World Journal of Agricultural Sciences 7 (6): 743-754, 2011
ISSN 1817-3047

Morphological and Physiological Characterization of *Colletotrichum musae* the Causal Organism of Banana Anthracnose

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Abstract: Sixteen isolates of *C. musae* were collected from different banana growing areas of Tamil Nadu and their pathogenicity was proved under laboratory conditions. Effect of different pH levels, temperature, light intensity and media were tested against the growth of *C. musae* under *in vitro*. Results indicated that the growth of *C. musae* was maximum at pH range of 6.50-7.00 and temperature range of 25-30°C. Exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness resulted in the maximum mycelial growth of *C. musae* compared to the 24 hr exposure to either continuous light or dark. Among the different media tested, Potato dextrose agar medium supported significantly the maximum growth of all the sixteen isolates of *C. musae*. Further, the strains were found to vary morphologically between the isolates under the study.

Key words: Banana · *Colletotrichum musae* · pH · Temperature · Light and Media

INTRODUCTION

Banana anthracnose caused by *Colletotrichum musae* (Berk. and Curt) Arx. is considered as one of the most important diseases of banana in the global level and is one of the major constraints to banana production [1,2]. It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption, thereby causing severe loss to farmers and traders. *Colletotrichum musae* is the most important pathogen on wounded green and ripe banana fruits [3,4]. Occasionally, the fungus invades necks of green fingers when damaged by flexing. Lesions are sunken and covered with salmon-colored acervuli [5]. Infections stimulate ripening of fruits and lesions elongate with ripening. On ripening fruits, sunken brown spots develop with orange acervuli [4]. Jinyoung Lim *et al*. [6] studied the cultural and morphological characters of *C. musae.* They observed the colony was loose with white aerial mycelium, which later became orange in color. Several black, acervulus-like masses developed on the culture plates after incubation for 10 days at 25°C with dark-orange drops of conidia. Conidia were aseptate, hyaline, mostly ellipsoid, ranging from 10-18 µm and 5-9 µm (average of 14.5-6.9 µm) in size. Photita *et al*. [7] reported that the *C. musae* isolates were pathogenic on banana. The cultures were distinct with fast growing sparse aerial mycelium, white, with copious cinnamon conidial masses, elliptical shape conidia and setae absent. coloured acervuli.

The growth characters of different isolates of *Colletotrichum* spp. varied on different solid media. The growth and sporulation of the *C. capsici* infecting chilli was maximum on PDA and Das Gupta's standard medium [8]. PDA supported the maximum growth of *C. gloeosporioides* [9,10]. Anand *et al*. [11] reported that the isolate of *C. capsici* produced white colonies on Richard's agar, ripe chilli fruit extract agar, oat meal agar and PDA and it produced greyish white, whitish black, blackish white and black coloured colonies on Czapek Dox agar, chilli leaf extract agar, green chilli fruit extract agar and radish dextrose agar, respectively. Manjunath [12] reported that *C. gloeosporioides* produced black coloured colonies on water agar, white coloured on Richards, oat meal agar, PDA, host leaf extract and Walksman's agar, blackish white colonies on nutrient agar, greyish white on Czapek Dox agar and dark white on Martin's Rose Bengal agar médium and reddish white on King's B agar medium. The metabolic and catabolic activity of an organism varies depending on the hydrogen ion concentration existing in the surrounding environment. Hence, pH plays a vital role

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in deciding the nature and activities of microorganisms [13]. *C. gloeosporioides* exhibited the maximum mycelial growth at pH 7 [14,15].

Temperature affects the physiological function of the fungi, which in turn affect the phenotypic expression. For each fungus, there is a particular temperature below which it will not grow. Likewise, there is a particular temperature above which the growth ceases. A temperature of 25°C was reported to be the optimum for the growth of *C. gloeosporioides* on mango, almond and avocado [16-17]. Nandinidevi [10] reported that the mycelial growth of *C. gloeosporioides* isolated from anthurium was maximum at 25°C compared to incubation of the fungus at 30°C. Manjunath [12] reported that the optimum growth of C 1 isolate of C. *gloeosporioides* isolated from noni was at 25-28°C. Mishra and Siradhana [19] reported that, the diurnal exposure favored the growth and sporulation of *C. graminicola*.

