viz*., CI-2 (10.20 µm), CI-33 (10.30 µm) CI-24 (10.50 µm), CI-23 (10.55µm), CI-25 (10.95 µm), CI-31(10.60 µm), CI-21 (11.00 µm) and CI-32 (11.05 µm). However in terms of width of conidia, there were significant differences among isolates with average breadth of conidia varied from 3.50 to 4.65 µm. Isolate CI-30 had maximum breadth and isolate CI-3 had minimum breadth. The quotient value (Average conidial length divided by average conidial breadth) varied from 2.14 to 3.85. Six isolates (CI-14, CI-11, CI-3, CI-6, CI-18 and CI-15) had quotient value of 3.5 or above indicating that spores had the curve and rounded ends. Isolates of *C. lindemuthianum*, the conidia exhibited variation in length (9.85-14.85µm) and width (3.50-4.65µm). These values are in agreement with other data described in the literature for these species (Roca *et al*. 2003) [36].

4. Shape of conidia: Conidia were hyaline, single celled but variation was noticed with respect to shape of conidia of different isolates and most of the isolates possessed the cylindrical with one or both ends are pointed (12 isolates) (Table-6). The variation in shape of conidia is due to production of secondary conidia directly from germinating primary spores. These results are in accordance with findings of Cannon *et al*. (2000) [8] and Enyiukwu *et al*. (2014) [14] who stated that many species of *Colletotrichum* produce secondary conidia in culture and these may vary morphologically when compared to those produced in conidiomata.

5. Grading of conidia: CI-23 produced fusiform conidia whereas the remaining all isolates produced four different types of conidia (Table-7). Out of 36 isolates, 12 isolates produced cylindrical conidia with one or both ends are pointed (33.33%); nine isolates produced spindle shaped conidia, with one pointed end and other rounded end (25.00%); seven isolates produced straight conidia with both ends are rounded (19.44%) and seven isolates produced straight conidia but centre have curve (19.44%).

6. Oil globules: The conidia of *C. lindemuthianum* isolates contain zero to three oil globules per conidia. Most of the isolates under study contained two oil globules per conidia (Table-6).

7. Sporulation rate: The sporulation rate of a pathogen affects its dispersion capacity, which subsequently affects the spatial distribution of the species (Pereira *et al*. 2006) [29]. Moreover, it is reported that *C. lindemuthianum* has limited dispersion capacity, which occurs preferentially between different parts of the same plant and occasionally between adjacent plants (Souza *et al*., 2010) [42]. The data pertaining to the sporulation potential of isolates varied widely and ranged

from 3.5 x 10 ^{4} to 10.5 x10^4 conidia/5.00 mm^2 colony areas. Out of 36 isolates screened, 27 isolates (75.00%) recorded good sporulation capacity (4.0 to 9.0 x 10^4) followed by five isolates (13.88%) with average spore producing ability (1.5 to 4 x10^4). Four isolates (11.11%) recorded excellent spore production ability above 9.0x10^4 spores (Table-6).

Table 6: Morphological variability of different isolates of *Colletotrichum lindemuthianum*

Isolate code	Conidia size (µm)			Shape	Number of oil globules	Sporulation (x10^4)	Setae
	Length	Width	L/W ratio				
CI-1	12.15	3.85	3.16	Spindle shape, one end is pointed and other end is round	1	5.0	+
CI-2	10.20	4.15	2.46	Straight both ends are rounded	2	6.0	+
CI-3	12.95	3.50	3.70	Straight both ends are rounded	0	7.5	+
CI-4	11.30	4.15	2.72	Cylindrical one or both ends are pointed	2	10.0	+
CI-5	11.95	3.60	3.32	Straight but centre have curve	2	6.5	+
CI-6	12.95	3.70	3.50	Straight but centre have curve	1	7.5	+
CI-7	11.35	3.70	3.07	Cylindrical one or both ends are pointed	2	5.5	+
CI-8	11.30	4.00	2.83	Spindle shape, one end is pointed and other end is round	3	3.5	+
CI-9	14.50	4.15	3.49	Cylindrical one or both ends are pointed	2	4.0	+
CI-10	14.00	4.54	3.09	Straight both ends are rounded	2	4.0	+
CI-11	14.85	3.95	3.76	Straight both ends are rounded	0	4.5	+
CI-12	11.20	4.00	2.80	Cylindrical one or both ends are pointed	1	8.5	+
CI-13	12.00	4.35	2.76	Straight both ends are rounded	2	6.5	+
CI-14	14.05	3.65	3.85	Cylindrical one or both ends are pointed	3	7.5	+
CI-15	13.30	3.60	3.69	Straight but centre have curve	2	9.0	+
CI-16	12.60	3.85	3.27	Cylindrical one or both ends are pointed	2	4.5	+
CI-17	12.15	3.75	3.24	Spindle shape, one end is pointed and other end is round	2	6.5	+
CI-18	13.10	3.65	3.59	Straight both ends are rounded	1	3.5	+
CI-19	12.85	3.90	3.29	Spindle shape, one end is pointed and other end is round	2	5.5	+
CI-20	12.80	4.25	3.01	Spindle shape, one end is pointed and other end is round	1	7.5	+
CI-21	11.00	4.40	2.50	Straight both ends are rounded	2	4.0	+
CI-22	9.85	4.60	2.14	Straight but centre have curve	2	7.0	+
CI-23	10.55	4.40	2.40	Fusiform	1	6.0	+
CI-24	10.50	4.00	2.63	Cylindrical one or both ends are pointed	2	8.5	+
CI-25	10.95	3.70	2.96	Straight but centre have curve	2	7.5	+
CI-26	12.00	3.45	3.48	Cylindrical one or both ends are pointed	1	4.5	+
CI-27	11.30	3.65	3.10	Spindle shape, one end is pointed and other end is round	1	5.5	+
CI-28	12.20	3.65	3.34	Cylindrical one or both ends are pointed	2	8.5	+
CI-29	12.80	4.35	2.94	Straight but centre have curve	1	8.0	+
CI-30	13.95	4.65	3.00	Cylindrical one or both ends are pointed	2	8.5	+
CI-31	10.60	4.45	2.38	Spindle shape, one end is pointed and other end is round	2	4.5	+
CI-32	11.05	4.45	2.48	Cylindrical one or both ends are pointed	1	10.0	+
CI-33	10.30	4.05	2.54	Spindle shape, one end is pointed and other end is round	2	10.5	+
CI-34	11.80	4.30	2.74	Spindle shape, one end is pointed and other end is round	1	8.5	+
CI-35	11.35	4.40	2.58	Cylindrical one or both ends are pointed	2	8.5	+
CI-36	12.05	4.60	2.62	Straight but centre have curve	2	7.5	+
CD	1.29	0.55				2.2	
SE(m)±	0.45	0.19				0.8	
CV	5.26	6.52				16.3	

