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Morphological and cultural variability among *Colletotrichum* spp. isolated from different host

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Abstract
Identification and characterization of various Colletotrichum diseases isolated from various crop such as soybean, sugarcane, chilli, turmeric, bean, mango and strawberry is studied. Three isolates of each crop was collected from different geographical location of chhattisgarh and was maintained for morphological variation such as in Setae, conidial shape, mycelium and growth pattern was observed. The various species confirmed morphological difference which includes *Colletotrichum acutatum, C. truncatum, C. capsicii, C. lindemuthiaum* and *C. falcatum* on the respective crop. The three isolate morphologically similar with *C. acuatum* produce falcate conidia, and developed dark grey colony color, The *Colletotrichum acutatum C. capsii* and *C falcatum* is easily differentiated with *C. gleosporoides* and *C frageriae* with the colony color and growth rate on Potato dextrose Agar plate. All the species differ from one another via acrevuli forming and non acervulli forming along with formation of setae. This was characteristics difference for the morphological variability undertaken in the study.

Keywords: Morphological, cultural, among, *Colletotrichum* spp.

Introduction
Anthracnose, a disease caused by *Colletotrichum* species, is a severe impediment to the shelf life and commercial viability of a variety of crops. The genus is important all throughout the globe, producing diseases in a wide range of commercial crops and ornamental plants. (Sutton, 1992; Than *et al*., 2008a-c; Hyde *et al*., 2009) [13, 14, 3]. Colletotrichum is a cause of a range of infections, including fruit rot and dieback. Leaf blight, red rot, and other diseases primarily attack seedlings, followed by shoot and fruit infection. The pathogen thrived and overwintered in the field mostly on fresh and dry leaves, as well as fresh saplings. anthracnose causes significant losses especially It results in significant losses, especially when the weather is favourable, such as rainy and warm conditions (Wang *et al*. 2015) [17]. Colletotrichum spp is a hemibiotrophic fungus that has a relatively short biotrophic phase accompanied by a very aggressive necrotrophic phase that kills large sections of the host plant's cells (O'Connell *et al*. 2012) [7]. A balance of the mechanical and hydrostatic pressure with sequential releasre of cuticle and cell wall degrading enzymes such as cutinase, pectinases, hemicellulases and cellulase is essential for the effective infection. (Huang 2001, Kubicek *et al*. 2014) [4, 6].
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.

Classification systematics of *Colletotrichum* spp.
Tode (1790) [18] was the first to Report *Colletotrichum* in the genus *Vermicularia*. Later in 1831 Cordo introduced it. *Colletotrichum* itself was introduced by Corda (1831) [19] and is now known to comprise "Coelomycetes" with a Glomerella teleomorph stage (Sutton, 1992; Shenoy *et al*., 2007; Hyde *et al*., 2009) [13, 20, 5] Deuteromycetes or fungi imperfecti with acervuli, pycnidia, or stromata as conidiomata are classified as coelomycetes. (Sutton 1980) [12] They are commonly found as conidial, spermatial/microconidial states, or anamorphs of ascomycetes or basidiomycetes, and might just as well be named ascomycetes (Thaung *et al*, 2008) [14]. Colletotrichum is the one genus that has got the greatest attention, owing to the diversity, spread, and severity of species as *C. acutatum, C. gloeosporioides, C. graminicola, C. capsici C. falcatum* etc. represent.

Colletotrichum is a phytopathogen with setose acervuli, moderately large cylindrical or falcate phialo conidia, and appressoria that attacks a wide variety of plant hosts. It includes about 40 plant parasite species and gives anamorphs of Glomerella with a significant reservoir of synonymy that makes precise and convincing systematic investigation difficult (von Arx and Müller 1954; von Arx 1957) [16, 15].

Material and Method

Survey and isolation of pathogen

The fungi that causes anthracnose disease was identified from diseased soybean, turmeric, mango leaves, sugarcane internode, bean, strawberry and chilli. After surface sterilisation 1% $Hgcl_2$, the stems were chopped into small 3-4 mm pieces and stored on potato dextrose agar media. When the pathogen began to proliferate, small pieces of agar containing fungal colonies were cut and injected into PDA and PDA broth, sterilized in autoclave at 121 ℃, which was then maintained in a rotatory shaker for 10-12 days. The mycelial mat was then collected and dried using filter paper before being stored at -20 ℃ for future use. The various *colletotrichium* species which are taken under studies include *C gleosporoides*, *C capsici*, *C lindemuthianum C. acutatum*, *C truncatum* and *Colletotrichum falcatum*.

