**Cultural and morphological characterization studies on *Colletotrichum* spp. causing Anthracnose disease of soybean in Telangana,India**

**Abstract**

**Aim:** A comprehensive study was conducted to characterize the morphological and cultural variability among Colletotrichum spp. isolates associated with anthracnose disease in soybean from major growing districts of Telangana, India.

**Study Design:** The present study was conducted at the Department of Plant Pathology, CIC, SRTC, IBT and ARS, PJTAU, Telangana*,* India during *Kharif* (June–October, 2023)

**Methodology:** Twenty-seven Colletotrichum isolates (C-1 to C-27) were obtained and characterized based on colony morphology, radial growth, mycelial dry weight and microscopic features such as conidial shape, size and sporulation with different media was recorded by using Completely Randomized Design.

**Results:** Considerable variation was observed among the isolates in colony pigmentation, growth patterns and microscopic morphology with isolate C-17 showing the highest mycelial growth (88.5 mm), sporulation (10.0 × 10⁴ conidia/ml) and biomass production. Further, the growth behavior of isolates was evaluated on four different solid media (PDA, CDA, CMA and SA). Czapek Dox Agar supported the highest average mycelial growth across several isolates whereas Sabouraud Agar showed variable support depending on the isolate.

**Conclusion:** Statistical analysis confirmed significant differences among isolates, media and their interactions. These findings indicate substantial phenotypic variability among Colletotrichum isolates which may influence pathogenicity and environmental adaptability and highlight the importance of further molecular characterization for accurate taxonomic placement.

**Keywords:** Anthracnose, Colletotrichum, Soybean, Variability

1. **INTRODUCTION**

Soybean (*Glycine max* (L.) Merr.), often referred to as the "Yellow Jewel" or "Golden Bean" is one of the most significant oilseed crops in India originating from Eastern Asia, particularly China, soybean has spread to various parts of the world including India. Globally it is cultivated on approximately 146.70 million hectares with a total production of 420.77 million tons and an average productivity of 2870 kg/ha (Anonymous, 2024). The major soybean producing countries are the USA, Brazil, Argentina, China and India. In India, soybean is cultivated over an area of 11.30 million hectares producing about 10.93 million tons with a productivity of 960 kg/ha. Madhya Pradesh is the leading producer in the country earning it the title of the “Soya State”. It is followed by Maharashtra, Rajasthan, Karnataka and Telangana (Hartman et al., 2016). Soybean is crucial globally as a source of vegetable oil and proteins for both human consumption and animal feed (Pagano and Miransari, 2016). However, various diseases severely affect soybean production worldwide. Among the prominent diseases, *Colletotrichum* species causing anthracnose, *Fusarium* wilt, root rot caused by *Macrophomina phaseolina* and soybean mosaic virus (SMV) are the most commonly observed in soybean fields (Singh *et al*., 2020). Anthracnose symptoms on egg plant as small and pale-yellow irregular spots that rapidly enlarged and turned to dark brown and measured up to 3-9 mm Kumar et al. (2010). Anthracnose symptoms on soybean caused by *C. gloeosporioides*, it produced pinkish brown lesions on the pods, and the formation of dark lesions on the leaves and stems, sometimes followed by stem girdling, die back, and distorted growth Mahmodi et al. (2013). Cultural, morphological and pathogenic variability could be preliminary parameters for characterizing the fungal isolates. Variation in races or pathotypes is essential for the understanding of the population. Currently about forty Colletotrichum species were accepted based on detailed studies on morphology, cultural characters and pathogenic abilities (Cannon et al., 2000). Pereira et al. (1998) observed that, bean pod immersed (BPI) medium produced more conidia of *C. lindemuthianum*. Kumar et al. (2017) tested twelve isolates of C. gloeosporioides on four media, among the four media, PDA supported significantly for maximum growth. Drijifhout and Jansen (1989) evaluated the effect of culture media on spore production of *C. lindemuthianum*. Malt extract agar (MEA) gave the highest average spore production, over the ten races considered followed by neopeptone glucose agar (PGA), bean pod agar (BEA) and Czapek‟s dox agar (CDA). Maibam et al. (2015) estimated the yield loss due to anthracnose under protected and non-protected conditions using a susceptible variety Manipuri local -J and reported that reduction in pod weight varied from 9.1 to 11.2%, pod length from 4.3 to 6.6%, breadth 4.5 to 8.8% and thickness 16.6 to 30.5% according to disease severity rating. Telangana, a state in southern India, has emerged as an important soybean growing region. With an increasing area under soybean cultivation, farmers in Telangana face several challenges, including biotic and abiotic stress factors affecting crop productivity. Among the biotic stresses, fungal diseases pose a significant threat to soybean production with anthracnose caused by *Colletotrichum* spp. being a major concern. Despite the increase in production due to its diverse applications in food, feed and industry, soybean cultivation still faces several biotic and abiotic challenges. Among the biotic stresses, anthracnose poses a significant threat, as it infects both plants and seeds potentially leading to yield losses of upto 100% (Hartman et al., 2016).

