Symptomatology and morpho-cultural characterization of *Colletotrichum musae*, a causal agent of anthracnose disease in banana

Abstract

The study focused on the identification and characterization of *Colletotrichum musae* as a significant post-harvest fungal pathogen causing anthracnose disease in bananas. Symptoms included sunken, water-soaked spots on infected tissues, leading to soft, red-brown to black-coloured spots with irregular shapes. The pathogen was isolated and cultured on Potato dextrose agar medium, and its pathogenicity was confirmed by inoculating healthy banana fruits, resulting in typical anthracnose-like spots. Cultural characteristics of *C. musae* on PDA revealed whitish to black colonies, and microscopic examination showed oblong to cylindrical, single-celled conidia. Acervuli, which were salmon-coloured initially and later turned dark brown to black, were observed on the lesions. The fungus exhibited maximum growth and sporulation on PDA, followed by oat meal agar and Richard's agar. Additionally, the study highlighted the morphological features of *C. musae*, including septate, irregularly branched, and vacuolated mycelium. The findings contribute to understanding the pathology and characteristics of *C. musae*, providing valuable information for managing post-harvest anthracnose in bananas.

Keywords: Post harvest pathogen, *Musa* sp., fruit decay, *C. musae*, isolation, Kochs postulates

1. INTRODUCTION

Banana (*Musa paradisica* L.) is an important fruit crop in tropical and sub-tropical regions. The term banana was introduced from the Guinea Coast of West Africa by the Portuguese while; the term 'Plantain' (for cooking bananas) was derived from 'Plantano' of the Spaniards. The banana, which is a member of the *Musaceae* family, is India's and the world's most significant fruit crop. It is renowned for its age and has a long history that dates back to the beginning of civilization in India. Due to its socioeconomic importance and variety of uses, the banana crop is known as "Kalpataru" (Plant of Heaven) and defines the socioeconomic standing of the farmers. It is known as "Poor man's apple" and is inexpensive and nutrient-rich. Unripe fruit is consumed as a starchy food. Banana is cultivated in nearly 120 countries in the world. Banana is the fourth largest fruit crop of the world. Major banana producing countries are India, China, Indonesia, Brazil, Ecuador, Philippines, Guatemala, Angola, United Republic of Tanzania, Colombia, Costa Rica. In world, 5.6 million ha of land are dedicated to banana with production of 119.8 million metric tons [1]. India being the largest producer at 30.5 million metric tons on 866,000 ha. China comes second with 12 million metric tons per year on 358,924 ha. with 7.2 million metric tons of production per year, Indonesia is the third largest producer of banana [1]. In terms of production among fruits, bananas top the list in India. After mango and citrus, it is the third largest in terms of area. India produces 324.54 lacs MT of bananas annually from an area of 8.84 lac

ha. Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu, Karnataka, Uttar Pradesh, Bihar, Madhya Pradesh, West Bengal, Assam, Chhattisgarh are the top banana producing states of India. The production of Andhra Pradesh is 58.38 lakh MT and share is 17.99% Maharashtra ranks second with production of 46.28 lakh MT and share of 14.26%. Gujarat being third on position with production of 39.07 lakh MT and share of 12.04 % of India's total production [13]. In addition to being a great source of potassium, vitamin A, vitamin C, vitamin B-6, and fiber, bananas are also low in fat, cholesterol-free, and salt. Certain blood pressure drugs reduce the body potassium reserves. The potassium balance is restored by consuming one banana each day. At least five servings of fruits or vegetables per day are advised in order to maintain a healthy diet and reduce the risk of cancer. According to a recent study, consuming nine or ten servings of fruits and vegetables per day, together with three portions of low-fat dairy products, can significantly lower blood pressure. The ripe banana fruits are edible, delicious and very nutritious. Banana is a rich source of vitamin A and fair source of vitamin C, B and B1. The fruits are also rich in carbohydrates, magnesium, sodium, potassium and phosphorus. It contains 17 mg calcium, 36 mg phosphorus, 27 g carbohydrates and 1 g protein in 100 g fruit. From the nutritional point of view banana has a calorific value of 116 calories per 100 g fruit. The food value is about three times that of wheat. It makes healthy and salt free balanced diet than many of the fruits [9]. The lack of proper storage and transportation infrastructure makes post-harvest losses more common in underdeveloped nations [18]. For many years, mycologists have focused their attention on the microbes responsible for fruit rotting after harvest. Fruit deterioration after harvest results in enormous losses. According to [8, 10]. infections cause 20 to 25 percent of harvested fruits to rot during post-harvest handling. Banana fruits are extremely prone to spoilage. At room temperature, bananas have a limited shelf life as climacteric fruit. Without any restrictions on the use of ripening agents, banana fruits are professionally ripened and kept by fruit merchants. Due to inappropriate handling, inadequate storage, and post-harvest infections, there is a 25 to 30 % post-harvest loss of bananas [8, 10]. Fruits like bananas suffer significant post-harvest losses in tropical nations like India. The cultivated banana is prone to a wide range of diseases, mostly fungi that affect the plant's numerous parts from the root to the fruit. During storage, banana fruits deteriorate through the activity of microorganisms and their activity is favoured by the changing physiological state of the fruit. Banana fruit suffers from many serious diseases such as anthracnose, crown rot, finger rot, white rot, cigar end rot etc. Due to all these diseases storage of banana is difficult. The two primary postharvest rots of banana fruits are anthracnose and crown rot. The fungus *C. musae* can cause both crown rot and anthracnose; in addition, crown rot diseases may also be caused by fungal pathogens in the genera *Fusarium*, *Acremonium*, *Verticillium* and *Curvularia* [25, 26]. The fungus *Colletotrichum* has been the most notorious fungal pathogen, which causes severe rots which rapidly deteriorating fruit quality and rendering the fruit completely to a rotten with sticky mass tickling from the infected pulpy banana [27].