The phase of growth is either stationary or accelerated or declining or autolysis. Yashodha *et al.* [20] studied the growth phases of *C. gloeosporioides* causing anthracnose of arecanut. They noticed that the fungus reached maximum growth after ten days of inoculation beyond which autolysis occurred. Vegetative growth of *C. gloeosporioides* isolated from stylosanthes reached maximum on 14th day after inoculation and after that autolysis was noticed [21].

MATERIALS AND METHODS

Collection and Establishment of Isolates of *C. musae:* During the survey, anthracnose diseased samples were collected from different market places of Tamil Nadu *viz.,* Coimbatore, Madurai, Erode, Tirunelveli and Kancheepuram. The samples were first examined to confirm the presence of the fungus. The diseased tissues were teased with a sharp blade on a glass slide having a drop of clear water and covered with a cover slip to confirm the presence of fungal spores under the binocular research microscope (10 X). After confirming the presence of fungal spores, isolation was carried out in the laminar flow chamber under aseptic conditions following a standard tissue isolation method [22]. The infected tissue of fruits which showed typical symptoms were cut into small bits measuring about 2 mm and surface sterilized in 0.1 per cent mercuric chloride solution for one minute and washed repeatedly thrice in sterile distilled water to remove the traces of mercuric chloride. Then surface sterilized tissues were transferred to sterile Petri plates containing PDA medium under aseptic conditions. The inoculated Petri plates and slants were incubated under sterilized bell jar at room temperature (28 ± 2°C) and observations were taken at regular intervals.

Identification of the Pathogen: The pathogen was identified up to species level based on their cultural and morphological characters. A loop full of fungal culture grown on PDA plates were taken on a glass slide and observed with image analyzer under 100 X magnifications for the presence of conidia and conidiophore. After confirming the spores, the cultures were purified by single spore isolation technique.

Pathogenicity Test: In order to prove Koch's postulates, pathogenicity test was carried out. Fully matured green unripe banana fruits were collected from the field, washed thoroughly under running tap water. The fruits were blot dried and surface sterilized with 70 per cent ethanol. The fruits were injured (pinprick) with sterilized needle and the spore suspension (5 x 10^5 spores/ml) of the pathogen was prepared using a seven days old PDA culture by grinding it with sterile distilled water in a pestle and mortar and sprayed over the fruits. The fruits inoculated with sterile distilled water after pin prick served as control. The inoculated fruit surface was covered with moist cotton and the fruits were kept inside the moist chamber. Five fruits were used for each method. The infection was recorded after seven days.

The fungus was reisolated from the artificially inoculated fruits showing typical anthracnose symptoms and the culture obtained was confirmed for its morphology and colony characters.

Isolates of *C. musae* Used in the Study: Sixteen different obtained isolates of *C. musae* used in present study are tabulated as follows:

Isolate	Variety	Place of collection
C1	Nendran 1	Coimbatore
C2	Nendran	Erode
C3	Nendran	Tirunelveli
C4	Karpooravalli	Erode
C5	Robusta	Coimbatore
C6	Rasthali	Coimbatore
C7	Nadan	Madurai
C8	Nendran 2	Coimbatore
C9	Hill banana	Madurai
C10	Karpooravalli	Coimbatore
C11	Nadan	Erode
C12	Poovan	Tirunelveli
C13	Nadan	Coimbatore
C14	Poovan	Kancheepuram
C15	Poovan	Erode
C16	Poovan	Coimbatore