1. **MATERIALS AND METHODS**
	1. **Collection of diseased samples**

Sampling for this study was conducted across major soybean growing districts of farmers’ fields in Telangana. From each field, petiole, stem, leaf and pod exhibiting symptoms of anthracnose disease were collected. The collected samples were stored in sterile polyethylene bags and subsequent brought to laboratory for further investigation.

* 1. **Isolation and maintenance of pathogen**

*Colletotrichum* isolates were obtained from lesions on leaf, petiole, pods and seed of soybean from major growing districts of Telangana. Small tissue pieces (5 × 5 mm) were excised from margins of diseased plant tissue of soybean. The samples were surface sterilized by immersion in 1% sodium hypochlorite solution for 3-5 minutes followed by rinsing two to three times with sterile distilled water. Sterilized tissues were then placed on Potato Dextrose Agar (PDA) plates and incubated at room temperature (25+2°C) with regular observations for fungal growth. Emerging fungal hyphae from the tissue margins were aseptically transferred to PDA slants for further development. Fungi were identified based on sporulation characteristics and pure cultures were maintained on PDA slants at 4°C for long-term storage.

### ****2.3. Cultural and morphological characterization of**** Colletotrichum ****isolates****

### ****2.3.1. Cultural characterization on PDA medium****

The cultural characteristics of *Colletotrichum* isolates were evaluated on Potato Dextrose Agar (PDA) medium. Each isolate was inoculated onto PDA plates and incubated at (25+2°C) for 15 days. Observations were recorded regularly for colony diameter to determine radial growth and calculate the growth rate (mm/day). Colony morphology including colour (upper and lower surfaces), texture, margin type and zonation patterns was carefully documented. After the incubation period, mycelial dry weight was determined by harvesting the fungal biomass on pre-weighed filter paper drying it at 60°C until a constant weight was reached and calculating the net dry mass in milligrams.

**2.3.2. Microscopic and morphological characterization**

For microscopic evaluation, conidia were harvested from 15-day old cultures. Spore suspensions were prepared and examined under a stereobinocular microscope (Carl Zeiss, Model US 300). Conidial morphology was characterized by measuring the length, width and length to width (L/W) ratio using a calibrated eyepiece micrometer at 100X magnification. The number of oil globules per conidium was recorded and the presence of diagnostic features such as setae and acervuli was noted. Sporulation was quantified and expressed as spore concentration in units of 10⁴ conidia/ml. The integrated cultural and microscopic assessments enabled detailed identification and differentiation of the *Colletotrichum* isolates based on phenotypic variability.

1. **RESULTS AND DISCUSSION**

**3.1. Collection of diseased samples**

Twenty-seven *Colletotrichum* isolates (C-1 to C-27) were obtained from typical anthracnose lesions found on petioles and pods of soybean and were characterized based on colony morphology, pigmentation, mycelial growth and dry weight on PDA medium and are presented in Table 1.

Agostini and Timmer (1992) and Freeman et al. (1998) suggested that *Colletotrichum* spp., can easily be isolated by incubating infected seeds, plant parts *etc.,* on sterilized moist blotter or PDA plates or often disinfecting with 0.1N NaOCl.

* 1. **Isolation and maintenance of pathogen**

The observations included colony colour, growth pattern, margin type, upper and lower surface pigmentation of the Petri plate, radial mycelial growth (mm), daily growth rate (mm day⁻¹) and mycelial dry weight (mg). Most isolates exhibited regular growth patterns with colony colours ranging from whitish grey, blackish grey, olive green to velvety shades of black and grey. Margins were mostly regular and some of the isolates (C-6, C-8, C-9, C-10, C-20, C-22, C-23 and C-27) showed irregular margins (Table 1). Upper surface colours varied widely including white, greyish, pink, chocolate brown and olive brown while the lower surfaces were predominantly yellowish black or brownish black. The maximum radial mycelial growth was recorded in isolate C-5 (88.65 mm) followed closely by C-4 and C-17 (88.5 mm) while the minimum was observed in C-26 (54.75 mm). Correspondingly, growth rates ranged from 3.65 mm day⁻¹ (C-26) to 5.91 mm day⁻¹ (C-5). Mycelial dry weight varied significantly with the highest observed in isolate C-20 (239.5 mg) and the lowest in C-4 (180.1 mg) (Table 4).

Alam and Rudolph (1993) isolated several strains of *C. lindemuthianum* from anthracnose infected bean plants (*P. vulgaris*) collected from fields at different locations in Turkey. While, Dillard and Cobb (1993) isolated *C. lindemuthianum* from anthracnose infected plant debris of bean (*P. vulgaris*).

* 1. **Cultural and and morphological characterization of Colletotrichum isolates**

### ****3.3.1. Cultural characterization on PDA medium****

The morphological characterization of 27 fungal isolates (C-1 to C-27) revealed considerable variation in conidial size, shape, length-to-width (L/W) ratio, number of oil globules and sporulation. All isolates exhibited sporulation (+) and presence of setae was confirmed in each isolate.