Eight *Colletotrichum* species have been reported on banana outside of China: *C. musae*, *C. siamense*, *C. chrysophilum*, *C. tropicale*, *C. theobromicola* [37], *C. karstii* [38], *C. paxtonii* [39], and *C. gloeosporioides* [23]. The three species *C. musae*, *C. gloeosporioides* [24], and *C. scovillei* [39] have been reported as pathogens causing banana anthracnose in China. Six *Colletotrichum* species found

on banana (*C. musae*, *C. gloeosporioides*, *C. siamense*, *C. tropicale*, *C. chrysophilum*, and *C. theobromicola*) have been placed in the *C. gloeosporioides* species complex [40], *C. paxtonii* and *C. scovillei* have been placed in the *C. acutatum* species complex [38], and *C. karstii* belongs to the *C. boninense* species complex [38]. Recently, Huang [41] five species namely *C. fructicola*, *C. cliviicola*, *C. siamense*, *C. karstii*, and *C. musae* have been reported on banana causing anthracnose.

One of the most significant postharvest diseases of bananas is anthracnose, which is typically brought on by the fungus *C. musae* (Berk. & Curt.), which affects both mature and damaged green fruits [27]. Additionally, banana tip rot, crown rot, and blossom end rot have all been caused by *C. musae* [28, 29, 30, 311, 32, 33, 34, 35]. The disease typically develops during extended periods of storage and transit that are characterized by low temperatures and high humidity. Banana anthracnose is characterized by brown patches that develop into depressed lesions with acervuli that are orange or pink in colour. The disease won't be seen until the fruit ripens. The following objectives guided the planning and execution of the current investigations on post-harvest fruit rot of bananas in light of the disease's significance [27].

2. MATERIALS AND METHODOLOGY

2.1 Source of disease sample

The diseased fruit sample of banana showing typical circular to angular, light to dark brown spots with a dark red or blackish margin on the fruit spots collected from Dhule market. The infected fruit were brought into the laboratory, placed in blotting papers under pressure with herbarium press and preserved for further investigations. The fresh infected fruits were subjected to microscopic examination and tissue isolation for the causal agent showing typical symptoms of fruit rot were cut with sharp sterilized blade, put in distilled water drop on clean glass slides, covered with cover slip and mounted initially under low power objective lens of the research microscope to observe the test pathogen structures like mycelium, spores, conidiophores, fruiting body, acervuli, setae, conidia, etc. if any further, from the rotted fruits of banana, fungus growth was scrapped gently with needle, put on clean glass-slide in a drop of distilled water, covered with cover slip and observed under research microscope.