Cultural Characters of the Isolates of C. *musae*

Growth Characters on Solid Media: Different solid media mentioned below were used for assessing the growth of isolates of *C. musae*. The mycelial diameter as well morphological character of mycelia on different media was recorded. The composition and preparations of the following media were obtained from "Ainsworth and Bisby's Dictionary of the fungi" by Ainsworth [23] and Plant Pathological methods, fungi and bacteria by Tuite [24]. Each culture medium was prepared in one liter of water and autoclaved at 120°C at 15 psi for 20 min. These were cooled to 45°C and then poured in 9 cm Petri dishes for solidification. Potato Dextrose Agar (PDA) Medium (Potato 250 g, Dextrose 20 g and Agar agar 20 g), Oat meal agar (Oat meal 40 g and Agar agar 20 g) [25], Water agar (Agar agar 20 g), Richards agar (Sucrose 50 g, Potassium nitrate 10g, Magnesium sulphate 2.5 g, Ferric chloride 10 ml and Agar agar 20 g) [26], Czapek dox Agar Medium (Sodium nitrate 2 g, Potassium nitrate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 3 g, Sucrose 30 g and Agar agar 20 g) [27], Martin's Rose Bengal agar medium (Magnesium sulphate 0.20 g, Dipotassium hydrogen phosphate 0.90 g, Ammonium Nitrate1.00 g, Potassium chloride 0.15 g, Glucose 3.00 g, Penta chloro nitrobenzene 0.20 g, Rose Bengal 0.20 g, Chlorothallonil 0.20 g and Agar agar 20 g) and Waksman's Agar Medium (Glucose 10 g, Peptone 5 g, Potassium dihydrogen phosphate 1 g, Magnesium sulphate 0.5 g and Agar agar 26 g).Three replications were maintained for each media. Colony diameter was measured ten day after inoculation. The different colony characters were recorded in each medium by visual observation.

Influence of Abiotic Factors on the Growth of Pathogen

Effect of pH on the growth of *C. musae:* The effect of pH on the growth of the pathogen was studied as per the method followed by Kiryu [28] using PDA medium. Different pHlevels *viz.,* 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were used. The pH levels of the medium were adjusted in a digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. The media with different pH levels were sterilized, cooled and poured in the sterilized Petri plates in 20 ml quantities and allowed to solidify. The eight mm disc of pathogen was placed on the centre of the Petri plates. The plates were incubated at room temperature (28 ± 2°C) for ten days. The diameter of the mycelial growth was recorded. Three replications were maintained for each treatment.

Effect of Temperature on the Growth of *C. musae:* The effect of temperature on growth of the pathogen was studied. Different temperatures maintained for the growth of pathogen on PDA were 5, 10, 15, 20, 25 and 30°C. Mycelial disc of 8 mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in incubator and temperature was adjusted to required level. The mycelial growth was recorded on seventh day after inoculation.

Effect of Light Intensity on the Growth of *C. musae:* The effect of light on the growth of pathogen was studied by exposing the inoculated culture to alternate cycles of 24 h light, 24 h dark and 12 h light and 12 h dark in an environment chamber maintained at room temperature (28 ± 2°C). Mycelial disc of eight mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelial growth was recorded on seventh day after inoculation.

Growth Phase Study: The growth phase study was conducted on potato dextrose broth (PDB). Thirty ml of broth was pipetted out into each 100 ml flasks. The flasks containing potato dextrose broth were then sterilized at 121°C for 20 min. at 15 lb pressure and then inoculated with 8 mm disc of pathogen. The inoculated flasks were incubated at 28 ± 2°C. A set of three flasks were harvested starting from first day up to 16th day.

The culture filtrate was filtered through Whatman No.4 filter paper. Before filtering, filter paper was dried to a constant weight in hot air oven at 50°C. The weight of dry mycelial mat was recorded.

RESULTS

Banana fruits showing typical symptoms of anthracnose were collected during the survey from different banana growing areas of Tamil Nadu. The cultures of 16 isolates of *C. musae* were isolated and purified. The isolates were maintained on PDA slants for further studies.

Identification of the Pathogen: Pathogen associated with anthracnose disease was isolated on PDA medium and it was identified as *C. musae* (Berk. and Curt.) Arx based on the morphological and cultural characteristics of the fungus.