Tozze Junior et al. (2006) isolated *Colletotrichum* spp., from hot pepper, sweet pepper and garden egg plant and observed morphological and physiological variability among the isolates. Morphological characterization was based on the size and shape of conidia and the shape of appresoria of 30 isolates.

**3.3.2. Microscopic and morphological characterization**

The length of conidia ranged from 24.25 µm (C-9) to 28.95 µm (C-17). The width ranged from 3.15 µm (C-23) to 4.1 µm (C-27). The highest L/W ratio was recorded in C-10 (8.24) and C-23 (8.22) indicating more slender conidia while the lowest was in C-13 (6.39), denoting shorter and broader conidia. Conidial shapes were diverse with both straight and fusiform forms predominant. Several isolates (*e.g.,* C-2, C-4, C-6, C-10, C-14, C-17, C-20, C-23 and C-25) exhibited typical fusiform conidia with pointed tips. The isolates C-5 and C-11 exhibited straight conidia with rounded tips while isolates like C-13 and C-15 showed curved or bulbous middle regions indicating possible taxonomic or physiological diversity among the isolates. The number of oil globules per conidium ranged from 1 to 3 with most isolates containing 2 globules. The highest oil globule count was recorded in C-17 (3 globules). Oil globules can be indicative of energy reserves and may relate to the metabolic activity or stress tolerance of the isolates. The sporulation capacity ranged from 3.0 (C-4, C-8 and C-21) to 10.0 (C-17). The isolate C-17 which had the highest conidial length and oil globules also exhibited the highest sporulation suggesting a potential correlation between conidial morphology and reproductive efficiency. On the other hand, C-4 and C-8 showed lower sporulation despite typical fusiform conidial shapes. All isolates produced setae, which are often associated with protection against environmental stress and could play a role in host infection.The critical difference (CD) at 1% significance was 2.01 µm for length and 0.29 µm for width with standard errors (SE) of ±0.71 µm and ±0.10 µm, respectively. These values confirm that the observed variation in conidial measurements among isolates is statistically significant (Table 2).

Pria et al. (1997) tested different culture media for induction of sporulation in *C. lindemuthianum* and reported that; bean pod agar was best medium for growth and sporulation when incubated at 20°C in dark condition for 20 days.

The growth characteristics of 27 fungal isolates (C-1 to C-27) were studied on four different solid media: Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Corn Meal Agar (CMA) and Sabouraud Agar (SA). The mycelial growth (mm) and growth rate (mm/day) were recorded and analyzed to evaluate cultural variability.

### Significant variation in mycelial growth was observed among isolates and across media. The **mean mycelial growth** across isolates ranged from **60.13 mm (C-11)** to **87.38 mm (C-17)**with the highest growth observed in **C-17 (87 mm on PDA and 89.5 mm on SA)** suggesting robust mycelial growth irrespective of media. Among the media, CDA showed the highest average growth for most isolates (*e.g.,* C-2, C-4, C-21, C-22) indicating its rich nutritional content is favorable for fungal growth. CMA and PDA also showed considerable growth while SA showed slower growth particularly in isolates like C-10 (57 mm) and C-18 (49 mm). The lowest mycelial growth was noted in C-11 (51 mm on PDA) and C-22 (41 mm on CMA). The statistical analysis revealed that differences due to isolates (CD at 1% = 2.739 mm), media (CD at 1% = 1.054 mm) and their interaction (CD at 1% = 5.477 mm) were statistically significant confirming true biological variation. The growth rate showed a trend similar to mycelial diameter. The mean growth rate ranged from 4.01 mm/day (C-11) to 5.83 mm/day (C-17). Again, C-17 exhibited the highest growth rate across all media, peaking at 5.97 mm/day on SA, highlighting its adaptability and rapid colonization ability. Among the media, CDA and SA showed relatively higher mean growth rates. Several isolates such as C-13, C-21, C-26 and C-27 also demonstrated consistently high growth rates (>5.4 mm/day), indicating strong adaptability. The lowest growth rates were found in C-10 (mean 4.43 mm/day) and C-11 (mean 4.01 mm/day). The critical difference (CD) at 1% for growth rate due to isolates, media and interaction were 0.186 mm/day, 0.072 mm/day, and 0.372 mm/day, respectively which confirms that these differences are statistically significant (Table 3).

Growth and cultural characteristics of *C. lindemuthianum* was studied in different media by Rajesha and Mantur (2014) and observed the significant differences among the media tested. The maximum mean colony growth (81.00 mm) was recorded in potato dextrose agar which was statistically on par with Richard‟s agar (79.00 mm) and significantly different from Brown‟s agar (72.33 mm) and Czapek‟s dox agar (69.67 mm). The least mean colony diameter (60.00 mm) was noticed in Sach‟s agar medium.