2.2 Isolation

The pathogen *C. musae* was isolated from naturally infected banana fruit showing typical symptoms circular to angular / irregular ring, light to dark brown spots with a dark red or blackish margin were used to isolate the pathogen by tissue segment method [16] on potato dextrose agar (PDA) medium. Then infected portion was cut into small pieces of about 0.2 to 0.5 cm along with some healthy portion with sterile scalpel. The tissue pieces were surface sterilized with 0.1 % mercuric chloride solution for 30 sec. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite. After that excess water was removed with sterilized blotting paper then the pieces were transferred on to Petri plate containing 20 ml sterilized solid PDA medium under aseptic condition. Plates were incubated at 25 ± 2º C and observed periodically for growth of the fungus. Sub cultures were made from the periphery of the mycelium growth which appeared after

3-4 days, the pathogen was purified and its pure culture was maintained on agar slant in test tubes and stored in refrigerator for further studies.

2.3 Identification

Pure culture of the test pathogen was obtained and inoculated aseptically on autoclaved PDA in Petri-plates were incubated 25 ± 2°C. One week after incubation, the test pathogen was fully developed, of which cultural, morphological and microscopic characteristics were studied with the help of published literature and confirmed.

2.4 Pathogenicity

To prove Koch's postulate, mature and semi ripen healthy banana fruits were collected from banana fruit market of Dhule district and were bought to the laboratory. The fruits were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 seconds followed by three subsequent washings with sterilized water and separately were inoculated with isolated fungus with following different methods.

A. Pin pricking injury method: A sterile pin was dipped in fungal suspension and pricked immediately on fruit at different places on peel.

B. Cork borer wounding method: For inoculation in fruits, a hole of 2 mm diameter and 2 mm depth was made with help of sterilized cork-borer. The fungal inoculation was placed in the hole and it was plugged with the same host tissue corked out earlier.

C. Control (without injury): The un-inoculated healthy mature banana fruits were kept as control. The symptoms of anthracnose of banana were studied. The test fruits were inoculated with the *C. musae* and expression of the characteristic symptoms were recorded.

2.5 Morpho-cultural characterization of *C. musae*

Isolates of *C. musae* were purified through tissue segment method on PDA slants and stored in refrigerator (4º C). These isolates were separately grown on PDA in Petri plates for seven days to provide inoculation in this study. Cultural studies for isolates were conducted on PDA in Petri plates (90 mm). The plates were inoculated centrally with 5 mm culture discs taken from periphery of 7-day old culture and incubate at temperature of 25 ± 2° C. Cultural and Morphological characters of the fungus were studied by observing cotton blue stained slides under compound microscope. After calibrating the microscope, measurement of conidia, hyphae were made with the help of ocular microscope. The shape, size, and colour of conidia and mycelial characters of the pathogen were examined after incubation at 25° C for 10 days on PDA. Observations on colony diameter and colony characteristics were recorded after a week of incubation and that of sporulation, after 10–12 days of incubation. The sporulation was graded as per the scale [21] given below.

List 1: Grading scale for the sporulation

Score	Grade	Conidia / Microscopic field 10X
++++	Excellent	More than 50

+++	Good	31-50
++	Moderate	11-30
+	Poor	1-10
-	No Sporulation	0

2.6 Effect of different cultural growth media

To study the effect of different solid culture media on colony, morphology and mycelial growth of *C. musae*, five culture media *viz*., Potato dextrose agar, oat meal agar, czapeck's agar, richard's agar and host leaf extract agar was used. All the media were sterilized at 15 lbs pressure (121 ^{0}C) for 15 minutes.

3. RESULT AND DISCUSSION

3.1 Symptomatology

The typical symptoms (Fig 1 A and B) induced by *C. musae* on banana fruits, lesions are sunken and covered with salmon coloured acervuli. On ripening fruits, sunken brown spots develop with orange acervuli. The fungus produces sunken, water-soaked spots, rapidly expanded on infected tissues, become soft on full expansion of red brown to black coloured, round to oval, regular to irregular and brownish red to black spots. The banana anthracnose disease symptoms appeared as black, circular sunken lesions which were distributed all over the fruit surface. Under favourable moist conditions, the disease-causing fungus started developing acervuli, sometimes as concentric rings and pinkish conidia. More severely disease affected fruits rotten and appeared complete black in colour. Similar symptoms were reported by [4, 15]. They described the symptoms of anthracnose caused by *C. gleosporioides* as sunken, water-soaked spots, rapidly expanded on infected plant tissues, which become soft on full expansion of red brown to black coloured, round to oval, regular to irregular and brownish red to black spots. Also, as per the recent study by Zakaria [22] the anthracnose pathogens affect fruits, leaves, blossoms, twigs, and branches.