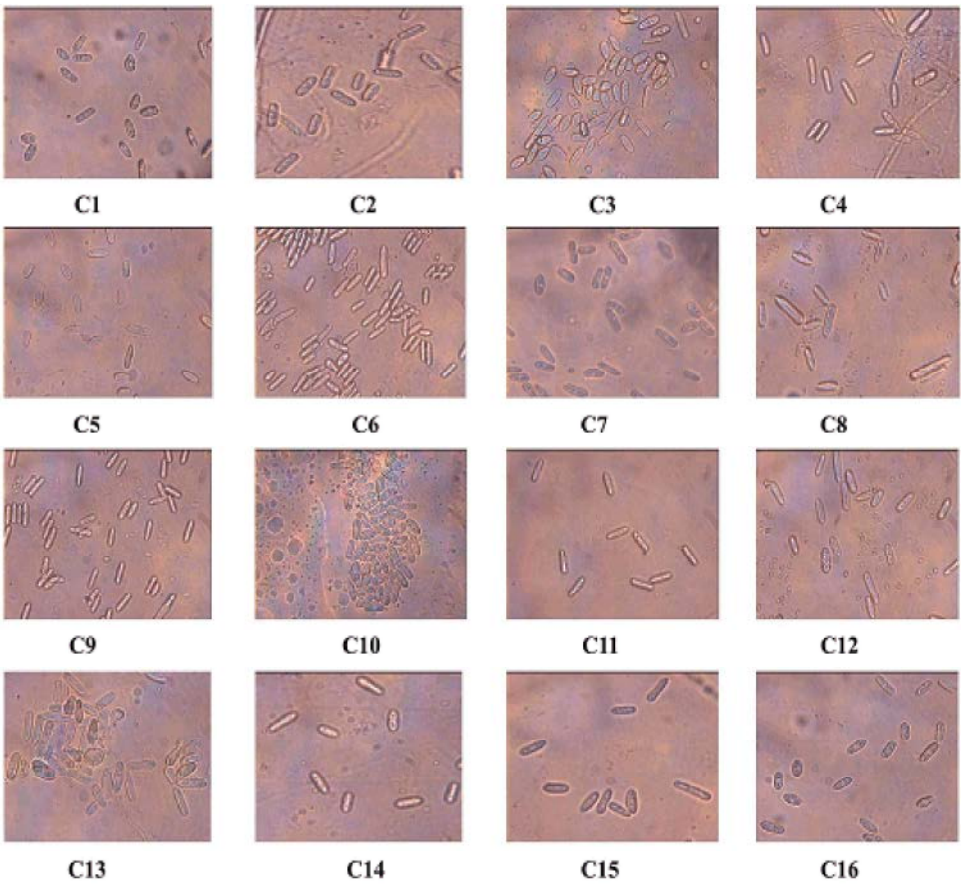

Fig. 1: Morphological characters of *C. musae* isolates

Table 1: Conidial characteristics of different isolates of *C. musae*

Isolate	Length (µm) *	Width (µm) *	Colour	Shape
C1	10.94 ^{e}	7.00 ^{b}	Hyaline	Cylindrical
C2	11.80 ^{cde}	7.10 ^{a}	Hyaline	Cylindrical
C3	14.28 ^{ab}	5.28 ^{l}	Hyaline	Cylindrical
C4	13.27 ^{abc}	6.36 ^{g}	Hyaline	Cylindrical
C5	10.23 ^{e}	5.31 ^{k}	Hyaline	Cylindrical
C6	14.71 ^{a}	6.86 ^{d}	Hyaline	Cylindrical
C7	11.25 ^{de}	7.00 ^{b}	Hyaline	Cylindrical
C8	12.76 ^{bcd}	5.87 ^{h}	Hyaline	Cylindrical
C9	10.34 ^{e}	5.00 ^{n}	Hyaline	Cylindrical
C10	11.28 ^{de}	5.37 ^{j}	Hyaline	Cylindrical
C11	10.28 ^{e}	7.00 ^{b}	Hyaline	Cylindrical
C12	10.23 ^{e}	6.57 ^{c}	Hyaline	Cylindrical
C13	14.17 ^{ab}	6.37 ^{f}	Hyaline	Cylindrical
C14	13.40 ^{abc}	6.87 ^{c}	Hyaline	Cylindrical
C15	14.18 ^{ab}	5.72 ^{i}	Hyaline	Cylindrical
C16	13.28 ^{abc}	5.17 ^{m}	Hyaline	Cylindrical

*Mean of ten conidia

In a column, means followed by a common letters are not significantly different at the 5 % level by DMRT

Morphological Characters of the Isolates of *C. musae:* The morphological characteristics of different isolates of *C. musae* on PDA were studied. The results revealed that all the isolates of *C. musae* produced hyaline cylindrical conidia. Significant variations were observed with respect to conidial dimensions among the isolates. The length of conidia ranged from 10.2-14.7 µm. Highest length of conidia was observed in C6 isolate (14.7 µm) followed by C3 isolate (14.3 µm) and shortest conidia was recorded in C5 and C12 isolates (10.2 µm). Width of the conidia ranged from 5.0 to 7.1 µm. Isolate C2 recorded the highest width of conidium (7.1 µm) and was followed by C1, C7 and C11 (7.0 µm). Lowest width was observed in C9 isolate (5.0 µm) [Table 1; Figure 1]. Jinyoung Lim *et al*. [6] reported that the conidia of *C. musae* isolates were aseptate, hyaline, mostly ellipsoid, ranging from 10-18 µm and 5-9 µm (average of 14.5-6.9 µm).