Mota et al. (2016) reported the growth of *Colletotrichum* species on M3 culture medium. *C. lindemuthianum* group showed grey to black color at the beginning of growth and were totally black at 15 days. Conidia were cylindrical and size was ranged from 12.58 μm (LV 228) to 17.47 μm (LV 145 (length) and 3.78 μm (LV 134) to 6.09 μm (width). On the basis of conidial morphology, their size, acervuli and setae, the representative sample was identified tentatively as *C. lindemuthianum* and the morphological characteristics were tallied with Mordue (1971). The descriptive characters of the fungus *C. lindemuthianum* were similar to the descriptions of Desai and Prasad (1955), Barnett and Hunter (1972), Sutton (1973), Kulshrestha et al. (1976), Sutton (1980), von Arx (1981). These characters further supported by findings about spore dimensions in culture (Wijesekara and Agarwal, 2006 and Khan et al., 2010).

**Table 1. Morphological characters of *Colletotrichum* spp on PDA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate code** | **Colony characters** | **Colony colour**  |  **Mycelial dry weight**  |
| **Growth pattern**  | **Mycelial growth**  | **Growth rate (mm day-1)**  | **Margin**  | **Upper side of Petri plate**  | **Lower side of Petri plate**  | **(mg)**  |
| **(mm)**  |   |
| C-1 | Whitish grey  | 87.00 | 5.80 |  Regular  | White  | Yellowish black | 216.00 |
| C-2 | Blackish grey | 87.75 | 5.85 |  Regular  | White  | Yellowish black | 225.00 |
| C-3 | Whitish grey | 88.20 | 5.88 |  Regular  | Grayish white  | Yellowish black | 222.15 |
| C-4 | Whitish grey  | 88.50 | 5.90 |  Regular  | Whitish grey  | Yellowish black | 180.10 |
| C-5 | Velvety grey  | 88.65 | 5.91 |  Regular  | Chocolate brown  | Yellowish black | 231.2 |
| C-6 |  Velvety black | 73.50 | 4.90 |  Irregular  | Olive brown  | Yellowish black | 230.2 |
| C-7 | Olive green  | 72.75 | 4.85 |  Regular  | White  | Black | 210.3 |
| C-8 | Velvety black | 75.00 | 5 |  Irregular  | White  | Brownish black | 210.65 |
| C-9 | Velvety grey | 67.50 | 4.5 |  Irregular  | Whitish brown  | Yellowish | 230 |
| C-10 | Blackish white | 66.75 | 4.45 |  Iregular  | Whitish black  | Yellowish black | 212.35 |
| C-11 | Whitsh grey  | 82.5 | 5.5 |  Regular  | Pink  | Yellowish black | 230 |
| C-12 | Greyish | 70.5 | 4.7 |  Regular  | Whitish grey  | Yellowish black | 227.5 |
| C-13 | Blackish grey  | 69 | 4.6 |  Regular  | Olive brown  | Yellowish black | 238.3 |
| C-14 | Olive green  | 66.75 | 4.54 |  Regular  | Light brown  | Yellowish black | 220.65 |
| C-15 | Greyish white  | 69 | 4.6 | Regular | Black  | Yellowish black | 228.2 |
| C-16 | Black brown | 84 | 5.6 | Regular  | Whitish black  | Black | 212.1 |
| C-17 | Blackish white | 88.5 | 5.9 |  Regular  |  Creamy white  |  Brownish black  | 232.5 |
| C-18 | Olive green | 84 | 5.6 |  Regular  | Grayish white with purple tinge  | Brownish black  | 235.5 |
| C-19 | Black grey  | 82.5 | 5.5 |  Regular  |  Brownish white  | Brownish black  | 220.5 |
| C-20 | Olive grey  | 82.5 | 5.5 |  Irregular  | White  | Yellowish | 239.5 |
| C-21 | Whitish grey  | 81 | 5.4 |  Regular  | Blackish white  | Yellowish | 235.1 |
| C-22 | Blackish white  | 85.5 | 5.7 |  Irregular  | Olive brown  | Yellowish | 220.1 |
| C-23 | Black | 87.75 | 5.65 |  Irregular  | Light grayish white  | Black | 219.5 |
| C-24 | Whitish grey  | 56.25 | 3.75 | Regular  | White  | Black | 220.1 |
| C-25 | Greyish white  | 73.45 | 4.91 |  Regular  | White  | Black | 210.6 |
| C-26 | Grey  | 54.75 | 3.65 |  Regular  | Blackish white  | Black | 225.25 |
| C-27 | Black  | 77.25 | 5.15 | Irregular  | White with light grey  | Yellowish black | 238.5 |
| CD  | - | 6.11 | 0.41 | - | - | - | 17.53 |
| SE(m)± | - | 2.15 | 0.14 | - | - | - | 6.17 |
| CV (%) | - | 4.80 | 4.80 | - | - | - | 4.79 |