3.2 Isolation of pathogen

Applying tissue isolation technique [19], anthracnose caused by *C. musae*, were successfully isolated aseptically from the naturally rotted banana fruit samples showing typical symptoms on fruits, using Potato dextrose agar (PDA) medium and incubated at 25 ± 2^{0}C. Fungi were identified by microscopic observations of the morphological characters. The test fungi were transferred on fresh PDA plates and purified through frequent sub-culturing. The purified culture was maintained on fresh agar slant and preserved in the refrigerator at 4 ^{0}C for further studies. *C. musae* produced initially white, cottony mycelium, which gradually turned greyish white to dark gray or white in colour. White to light gray coloured colonies was produced. Culture plates were white to light brown in colour (Fig 1 C). Aged culture appeared completely light brown with no aerial mycelium. A piece of sporulating mycelium was mounted in lactophenol cotton blue and observed under the light microscope. The acervuli were usually setose or glabrous, round to elongate or irregular in shape, conidia were hyaline, cylindrical, aseptate. Similar results were reported by [10, 13]. They enumerated the morphological characters of *C. gloeosporioides.*

3.3 Identification of the pathogen

Identification of the pathogen based on typical symptoms on fruits, cultural and morphological characteristics, microscopic observations and pathogenicity test, the test pathogen was identified as *C. musae*. It produced septate mycelium. Later it produced conidiophores arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends, one to two oil globules were observed in the conidium, setae were black in colour. Based on the colony and morphological characters of conidia and conidiophores the identity of the causal organism was confirmed as *C. musae* (Fig 1 D, E and F). Similar characters were reported by [13, 14]. They reported that the mycelium is immersed, branched and consists of rather narrow, sparsely septate, hyaline hyphae which turn slightly darken with age.

3.4 Pathogenicity test

Pathogenicity test was carried out as described in material and methods by pin pricking method of pure culture of the fungus to the healthy banana fruits (Fig, 2). The symptoms developed after 5 days of incubation as typical spots resembling anthracnose infection. Re-isolation of the test pathogen was done on Potato dextrose agar (PDA). Re-isolated fungus was compared with the original culture obtained from naturally infected banana fruits for their morphological characters. The re-isolated culture was identical to that of original culture used for isolation. The control fruits which were not inoculated with the fungus did not show any symptom of the disease. Similar results were reported by [7]. He proved the pathogenicity of *C. gloeosporioides* on mango and banana. In pathogenicity studies, the conidial suspension of *C. musae* was sprayed on healthy fruits and typical symptoms were noticed, seven days after incubation.

3.5 Cultural and morphological characters of *Colletotrichum musae.*

3.5.1 Cultural characters

The growth of *C. musae* was studied based on the cultural and morphological characters. The observations were recorded for colony diameter, colony colour, mycelial growth and sporulation on Potato dextrose agar media were recorded at 8 days after inoculation. The cultural characters of *C. musae* are presented in (Table 1-2, Fig, 3). The result revealed that potato dextrose agar culture media resulted with maximum mycelial growth of *C. musae*. However, at 8 days of inoculation, the maximum mycelial growth was recorded on Potato dextrose agar (90.00 mm). The observations on colony pigmentation were taken at 8 days after incubation. *C. musae* produced whitish to black colonies on PDA. The pathogen was also observed for their colony appearance. The good growth of pathogen and smooth margin was observed on PDA. The PDA culture media tested exhibited a wide range of sporulation of the test pathogen. However, excellent sporulation (++++) was recorded on PDA.

3.5.2 Mycelial growth & sporulation on different media

The fungus growth on five different solid media indicated that the maximum radial growth of *C. musae* was observed in potato dextrose agar, oat meal agar, czapeck's agar & richard's agar and the least growth was observed on host leaf extract medium. Colonies were whitish to pale red on PDA, RA and CDA. On OMA, the fungus produced greyish to pink colonies. The fungus produced greyish brown colonies on host leaf extract agar. The fungus showed aerial fashion mycelial growth on PDA, CDA, RA and OMA. Sporulation was found to be maximum in on PDA. These results were in conformity with the findings of [8, 9, 10, 20] and who recorded cultural and morphological characters of different *C. musae* isolates. Morphological observation of the fungus was recorded by adopting slide culture technique. The observations of different morphological structure of the *C. musae* are presented in (Table, 2, Fig, 3).