Cultural Characters of the Isolates

Growth Characters on Solid Media: The growth characters of *C. musae* isolates were studied on seven different solid media. The colony diameter and colony colour were considered as growth characters. The results showed that all the seven media tested supported the mycelial growth of all the isolates of *C. musae*.

Table 2: Effect of different solid media on the growth of *C. musae* isolates

	Mycelial diameter (mm)						
Isolate	Water agar	Oat meal agar	Richards agar	PDA	MRB	Czapek Dox agar	Walksman's agar
C1	8.80 ^{ef}	80.00 ^{k}	88.05 ^{a}	89.00 ^{c}	40.00 ^{e}	65.55 ^{m}	79.00 ^{g}
C2	10.00 ^{b}	83.00 ^{d}	84.10 ^{b}	89.61 ^{a}	49.00 ^{bc}	70.05 ^{d}	78.80 ^{d}
C3	10.50 ^{a}	82.00 ^{f}	74.90 ^{cef}	89.10 ^{b}	42.00 ^{cde}	65.50 ^{n}	76.02 ^{n}
C4	8.50 ^{ghi}	81.45 ^{h}	82.25 ^{b}	89.00 ^{c}	44.00 ^{bcde}	66.00 ^{l}	76.12 ^{m}
C5	8.31 ^{ij}	81.62 ^{g}	76.60 ^{fg}	89.00 ^{c}	42.95 ^{bcde}	69.93 ^{e}	81.05 ^{c}
C6	8.75 ^{hij}	81.00 ^{i}	81.14 ^{cd}	81.06 ^{n}	43.00 ^{bcde}	66.61 ^{i}	76.20 ^{l}
C7	9.14 ^{cd}	81.51 ^{h}	81.17 ^{fg}	86.03 ^{m}	44.99 ^{bcde}	67.35 ^{h}	79.30 ^{f}
C8	8.70 ^{fg}	78.95 ^{n}	76.30 ^{ef}	86.40 ^{k}	43.41 ^{bcde}	69.24 ^{g}	81.80 ^{b}
C9	9.35 ^{c}	83.24 ^{c}	78.34 ^{de}	88.47 ^{e}	48.75 ^{ab}	70.51 ^{o}	79.42 ^{e}
C10	9.00 ^{de}	79.64 ^{l}	79.25 ^{cde}	87.25 ^{i}	41.73 ^{de}	62.47 ^{c}	78.62 ^{h}
C11	9.24 ^{cd}	83.25 ^{b}	80.21 ^{g}	86.25 ^{l}	41.14 ^{de}	61.06 ^{p}	78.10 ^{j}
C12	8.54 ^{ghi}	85.20 ^{a}	75.24 ^{cde}	87.54 ^{g}	42.17 ^{cde}	66.03 ^{k}	77.02 ^{k}
C13	8.65 ^{fgh}	79.54 ^{m}	80.64 ^{cde}	88.75 ^{d}	46.30 ^{bcde}	66.40 ^{j}	75.12 ^{o}
C14	8.41 ^{hij}	82.55 ^{e}	80.25 ^{cde}	87.34 ^{h}	48.34 ^{bcd}	71.47 ^{b}	83.05 ^{a}
C15	8.23 ^{j}	80.20 ^{j}	79.95 ^{cde}	88.23 ^{f}	50.00 ^{b}	72.25 ^{a}	76.20 ^{m}
C16	9.14 ^{cd}	83.25 ^{c}	81.75 ^{bc}	86.55 ^{j}	42.24 ^{cde}	69.25 ^{f}	78.23 ^{i}
Mean	8.95	81.65	80.00	87.47	44.37	67.47	78.37

* Mean of three replications

In a column, means followed by a common letters are not significantly different at the 5 % level by DMRT