Table 1: List of locations investigated for gathering naturally occurring anthracnose symptoms in order to examine the pathogen's variability

S. No.	Host	Place of collection	Latitude	Longitude	Plant stage	Isolate name
1.	Chilli	Ajirma	26.05	74.02	Matured Fruit	C1
2.	Soybean	CoA, Raipur	21.25	81.62	Pod maturation	C2
3.	Turmeric	KVK, Ambikapur	23.12	83.18	Leaf	C3
4.	Bean	Surajpur	23.21	82.86	Pod maturation	C4
5.	Sugarcane	KVK, Ambikapur	23.12	83.18	Stem	C5
6.	Mango	RMD CARs, Ambikapur	23.12	83.18	Leaf	C6
7.	Strawberry	KVK, Ambikapur	23.12	83.18	Leaf	C7

The genus Colletotrichum causes anthracnose diseases in large number of agricultural and horticultural crops. And is one of the top 10 plant pathogenic fungi based on economic and scientific investigation (Dean *et al*. 2012) [21]. Earlier it was thought that the genus Colletotrichum is a host specific fungal plant pathogen but later it was proved incorrect as single species has wide range of host crops.

In order to carry out the variability studies and to maintain the genetic purity of the isolates of Colletotrichum spp. single spore isolation technique was used. After the single spore isolation from the naturally infected pods the obtained cultures were then subjected to cultural variability studies.

Result and Discussion

Cultural variability

Colony growth rate: The Colletotrichum spp. isolates showed considerable differences in their grown rate when the cultures were grown on potato dextrose agar media. The readings were taken from 48 hrs after inoculation till 7th day and the growth rate of the cultures were recorded. Colletotrichum spp. isolate C3 showed high growth rate of about 88mm diameter after 7 days followed by C4 and C2 and C7 with growth rate 80 mm while slow growth rate was seen in isolate C1 with 58 mm followed by C5 and C6 with 62mm. The growth rate of all the isolates was as follow.

Table 2: Radial growth of Colletotrichum spp. Isolates on Potato Dextrose Agar Media during 7 days of inoculation

Radial growth Rate(mm)					
S. No.	Host	Sample	3rd day	5th day	7th day
1.	Chilli	C1	10	30	58
2.	Soybean	C2	22	40	80
3.	Turmeric	C3	22	70	88
4.	Bean	C4	44	70	86
5.	Sugarcane	C5	16	39	62
6.	Mango	C6	16	30	64
7.	Strawberry	C7	16	42	80

Table 3: Growth of *Colletotrichum* spp in a radial fashion: The three categories of Fast, medium, and slow growth were used to categorize the cultures

Colony growth Rate	Sample
Fast (85-90)	C3, C4
Medium (79-84)	C2, C7
Slow(below 78)	C1, C5, C6

Conidial morphology

Millions of spores or conidia are produced by *Colletotrichum* spp. isolates on potato dextrose agar. Hyaline, aseptate, falcate-shaped, smooth-walled conidia with acute ends (Fig.1). The spores from the seven-day-old culture were measured using microscopy. Using a research microscope (model Carl Zeiss US 300) with a 100X magnification, conidia of all *Colletotrichum* spp. isolates were seen, and their length and width were assessed. Among all the isolates of *Colletotrichum* spp. maximum conidial length was observed in isolate C2 (26.75µm) followed by C3 (16.52µm), C1 (15.52) whereas conidial length was minimum in sample C1 (4.52µm), C18 (23.1µm), C4 (23.76µm) respectively. Conidial width was maximum in isolates C3 (5.11 µm), C4 (5.03 µm) and minimum in sample C2 (2.91 µm), C7 (3.25 µm), C5 (4.42µm). Conidia of all the isolates of *Colletotrichum* spp. was observed to be non-septate, hyaline, falcate shaped to cylindrical shape and contains an oil droplet in the cytoplasm.

Variation in colour of conidial mass: Acervuli produce millions of spores in groups regarded as spore/ conidial mass. Differences were seen in the colour of the spore mass (Fig. 1) produced by the isolates of *Colletotrichum* spp.

- Conidial masses were white in colour in isolates-C1, C2
- Conidial masses were orange coloured in isolates-C3, C4
- Conidial masses were cream in colour in isolates-C5
- Conidial masses were pale purple in colour in isolates-C6, C7

Sporulation rate: The sporulation rate of a pathogen affects its dispersion capacity, which subsequently affects the spatial distribution of the species. Moreover, it is reported that *Colletotrichum* spp. has limited dispersion capacity, which occurs preferentially between different parts of the same plant and occasionally between adjacent plants.