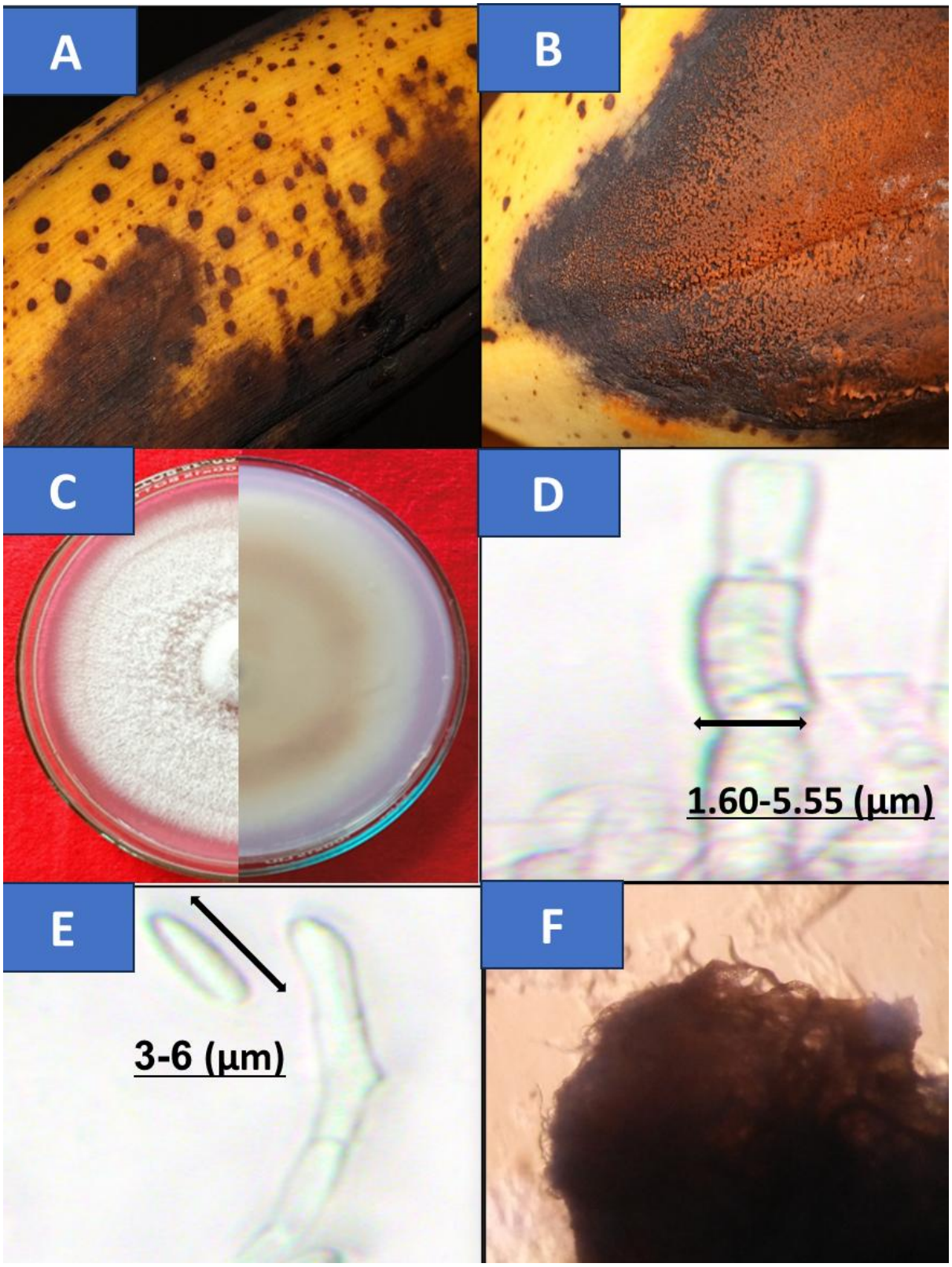

Fig: 1 Symptomatology and morpho-cultural characteristics A) Typical symptoms of anthracnose of banana on fruit B) Close up view of anthracnose spots C) Colony growth of *C. musae*: front and inverted view D) Mycelium E) Mycelium with conidia F) Acervulus structure (Note: The figures on scale bar is an average value of five replications)