Table 3: Cultural characteristics of *C. musae* isolates on different solid media

	Colony colour						
Isolate	Water agar	Oat meal agar	Richards agar	PDA	MRB	Czapek Dox agar	Walksman's agar
C1	White	Blackish white	Blackish white	Blackish white	Reddish white	Blackish white	Blackish white
C2	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C3	White	Blackish white	Blackish white	Blackish white	Reddish white	Blackish white	Blackish white
C4	Orange	Orange	Orange	Orange	Reddish orange	Orange	Orange
C5	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C6	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C7	Orange	Orange	Orange	Orange	Reddish orange	Orange	Orange
C8	White	Blackish white	Blackish white	Blackish white	Reddish white	Blackish white	Blackish white
C9	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C10	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C11	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C12	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C13	White	Blackish white	Blackish white	Blackish white	Reddish white	Blackish white	Blackish white
C14	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C15	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink
C16	Pink	Pink	Pink	Pink	Reddish slight pink	Pink	Pink

The highest mean colony diameter of 87.47 mm was recorded on PDA followed by oat meal agar (81.65 mm), Richards's agar (80.00 mm) and Walksman's agar (78.37 mm). A lowest mycelial growth of 8.95 mm was recorded on water agar [Table 2; Figure 2].

The isolates of *C. musae* exhibited variations in respect of colony colour. The isolates C1, C3, C8 and C13 produced white coloured colonies on water agar; blackish white colour on oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish white on Martin's Rose Bengal. The isolates C2, C5, C6, C9, C10, C11, C12, C14, C15 and C16 produced pink coloured colonies on water agar, oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish slight pink on MRB and the isolates C4 and C7 produced orange coloured colonies on water agar, oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish orange on MRB [Table 3].

1. Potato Dextrose agar
2. Oat meal Agar
3. Richards Agar
4. Walksman's Agar
5. Czapek Dox Agar
6. Martin's Rose Bengal agar
7. Water Agar

Fig. 2: Effect of different media on growth of *C. musae*

1 C1	7 C7	13 C13
2 C2	8 C8	14 C14
3 C3	9 C9	15 C15
4 C4	10 C10	16 C16
5 C5	11 C11	
6 C6	12 C12	

Fig. 3: Colony characters of *C. musae* on PDA

Colony Characters on PDA: The colony characters of *C. musae* isolates were studied on PDA medium because the maximum mycelial growth was observed on PDA medium. Blackish white coloured colonies were observed in the isolates C1, C3, C8 and C13. Ten isolates (C2, C5, C6, C9, C10, C11, C12, C14, C15 and C16) produced pink coloured colonies and isolates C4 and C7 produced light orange coloured colonies.

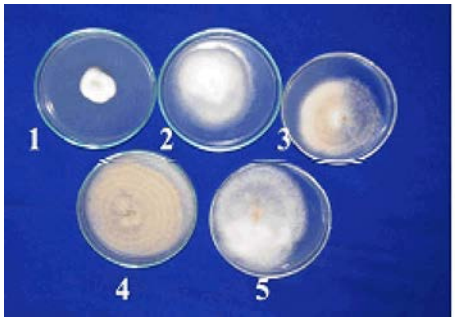

Fig. 4: Effect of pH on growth of *C. musae*

Significant variations were observed with respect to substrate colour. Four isolates (C1, C3, C8 and C13) produced the black colour, while C2, C5, C6, C9, C10, C11, C12, C14, C15 and C16 produced pinkish black. Isolate C4 and C7 recorded dark orange. Two isolates (C8 and C13) had irregular margin and the rest of isolates were having smooth margin.

Significant variations were observed with respect to topography of the colonies between different isolates of *C. musae*. Isolates C1, C3, C8 and C13 had flat mycelial growth and rest of the isolates recorded the raised fluffy growth. Isolate C8 produced concentric zonation, while all other isolates did not. Black pigmentation was noticed in isolates C1, C3, C8 and C13 whereas orange pigmentation was recorded in C4 and C7 and rest of the isolates produced pink pigmentation. Highest radial mycelial growth was noticed in C2 with 89.61 mm followed by C3 (89.10 mm) and C1 (89.00 mm). Least mycelial growth of 81.06 mm was recorded in isolate C6.