Cultural and morphological variability was noticed, and several of these are important for pathogen development and survival, such as mycelial growth rate, colony diameter, sporulation capacity, and per cent germination (Souza *et al.* 2007) [41]. *C. lindemuthianum* isolates from different localities had variation in cultural and morphological characters, especially radial growth and growth rate, colony colour, sporulation and shape. Similar observations were made by Denobys and Baudry (1995) [11] who argued that variation in the isolates may be inherent since isolates were collected from different locality; hence the morphological and cultural characters are influenced by environmental conditions through natural chance mutations which may be responsible for such variability. Some of the isolates of *C. lindemuthianum* showed somewhat overlap of characters such as mycelial growth, spore shape and sporulation potential among the thirty six isolates studied. This result was in agreement with a previous study by Hindorf (1973) [17], who found a morphometric overlap of conidial size within *Colletotrichum* species. Similarly Thind and Jhooty (1990) [43] successfully used morphological and pathological characteristics to categorize 150 isolates of *C. capsici* and *C. gloeosporioides* causing chilli anthracnose.

Table 7: Grouping of isolates of *C. lindemuthianum* based on cultural and morphological characters

S. No	Colony growth (mm)	Isolate code	No. of isolates	Average no. of conidia ml^{-1}	Isolate code	No. of isolates	Shape of conidia	Isolate code	No. of isolates
1	20 and less	NIL	0	< 1.0 X 10^4	Nil	0	Fusiform	CI-23	1
2	21 to 40	CI-5, CI-9 and CI-28	3	> 1.0 X 10^4 – 4.0 X 10^4	CI-8, CI-9, CI-10, CI-18 and CI-21	5	Straight both ends are rounded	CI-2, CI-3,CI-10, CI-11, CI-13, CI-18 and CI-21	7
3	41 to 60	CI-11, CI-19, CI-21, CI-22, CI-26 and CI-36	6	> 4.0 X 10^4 – 9.0 X 10^4	CI-1.CI-2, CI-3, CI-5, CI-6, CI-7, CI-11, CI-12, CI-13, CI-14, CI-16, CI-17, CI- 19, CI-20, CI-22, CI-23, CI-24, CI-25, CI-26, CI-27.CI-28. CI-29, CI-30, CI-31,CI-34, CI-35 and CI- 36	27	Cylindrical one or both ends are pointed	CI-4, CI-7, CI-9, CI-12, CI-14, CI-16, CI-24, CI-26, CI-28, CI-30, CI-32 and CI-35	12
4	61 to 80	CI-1,CI-2,CI-3,CI-4, CI-6, CI-7,CI-8, CI-10, CI-12, CI-13, CI-14,CI-15, CI-16, CI-17, CI-18, CI-20, CI-23, CI-24, CI-27, CI-29, CI-30 and CI -31	22	> 9.0 X 10^4	CI-4,CI-15,CI-32 and CI- 33	4	Spindle shape, one end is pointed and other end is round	CI-1, CI-8, CI-17, CI-19, CI-20, CI-27, CI-31, CI-33 and CI-34	9
5	Above 80	CI-25,CI-32,CI-33,CI-34 and CI-35	5	-	-	-	Straight but centre have curve	CI-5,CI-6, CI-15, CI-22. CI-25, CI-29 and CI-36	7

Conclusion

The present investigation on cultural and morphological variability of *C. lindemuthianum* causing anthracnose revealed 36 isolates identified based on conidial characters as per fungal descriptions in the literature. Thus *Colletotrichum lindemuthianum* is major pathogen responsible for anthracnose in field bean in Andhra Pradesh and showed different types cultural and morphological variability (colony morphology, conidial shape, radial growth, colony colour, colony reverse, mycelial growth pattern and sporulation) in the PDA. These results clearly indicated the existence of variability among the isolates. These parameters are limiting due to the fact that they cannot give details about variation in races or pathotypes and are influenced by the environmental conditions. Variation in pathotypes is essential for the understanding of the population dynamics of *C. lindemuthianum* for utilizing the breeding programme to develop the resistance.

Conflict of Interest: None declared

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