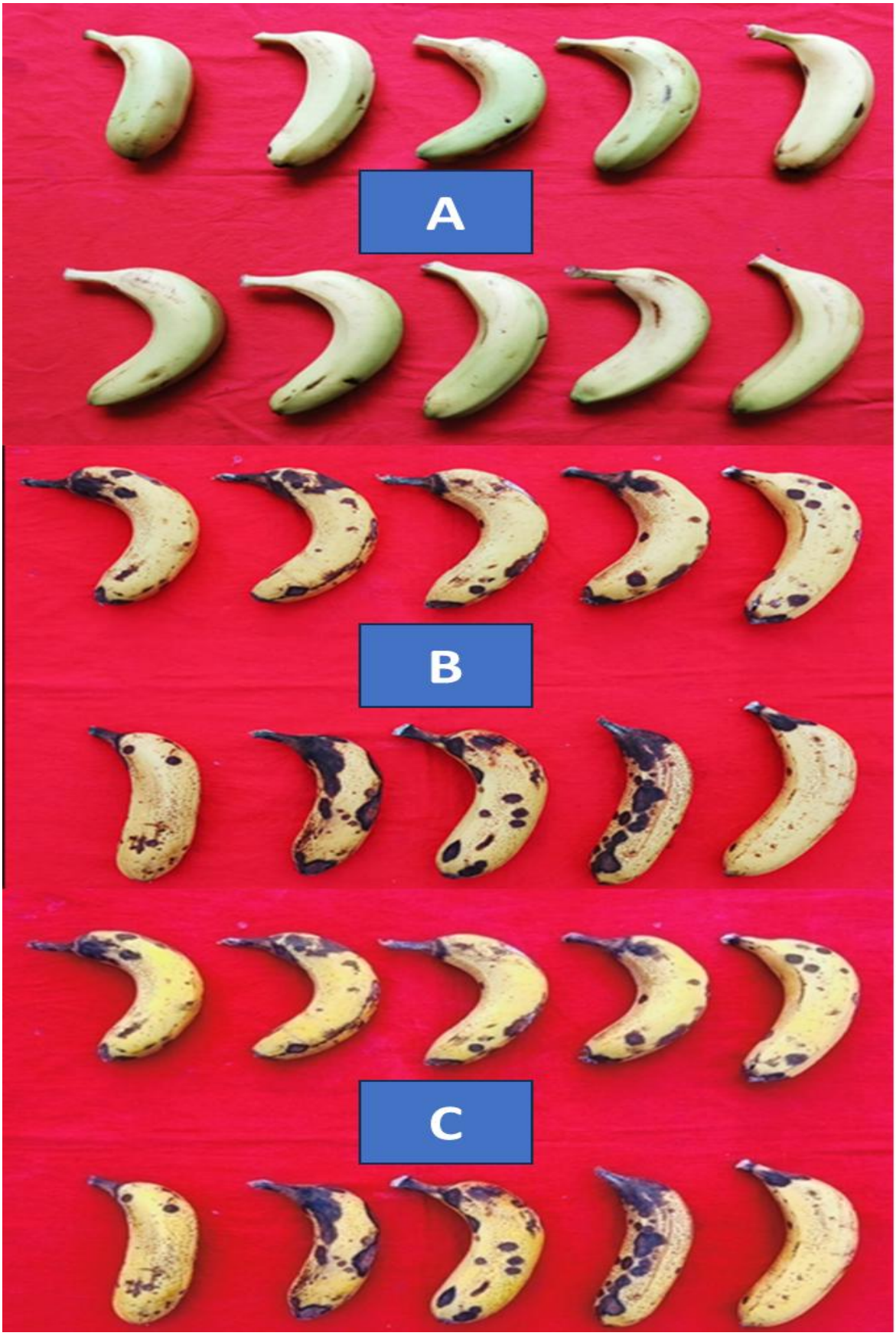

Fig: 2 Kochs postulate assay A) Healthy banana fruits B) symptoms expression after 3 days and C) After 5 days