Seven isolates (C5, C7, C10, C12, C14, C15 and C16) produced good sporulation while five isolates (C1, C2, C4, C6 and C9) produced medium sporulation and poor sporulation was recorded in the isolates C3, C8, C11 and C13 [Table 4; Figure 3].

Influence of Abiotic Factors on Growth of Pathogen

Effect of pH on the Mycelial Growth: Effect of pH on the mycelial growth of different isolates of *C. musae* was studied. The results revealed that the maximum mean mycelial growth of 87.52 mm was observed at pH 7.0 followed by pH 6.5 (85.97 mm) and pH 6.00 (79.85 mm). The lowest mean mycelial growth was recorded at pH 4.0 (34.54 mm). The pH below six and above seven was detrimental to the growth of pathogen [Table 5; Figure 4].

Effect of Different Temperature on the Mycelial Growth: Effect of different temperatures on the mycelial growth of different isolates of *C. musae* was studied.

Table 4: Cultural characters of *C. musae* isolates on PDA medium

Isolate	Colony colour	Substrate colour	Margin	Topography	Zonation	Pigmentation	*Colony diameter (mm)	Sporulation
C1	Blackish white	Black	Smooth	Mycelium flat growth	Without Zonation	Black	89.00 [c]	++
C2	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	89.61 [a]	++
C3	Blackish white	Black	Smooth	Mycelium flat growth	Without Zonation	Black	89.10 [b]	+
C4	Light Orange	Dark orange	Smooth	Raised fluffy growth	Without Zonation	Orange	89.00 [c]	++
C5	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	89.00 [c]	+++
C6	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	81.06 [n]	++
C7	Light Orange	Dark orange	Smooth	Raised fluffy growth	Without Zonation	Orange	86.03 [m]	+++
C8	Blackish white	Black	Irregular	Mycelium flat growth	Concentric Zonations	Black	86.40 [k]	+
C9	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	88.47 [e]	++
C10	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	87.25 [i]	+++
C11	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	86.25 [l]	+
C12	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	87.54 [g]	+++
C13	Blackish white	Black	Irregular	Mycelium flat growth	Without Zonation	Black	88.75 [d]	+
C14	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	87.34 [h]	+++
C15	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	88.23 [f]	+++
C16	Pink	Pinkish black	Smooth	Raised fluffy growth	Without Zonation	Pink	86.55 [j]	+++

+ Poor sporulation : 1-10 spores / microscopic field (100X); ++ Medium sporulation : 11-50 spores/ microscopic field (100X)

+++ Good sporulation : More than 100 spores/ microscopic field (100X)

* Mean of three replications, In a column, means followed by a common letter is not significantly different at the 5 % level by DMR

Table 5: Effect of pH on the mycelial growth of different isolates of *C. musae*

	Colony diameter (mm) at different pH							
Isolate	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
C1	32.30 [f]	52.50 [e]	64.50 [d]	66.80 [c]	70.40 [b]	87.90 [a]	89.07 [a]	69.60 [b]
C2	35.55 [h]	59.00 [g]	62.20 [f]	67.00 [e]	78.80 [c]	85.20 [b]	88.55 [a]	69.00 [d]
C3	30.05 [f]	58.80 [d]	61.80 [d]	68.90 [c]	77.75 [b]	87.28 [a]	87.05 [a]	69.61 [c]
C4	35.50 [g]	56.02 [f]	60.20 [e]	66.04 [d]	80.20 [b]	87.40 [a]	88.50 [a]	69.10 [c]
C5	36.00 [g]	56.12 [f]	61.70 [e]	66.16 [d]	83.50 [b]	86.20 [a]	86.00 [a]	69.00 [c]
C6	39.93 [g]	51.05 [f]	60.00 [e]	68.05 [d]	81.10 [c]	85.23 [b]	89.93 [a]	69.00 [d]
C7	36.61 [e]	56.20 [d]	64.80 [c]	66.30 [c]	80.00 [b]	86.40 [a]	86.61 [a]	65.06 [c]
C8	37.35 [f]	59.30 [e]	60.23 [e]	69.20 [c]	82.00 [b]	87.25 [a]	87.35 [a]	66.03 [d]
C9	39.24 [h]	51.80 [g]	61.50 [f]	69.30 [d]	81.40 [c]	86.00 [b]	89.24 [a]	66.40 [e]
C10	30.51 [f]	59.42 [e]	65.20 [d]	68.52 [c]	82.00 [b]	85.16 [a]	85.51 [a]	68.47 [c]
C11	32.47 [f]	58.62 [e]	61.80 [d]	68.82 [c]	83.02 [b]	86.10 [a]	84.47 [ab]	67.25 [c]
C12	31.06 [h]	58.10 [g]	63.20 [f]	68.50 [d]	75.04 [c]	84.00 [b]	89.06 [a]	66.25 [e]
C13	36.03 [f]	57.02 [e]	61.70 [d]	67.08 [c]	82.34 [b]	87.00 [a]	87.03 [a]	67.54 [c]
C14	36.40 [h]	55.12 [g]	62.36 [f]	65.16 [e]	78.15 [c]	82.06 [b]	88.47 [a]	68.75 [d]
C15	31.47 [f]	53.05 [e]	60.00 [d]	69.05 [c]	84.80 [b]	86.03 [ab]	87.25 [a]	67.34 [c]
C16	32.25 [f]	56.20 [e]	61.90 [d]	66.80 [c]	80.40 [b]	86.40 [a]	86.25 [a]	68.23 [c]
Mean	34.54	56.14	62.06	67.60	79.85	85.97	87.52	67.91