**Table 2. Cultural characteristics of *Colletotrichum* spp. on PDA**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Isolate code**  | **Conidia size (µm)**  | **L/W**  | **Shape**  | **Number of oil globules**  | **Sporulation**  | **Setae**  |
| **Length**  | **Width**  | **ratio**  | **(10-4)**  |  |
| C-1 | 27.15 | 3.85 | 7.05 | Straight round at middle and rounded tip one side and pointed tip on other side | 3 | 5 | + |
| C-2  | 26.20 | 3.25 | 8.06 | Fusiform shaped having rounded tip at one side, pointed tip on other side | 2 | 4 | + |
| C-3 | 27.35 | 3.36 | 8.14 | Straight having having rounded tip at one side, pointed tip on other side | 2 | 4 | + |
| C-4 | 26.10 | 3.35 | 7.79 | Fusiform having pointed tip both the sides  | 2 | 3 | + |
| C-5 | 25.25 | 3.50 | 7.21 | Straight and rounded tip both the sides | 2 | 6 | +  |
| C-6 | 26.5 | 3.8 | 6.97 | Fusiform having pointed tip one side and rounded tip on other side | 3 | 3 | +  |
| C-7 | 27.5 | 3.8 | 7.24 | Fusiform having pointed tip both the sides | 1 | 5.5 | +  |
| C-8 | 25.3 | 3.8 | 6.66 | Fusiform having pointed tip one side and rounded on other side | 2 | 3 | + |
| C-9 | 24.25 | 3.25 | 7.46 | Straight slightly curved in the pointed tips both the sides | 2 | 3.9 | + |
| C-10 | 27.6 | 3.35 | 8.24 | Fusiform both the tips are pointed  | 2 | 3.5 | + |
| C-11 | 26.15 | 3.95 | 6.62 | Straight in the middle and having rounded tips both the sides | 2 | 3.5 | + |
| C-12 | 25.5 | 3.8 | 6.71 | Straight slight curved in the middle and both the tips are pointed | 1 | 4.5 | + |
| C-13 | 24.6 | 3.85 | 6.39 | Straight and curved in the middle and pointed in the both the tips | 2 | 3.5 | + |
| C-14 | 27.55 | 3.5 | 7.87 | Fusiform and tips are pointed | 2 | 3 | + |
| C-15 | 24.5 | 3.8 | 6.45 | Straight ad curved in the middle and tips are pointed | 2 | 6 | + |
| C-16 | 25.5 | 3.5 | 7.29 | Straight and cylindrical having rounded tip one side and pointed tip on other side  | 1 | 9.5 | + |
| C-17 | 28.95 | 3.75 | 7.72 | Spindle shape and fusiform and tips are pointed | 2 | 10 | + |
| C-18 | 26.8 | 3.75 | 7.15 | Straight and both pointed ends and a few are dumbbell shaped | 2 | 7 | + |
| C-19 | 24.85 | 3.8 | 6.54 | Fusiform and tips are pointed | 1 | 9.5 | + |
| C-20 | 26.8 | 3.85 | 6.96 | Fusiform and tips are pointed  | 2 | 6.5 | + |
| C-21 | 26.15 | 3.85 | 6.79 | Fusiform and tips are pointed | 2 | 5.5 | + |
| C-22 | 24.55 | 3.5 | 7.01 | Straight and middle part bulbous curve  | 2 | 7 | + |
| C-23 | 25.88 | 3.15 | 8.22 | Fusiform and tips are pointed | 1 | 6 | + |
| C-24 | 24.8 | 3.8 | 6.53 | Straight and middle part bulbous curve and pointed tips | 2 | 6.5 | + |
| C-25 | 24.55 | 3.5 | 7.01 | Fusiform and tips are pointed | 2 | 7.5 | + |
| C-26 | 25.8 | 3.55 | 7.27 | Straight and middle part bulbous curve and pointed tips | 1 | 4.5 | + |
| C-27 | 27.7 | 4.1 | 6.76 | Straight and middle part bulbous curve | 2 | 3.5 | + |
|  CD 1% | 2.01 | 0.29 |  **-**  |  **-**  |  **-**  | 0.44 |  **-**  |
| SE (m)± | 0.71 | 0.1 |  **-**  |  **-**  |  **-**  | 0.16 |  **-**  |
| CV (%) | 4.68 | 4.78 |  **-**  |  **-**  |  **-**  | 5.01 |  **-**  |