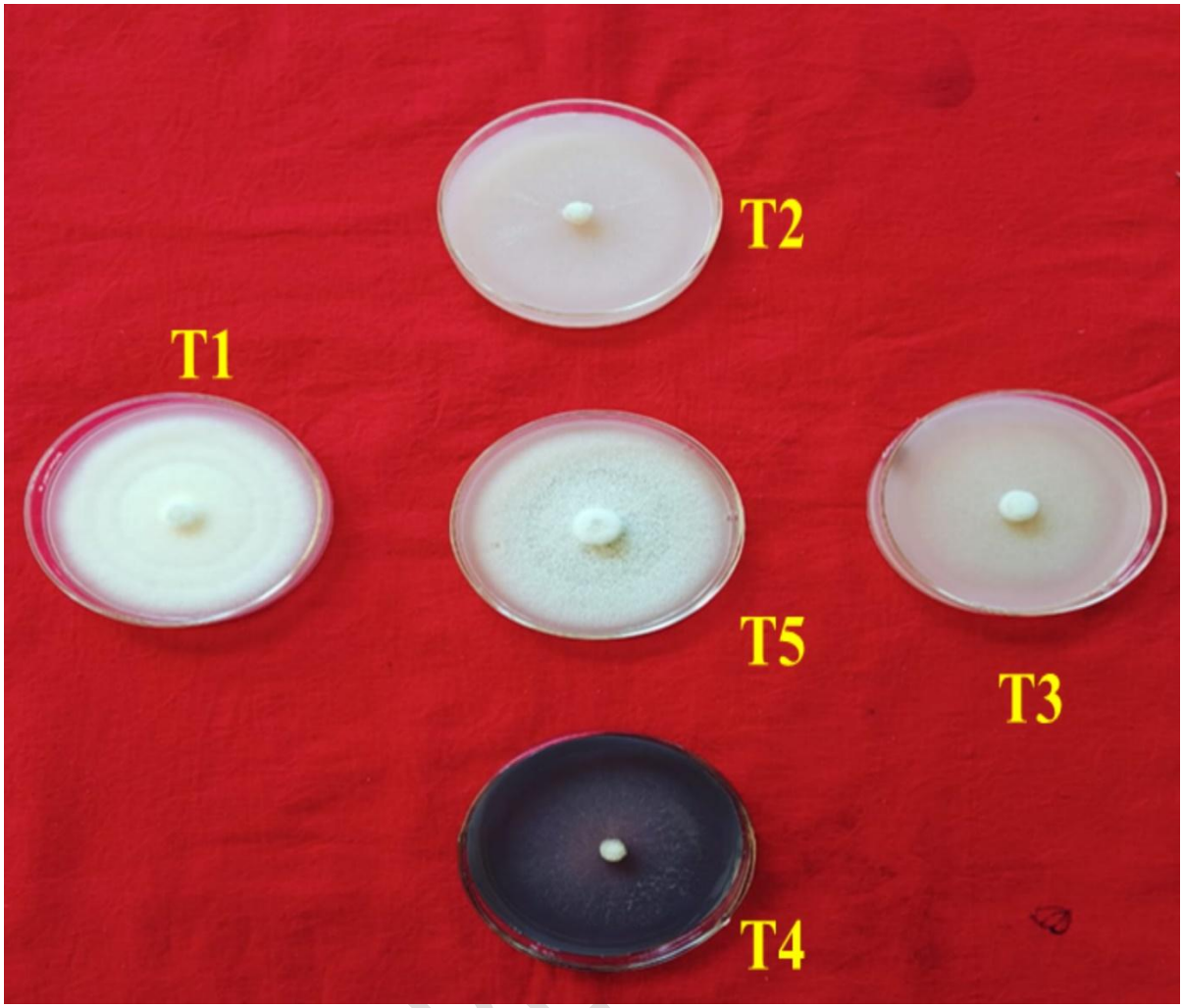

Fig: 3 *In-vitro* effect of different culture media on mycelial growth and inhibition of *C. musae*

Table 1: Cultural & morphological characteristics of *C. musae*

Sr. No.	Media	Mycelial colour	Margin of colony	Topography	Growth Character	Sporulation
1	Richard's agar (RA)	whitish-pale red	Irregular	Aerial mycelium	Moderate	++
2	Oat meal agar (OMA)	Greyish-pink	Irregular	Aerial mycelium	Good	+++
3	Czapek's dox agar (CDA)	Whitish-pale red	Irregular	Aerial mycelium	Moderate	++
4	Host leaf extract medium	Greyish brown	Irregular	Submerged mycelium	Poor	+
5	Potato dextrose agar	Whitish-pale red	Irregular	Aerial mycelium	Excellent	++++

Sporulation: ++++ = Excellent, **+++ =** Good, **++** = Moderate, **+ =** Poor, - = No