Table 4: Assessment of sporulation

Categories	Average no. of conidia/ml	Rating
Poor	< 1.5 x 10^4	+
Average	> 1.5 x 10^4 - 2.0 x 10^4	++
Good	> 2.0 x 10^4 - 3.0 x 10^4	+++
Excellent	> 3.0 x 10^4	++++

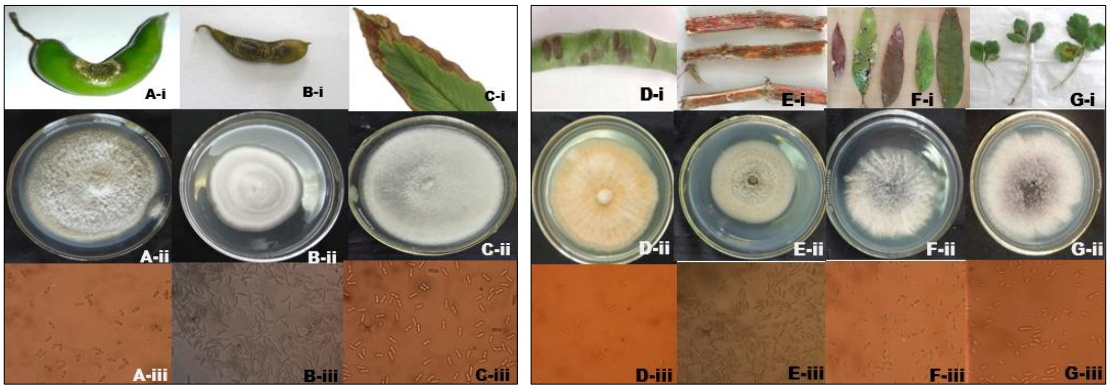
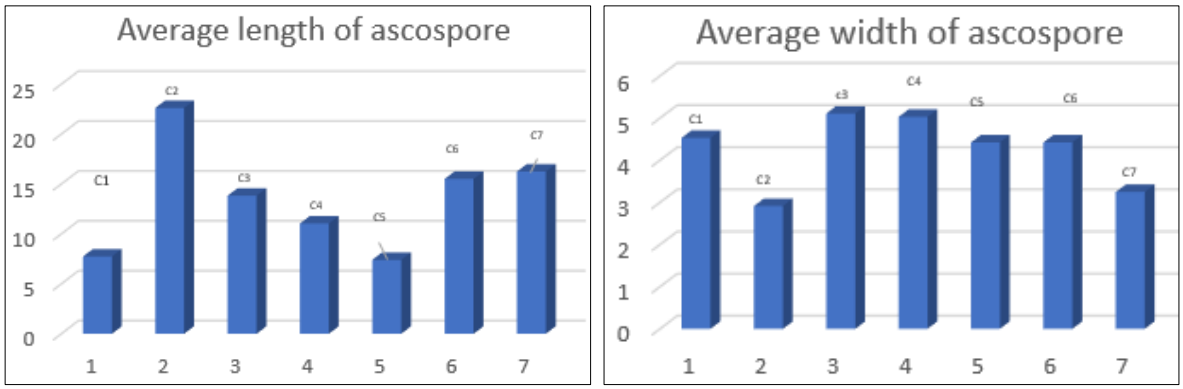


Fig 1: Morphological and cultural morphologies resulting from naturally occurring anthracnose affected by *Colletotrichum* spp from various Chhattisgarh locations A-i-iii Chilli Cylindrical with round ends, colony morphology on PDA, conidia; B-i-iii soybean, Colony morphology on PDA, Conidia; C -i-iii Turmeric, colony morphology on PDA, conidia; D-i-iii Bean, colony morphology on PDA, conidia; E-i-iii Sugarcane, colony morphology on PDA, conidia; F-i-iii Mango, colony morphology on PDA, conidia; G-i-iii Strawberry, colony morphology on PDA, conidia.

Fig 2: Setae and conidiophore of *Colletotrichum* spp.

Table 4: Colony features, conidial and setae dimensions in different samples of *Colletotrichum* spp.