|  |
| --- |
| **Table 3. Cultural variability on different solid media** |
|  |  |  |  |  |  |  |  |  |  |  |
| **Isolate code**  | **Mycelial growth (mm)**  | **Growth rate (mm/day)** |
| **PDA** | **CDA** | **CMA** | **SA** | **Mean** | **PDA** | **CDA** | **CMA** | **SA** | **Mean** |
| C-1 | 68.9 | 81.35 | 76.5 | 66 | 73.19 | 4.59 | 5.42 | 5.10 | 4.4 | 4.88 |
| C-2 | 75.2 | 84.5 | 85 | 79.5 | 81.05 | 5.01 | 5.63 | 5.67 | 5.3 | 5.4 |
| C-3 | 88.25 | 83.0 | 87.5 | 67.5 | 81.56 | 5.88 | 5.53 | 5.83 | 4.5 | 5.44 |
| C-4 | 54.9 | 86.0 | 74 | 69.5 | 71.1 | 3.66 | 5.73 | 4.93 | 4.63 | 4.74 |
| C-5 | 71.8 | 70.5 | 68 | 68 | 69.58 | 4.79 | 4.70 | 4.53 | 4.53 | 4.64 |
| C-6 | 69.2 | 71 | 69.5 | 70.0 | 69.93 | 4.61 | 4.73 | 4.63 | 4.67 | 4.66 |
| C-7 | 75.2 | 69.5 | 70.5 | 75.5 | 72.68 | 5.01 | 4.63 | 4.70 | 5.03 | 4.85 |
| C-8 | 81.0 | 64.5 | 81 | 58.0 | 71.13 | 5.40 | 4.30 | 5.40 | 3.87 | 4.74 |
| C-9 | 82.2 | 84.5 | 68 | 60.0 | 73.68 | 5.48 | 5.63 | 4.53 | 4.00 | 4.91 |
| C-10 | 85 | 72.5 | 51 | 57.0 | 66.38 | 5.67 | 4.83 | 3.40 | 3.80 | 4.43 |
| C-11 | 51 | 64 | 69.5 | 56 | 60.13 | 3.40 | 4.27 | 4.63 | 3.73 | 4.01 |
| C-12 | 54.5 | 76 | 81 | 58 | 67.38 | 3.63 | 5.07 | 5.40 | 3.87 | 4.49 |
| C-13 | 82 | 81 | 86 | 87.5 | 84.13 | 5.47 | 5.40 | 5.73 | 5.83 | 5.61 |
| C-14 | 59 | 82 | 76.5 | 73.5 | 72.75 | 3.93 | 5.47 | 5.10 | 4.90 | 4.85 |
| C-15 | 61 | 83.5 | 55 | 65.5 | 66.25 | 4.07 | 5.57 | 3.67 | 4.37 | 4.42 |
| C-16 | 80 | 80 | 81 | 70.0 | 77.75 | 5.33 | 5.33 | 5.40 | 4.67 | 5.18 |
| C-17 | 87 | 88 | 85 | 89.5 | 87.38 | 5.80 | 5.87 | 5.67 | 5.97 | 5.83 |
| C-18 | 80 | 74 | 79.5 | 49 | 70.63 | 5.33 | 4.93 | 5.30 | 3.27 | 4.71 |
| C-19 | 76 | 81 | 69.5 | 80 | 76.63 | 5.07 | 5.40 | 4.63 | 5.33 | 5.11 |
| C-20 | 84 | 75.5 | 70 | 76 | 76.38 | 5.60 | 5.03 | 4.67 | 5.07 | 5.09 |
| C-21 | 79 | 79.5 | 85 | 89 | 83.13 | 5.27 | 5.30 | 5.67 | 5.93 | 5.54 |
| C-22 | 78.65 | 88 | 41 | 79 | 71.66 | 5.24 | 5.87 | 2.73 | 5.27 | 4.78 |
| C-23 | 84 | 61 | 80 | 88 | 78.25 | 5.60 | 4.07 | 5.33 | 5.87 | 5.22 |
| C-24 | 85 | 69.5 | 82.5 | 80 | 79.25 | 5.67 | 4.63 | 5.5 | 5.33 | 5.28 |
| C-25 | 84.5 | 71 | 79 | 79.5 | 78.5 | 5.63 | 4.73 | 5.27 | 5.3 | 5.23 |
| C-26 | 82 | 73.5 | 85 | 88 | 82.13 | 5.47 | 4.90 | 5.67 | 5.87 | 5.48 |
| C-27 | 74 | 77 | 86.5 | 89 | 81.63 | 4.93 | 5.13 | 5.77 | 5.93 | 5.44 |
| Factors  | C.D. | SE(d) | SE(m) |  |  | C.D at | SE(d) | SE(m) |  |  |
| at 1% | 1% |
| Isolates | 2.739 | 1.388 | 0.982 |  |  | 0.186 | 0.094 | 0.067 |  |  |
| Media  | 1.054 | 0.534 | 0.378 |  |  | 0.072 | 0.036 | 0.026 |  |  |
| Interaction  | 5.477 | 2.777 | 1.963 |  |  | 0.372 | 0.188 | 0.133 |  |  |