Table 2: Morphological structure of *C. musae*

Sr. No.	Morphological structure	Measurement parameters (µm)	
		Length	Width
1	Mycelium	-	1.60-5.55
2	Acervulus	111-450	42-180
3	Setae	82-125	3-5

4	Conidia	6-18	3-6

The fungus produced profuse white mycelial growth on PDA, which later on turned dull white to slightly grey in colour. The mycelium was septate, irregularly branched and vacuolated. The young hyphae were slender, became broad on maturity and measured 1.60-5.55 µm in width. The fungus produced acervuli both on host as well as in culture. On fruit in the field, they were sub-epidermal while on artificially inoculated fruit they were prominent on the surface embedded in the mycelial growth. In culture, acervuli were produced in about 4 to 5 days after sub culturing and were firm on the medium. Most of the acervuli were produced within the mycelial mat. Initially, acervuli looked orange in colour, which later changed to dark brown to black in colour. They were globuse to saucer or irregular in shape. The acervuli (including setae) measured 111 to 450 µm X 42 to 180 µm in size. The base of acervuli was dark brown to black, while remaining portion was light brown in colour. Setae were irregularly arranged throughout the acervulus in the culture. They were ashy brown to dark brown, septate, stiff, straight or bending. At base, septation was at shorter distance, while at longer distance from mid to tip. The setae were wider at the base and tapering towards tip, which was obuse. The setae measured 82-125 µm in length and 3-5 µm in width. The conidia were oblong to cylindrical in shape. They were single celled, hyaline when single, but orange to light brown in mucilaginous masses or in acervuli. The conidia measured 6 to 18 µm in length and 3 to 6 µm in width. Similar results were reported by [10] They studied the cultural and morphological variability in *C. gloeosporioides*, causing anthracnose of mango and reported maximum radial growth on PDA (83.98 mm). Colonies colour of the isolates varied from bluish black to black in centre but variable towards margins. Size of conidia ranged between 12.13-21.83 µm x 4.34-7.67 µm.

3.5.3 Effect of different culture media

Studied the cultural characteristics and sporulation of *C. musae* by using five different culture media and results obtained are presented in Table,3 and Fig, 3. The cultural characters of *C. musae* were studied on five solid media as described in material and methods. Observations on radial growth, mycelial colour, margin of the colony, topography and sporulation on different media were recorded at 8 days after inoculation. The data on radial growth was analysed statistically and are presented in Table 3. The five different culture media were used are Richard's agar, oat meal agar, czapeck's agar, Host leaf extract and potato dextrose agar. Mycelial growth of test isolate was found significantly with various culture media, the mean mycelial growth range was recorded between 40.25 mm to 90.00 mm. However, Potato dextrose agar (PDA) had significantly higher mycelial growth (90.00 mm), followed by oat meal agar (85.75 mm) and richard's agar (80.67 mm) at par with czapack's agar (80.65 mm). The minimum mycelial growth was recorded on the host leaf extract medium (40.25 mm). Above mentioned results are also talking with [2, 11, 18] who too studied the effect of different medium on growth of *C. gloeosporioides* and stated that, highest sporulation was recorded in Richard's agar and malt extract agar.

Table 3: Effect of various cultural media on mycelial growth of *C. musae.*

Tr. No.	Culture Media	Mean colony diameter in (mm)	Sporulation
T_1	Richard's agar	80.67#	++
T_2	Oat meal agar	85.75	+++
T_3	Czapack's agar	80.65	++
T_4	Host leaf extract	40.25	+
T_5	Potato dextrose agar (control)	90.00	++++
	S.E. ± (m)	0.40	
	C.D at 1%	1.20	

#Average of three replications, Sporulation: **++++**= Excellent, +**++ =** Good, +**+**= Moderate, **+=** Poor, -= No

Conclusion

The present research concluded that, the pathogen, *C. musae* was successfully isolated on PDA medium from anthracnose diseased samples of banana fruits collected from various market locations. The pathogen was identified as *C. musae* based on symptomatology, cultural and morphological characteristics on PDA medium, microscopic observations and pathogenicity. The good growth of pathogen and smooth margin was observed on Potato dextrose agar. The pathogenicity of *C. musae* was proved by inoculating with spore suspension of pure culture of the fungus to the healthy banana fruits by pin pricking injury method. Among five different culture media tested for the growth and sporulation, the fungus produced maximum growth on potato dextrose agar, followed by oat meal agar.

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