S. No.	Sample	Host	Macroscopic assay			Microscopic Assay		
			Colony Morphology	Acervulus formation and Pigmentation	Growth Rate(average growth rate mm/day)	Spore Shape	Average Spore length (µm)	Average Spore Width (µm)
1.	C1	Chilli	Colony medium, thick, white Arial mycelium circular, concentric rings; reverse plate grey	Formation of acervulus; white color colony	Mean 1.844 SD+1.27	Cylindrical with round ends	15.52 SD+7.9	4.53 SD+ 0.20
2.	C2	Soybean	Colony medium to thick circular reverse plate greyish	Formation of acervulus with white color	2.38 SD+1.37	Falcate shape with pointed tip	22.62 SD+0.85	2.91 SD+0.267
3.	C3	Turmeric	Colony aerial white medium initially but later yellow to orange mass production, reverse plate white	No acervulus formation with orange color	3.144 SD+1.40	Cylindrical with round ends	16.25 SD+ 0.99	5.11 SD+0.305
4.	C4	Bean	Cottony pale orange aerial mycelium orange color mass reverse plate, orange	Orang pigmentation but non acervulus forming	3.33 SD+0.98	Cylindrical with round ends	13.83 SD+1.41	5.03 SD+4.42
5.	C5	Sugarcane	Cottony grey to white velvety mycelium with grey conidial mass, reverse plate; grey	Acervulus not observed	1.83 SD+0.96	Cylindrical with round ends	11.05 SD+1.83	4.42 SD+1.104
6.	C6	Mango	Cottony white margin with slight purple coloration reverse grey to pale purple	Pale purplish pigmentation non acervulus forming	1.86 SD+1.037	Cylindrical with clavate ends	7.7 SD+1.96	4.425 SD+0.46
7.	C7	Strawberry	Cottony aerial mycelium with white margins dark violet colonization reverse plate-violet color	Violet color pigmentation, non acervulus forming	2.41 SD+1.46	Cylindrical with clavate ends	7.4 SD+2.5	3.25 SD+0.314



Bar diagram 1: Bar diagram representing the variation in length and width of conidia produced by species examined during the studies

When cultured on potato dextrose agar, *colletotrichum* spp. isolated from different hosts displayed varying growth rates. The growth culture was monitored and readings were taken from 72 hours after inoculation to the 10th day. After 7 days, the *colletotrichum* spp. sample C4 isolated from bean grew to an 80mm diameter, followed by sample C3 from turmeric leaf, sample C7 from strawberry, and sample C2 from soybean. Sample C5, isolated from sugarcane, had the slowest growth, followed by sample C1, isolated from chilli, and sample C6, from mango.

Colony Colour: The colour of 10 days old culture differed amongst all *colletotrichum* spp isolates. Colors range from grey (C1) to dark grey (C5), greyish white (C2) to white (C3), orange (C4) and pale purplish (C6) to violet (C7).

Acervulus: Samples C1 and C2 produced acervulus, the asexual fruiting body of *Colletotrichum*. Following 10 days after inoculation, the rest of the sample showed mycelial growth (DAI) the maximum output was seen in *C. truncatum* species, followed by sample C1, *colletotrichum capsici*. Acervulus generated millions of spores in condial masses or clusters. In sample C3 isolated from turmeric, the colour of the conidial mass ranged from orange to cream, followed by sample C4 from bean. Sample C5 isolated from sugarcane had a greyish to black tint of conidial mass. The conidial morphology also showed difference on potato dextrose agar producing millions of spores. Conidia are hyaline, aseptate, falcate, smooth walled with acute ends. Micrometry was performed of 10days old culture the highest length was observed in sample C2 (isolate from soybean) with 23.31 µm showing falcate shape conidia followed with spore size 17-20 µm in sample C3 isolated from turmeric. The rest of the conidia where observed to be cylindrical in shape with greater width in sample C3 i.e 5.11 µm (from turmeric) followed within sample C4 with 5.03 µm.

Conclusion

Seven isolates derived from naturally infected plant parts were Sowing symptoms of anthracnose, die back, leaf spot, pod blight, fruit rot, isolated from chilli, turmeric soybean, sugarcane, mango and strawberry showed morphological studies indicating; difference in conidia and setae collected samples of *Colletotrichum* spp. Micrometry resolved the difference in size of the conidia of which varied from 24.51-7.1 µm×5.1-3.24 µm. conidia of the isolates were falcate, to cylindrical with acute tip to clavate base, hyaline, non-septate and oil droplet like appearance. The growth rate was determined by measuring the colony diameter after 10days (mm/day). Morphological characteristics are presented in table.

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