**Table 4. Dry mycelial weight on different media**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Isolate code**  | **Mycelial dry weight (mg)** | **Mean (mg)**  |
| **PDA** | **CDA** | **CMA** | **SA** |
| 1 | C-1 | 216.00 | 222.50 | 212.80 | 210.15 | 215.36 |
| 2 | C-2 | 225 | 231 | 210 | 206.8 | 218.20 |
| 3 | C-3 | 222.15 | 201.8 | 221 | 195.5 | 210.11 |
| 4 | C-4 | 180.1 | 236.6 | 205 | 209 | 207.68 |
| 5 | C-5 | 231.2 | 234.8 | 212.5 | 206.5 | 221.25 |
| 6 | C-6 | 230.2 | 240 | 214 | 207.45 | 222.91 |
| 7 | C-7 | 210.3 | 221.5 | 216.7 | 210.6 | 214.78 |
| 8 | C-8 | 210.65 | 218.5 | 214.2 | 208 | 212.84 |
| 9 | C-9 | 230 | 231.25 | 217.1 | 210 | 222.09 |
| 10 | C-10 | 212.35 | 214.75 | 208.6 | 206 | 210.43 |
| 11 | C-11 | 230 | 230 | 213.1 | 202.9 | 219.00 |
| 12 | C-12 | 227.5 | 231.8 | 212.5 | 189.1 | 215.23 |
| 13 | C-13 | 238.3 | 232 | 228 | 212.5 | 227.70 |
| 14 | C-14 | 220.65 | 236.5 | 221 | 213 | 222.79 |
| 15 | C-15 | 228.2 | 230 | 199.5 | 214 | 217.93 |
| 16 | C-16 | 212.1 | 218 | 216.8 | 201.5 | 212.10 |
| 17 | C-17 | 239.5 | 242.5 | 202 | 205.6 | 222.40 |
| 18 | C-18 | 235.5 | 240.1 | 222 | 213.8 | 227.85 |
| 19 | C-19 | 220.5 | 229.2 | 221 | 216.5 | 221.80 |
| 20 | C-20 | 239.5 | 242.5 | 224.4 | 211 | 229.35 |
| 21 | C-21 | 235.1 | 221 | 228 | 219 | 225.78 |
| 22 | C-22 | 220.1 | 232 | 226.7 | 212.65 | 222.86 |
| 23 | C-23 | 219.5 | 222.1 | 210.3 | 189.5 | 210.35 |
| 24 | C-24 | 220.1 | 226.8 | 222 | 201.5 | 217.60 |
| 25 | C-25 | 210.6 | 218.2 | 213.9 | 183 | 206.43 |
| 26 | C-26 | 225.25 | 227 | 214 | 215 | 220.31 |
| 27 | C-27 | 238.5 | 239 | 205.2 | 210 | 223.18 |
|  Mean (mg)  |   |   |   |   |   |  Mean (mg)  |
| Factors  | CD at 1 %  | SE (d)  | SE (m) ±  |   |   | Factors  |
| Media (A)  | 3.13 | 4.59 | 1.12 |   |   | Media (A)  |
| Isolate (B)  | 8.13 | 4.13 | 2.92 |   |   | Isolate (B)  |
| Interaction (A x B)  | 16.28 | 8.25 | 5.84 |   |   | Interaction (A x B)  |

**CONCLUSION**

The results of our study discloses that the morphological diversity among the fungal isolates particularly in conidial size, shape and sporulation suggests significant phenotypic variability that may correspond to ecological or pathogenic variability. Isolate C-17 stands out due to its superior traits in conidial size, oil globule content and sporulation indicating potential. The cultural variability among fungal isolates and their interaction with different media. The isolate C-17 stands out as the most vigorous, displaying superior growth across all media types. CDA emerged as the most supportive medium for mycelial growth but overall growth performance varied significantly by both isolate and media. The dry weight of mycelia was assessed to evaluate the biomass production capacity of 27 fungal isolates (C-1 to C-27) grown on four different media *viz*., Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Corn Meal Agar (CMA) and Sabouraud Agar (SA). The variability suggests that nutrient availability, strain-specific metabolic responses and media composition influence mycelial growth and could be pivotal in selecting optimal conditions especially in contexts such as pathogenicity. Further molecular identification would help confirm taxonomic placements and potential functional roles of these isolates.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, *etc.*) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

Agostini, J.P and Timmer, L.W. 1992. Selective isolation procedure for differentiation of two strains of *Colletotrichum gloeosporioides* from citrus. *Plant Disease*. 76: 1176- 1178.

Alam, M and Rudolph, K. 1993. Occurrence and characterization of the races of bean anthracnose (*Colletotrichum lindemuthianum*) in Turkey. *Phytopathologia Mediterranea*. 32 (3): 228-234.

Anonymous, 2024. Soybean Statistics ICAR, Available online: <https://icar.org.in> sites/default/files/2025-04/ICAR%20Annual%20Report%202023-24-english.pdf

Barnett, H.L and Hunter, B.H. 1972. Illustrated Genera of Imperfect fungi. 3rd edition. Burgess publishing Co. Minneapolis, USA. 231.

Cannon, P.F., Bridge, P.D and Monte, E. 2000. Linking the past, present and future of *Colletotrichum systematics*. In: D. Prusky, S. Freeman and M.D. Dickman (eds.) *Colletotrichum* host specificity, pathology and host pathogen interaction. American Phytopathological Society Press, Saint Paul. Minnesota. USA. 1-20.

Desai, M.V and Prasad, N. 1955. A new *Colletotrichum* from India. *Indian Phytopathology*. 8 (1): 52- 57.

Dillard, H.R and Cobb, A.C. 1993. Survival of *Colletotrichum lindemuthianum* in bean debris in New York State. *Plant Disease*. 77: 1233-1238.

Drijfhout, E and Jansen, J. 1989. Effect of culture medium on spore production and germination of races of *Colletotrichum lindemuthianum*. *Netherland Journal of Plant Pathology*. 95: 119-122.

Freeman, S., Katan, T and Shabi, E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Disease. 82 (6): 596605.

Hartman, G.L., West, E.D and Herman, T.K. (2016). Crops that feed the world 2. Soybean worldwide production, use, and constraints caused by pathogens and pests. *Food Security,* 3(1), 5-17.

Khan, A.M., Khan, Z.S and Nasreen, S. 2010. Efficacy of some fungicides against anthracnose on mungbean caused by *Colletotrichum lindemuthianum*. *International Journal of Plant Sciences*. 5(1): 70-73

Kulshrestha, D.D., Mathur, S.B and Neergaard, P. 1976. Identification of seed-borne species of *Colletotrichum*. *Freesia*. 11: 116-124.

Kumar, A., Singh, T.K., Prashant Kumar, Bose, U.S and Tiwari, R.K. 2017. Pathological and cultural variability in *Colletotrichum gloeosporioides* (Penz. and Sacc.) inciting anthracnose of mango. *International Journal of Current Microbiology and Applied Science*. 6(10): 2543-2550.

Kumar, K., Bhagat, S., Madhuri, K., Amarwsan, N and Srivastava, R.C. 2010. Morphological and molecular characterization of Colletotrichum species causing anthracnose disease in Bay Islands. *Indian Journal of Mycology and Plant Pathology*. 40 (3): 322-330.

Mahmodi, F., Kadir, J.B., Wong, M.Y., Nasehi, A and Puteh, A. 2013. First report of anthracnose caused by *Colletotrichum gloeosporioides* on soybean (*Glycine max*) in Malaysia. *Plant Disease*. 97(6): 841.

Maibam, N., Chandra, S., Baiswar, P., Majumder, D and Saikia, K. 2015. Host plant resistance and yield loss due to anthracnose caused by *Colletotrichum lindemuthianum* in French bean (*Phaseolus vulgaris*). *Indian Journal of Hill Farming*. 28 (1): 14-18.

Mordue, J.E.M. 1971. CMI description of Pathogenic Fungi and Bacteria, Sheet No. 315, 316, 317 Common wealth Mycological Institute, Survey, Kew, England. 2.

Mota, S.F., Barcelos, Q.L., Dias, M.A and Souza, E.A. 2016. Variability of *Colletotrichum* spp., in common bean. *Genetics and Molecular Research*. 15 (2): 7-15.

Pagano, M.C., and Miransari, M. (2016). The importance of soybean production worldwide. *Abiotic and Biotic Stresses in Soybean Production, 1*(1), 1-26.

Pereira, J.C.R., Batista, U.G., Gulmaraes, F.B and Misubuti, E.S.B. 1998. Effect of different culture media on the sporulation and inoculum potential of *Colletotrichum lindemuthianum*. *Summa Phytopathology*. 24 (2): 186-189.

Pria, M.D., Bergamin Filho, A and Amorin, L.1997. Evaluation of different culture media for sporulation of *Colletotrichum*. *Summa Phytopathologica*. 23 (2): 181183.

Rajesha, G and Mantur, S.G. 2014. Studies on morphological and cultural characters of *Colletotrichum lindemuthianum* inciting anthracnose of *Dolichos* bean. *Journal of Mycopathological Research*. 52 (1): 121-124.

Sharma, P., and Gupta, S.K. (2019). Management of soybean anthracnose through integrated approaches. *Journal of Oilseed Research, 36*(2): 110-118.

Singh, R., Tripathi, R.K., Mishra, R.K., and Pandey, S. (2020). Prevalence and management of major fungal diseases in soybean. *Indian Phytopathology, 73*(3), 1-12.

Sutton, B.C. 1973. Suprageneric classification of Deuteromycotina. Proceedings of International Symposium, Madras. 235.

Sutton, B.C. 1980. The Coelomycetes: Fungi imperfecti with pycnidia, acervuli, and stromata. Common wealth Mycological Institute, Kew, Surrey, England. 523-527.

Telenko, D.E.P., Babadoost, M., and Bradley, C.A. (2018). Anthracnose of soybean. *Crop Protection Journal. 32*(2): 145-152.

Tozze Junior, Mello, H.J., Massola, M.B.A and Junior, N.S. 2006. Morphological and physiological characterization of *Colletotrichum* sp. isolates from solanaceous crops. *Suma Phytopathology*. 32(1): 71-79.

Von Arx, J.A. 1981. The genera of fungi sporulating in pure culture, 3rd edn. Stechert Hafner, New York, p. 28.

Wijesekara, R.H.T and Agarwal, D.K. 2006. Taxonomic studies on five species of the genus *Colletotrichum*. *Indian Phytopathology*. 59(2): 203-209.