*Mean of three replications

In a column, means followed by a common letters are not significantly different at the 5 % level by DMR

Table 6: Effect of different temperatures on the mycelial growth of *C. musae* isolates

	Colony diameter (mm) at different temperature*						
Isolate	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
C1	25.00 ^{e}	42.25 ^{d}	53.50 ^{c}	72.25 ^{b}	90.00 ^{a}	90.00 ^{a}	43.50 ^{d}
C2	23.50 ^{f}	41.00 ^{e}	54.50 ^{c}	68.25 ^{b}	89.25 ^{a}	89.00 ^{a}	46.00 ^{d}
C3	24.45 ^{g}	46.75 ^{e}	62.00 ^{d}	72.50 ^{c}	88.25 ^{b}	90.00 ^{a}	43.54 ^{f}
C4	22.00 ^{e}	46.80 ^{d}	60.00 ^{c}	78.80 ^{b}	88.00 ^{a}	89.24 ^{a}	46.00 ^{d}
C5	21.65 ^{e}	43.25 ^{d}	60.25 ^{c}	68.35 ^{b}	89.00 ^{a}	89.50 ^{a}	42.35 ^{d}
C6	22.65 ^{g}	42.75 ^{f}	61.00 ^{d}	72.50 ^{c}	87.65 ^{b}	89.24 ^{a}	46.58 ^{e}
C7	22.60 ^{f}	49.00 ^{d}	61.30 ^{c}	78.30 ^{b}	90.00 ^{a}	90.00 ^{a}	47.45 ^{e}
C8	19.85 ^{g}	44.31 ^{e}	59.30 ^{d}	69.65 ^{c}	87.50 ^{b}	90.00 ^{a}	42.00 ^{f}
C9	20.00 ^{g}	46.00 ^{f}	57.80 ^{d}	76.80 ^{c}	87.34 ^{b}	89.34 ^{a}	48.00 ^{e}
C10	22.00 ^{e}	46.35 ^{d}	50.00 ^{c}	70.00 ^{b}	87.30 ^{a}	89.00 ^{a}	46.15 ^{d}
C11	24.25 ^{g}	42.50 ^{f}	54.41 ^{d}	75.80 ^{c}	85.00 ^{b}	89.00 ^{a}	48.00 ^{e}
C12	22.75 ^{g}	43.45 ^{f}	51.73 ^{d}	70.00 ^{c}	85.25 ^{b}	89.75 ^{a}	47.90 ^{e}
C13	27.00 ^{f}	41.00 ^{e}	50.50 ^{c}	64.41 ^{b}	88.37 ^{a}	90.00 ^{a}	46.75 ^{d}
C14	27.31 ^{g}	41.65 ^{f}	53.25 ^{d}	61.73 ^{c}	84.00 ^{b}	88.75 ^{a}	48.90 ^{e}
C15	25.00 ^{f}	42.65 ^{e}	58.20 ^{c}	70.50 ^{b}	87.25 ^{a}	87.75 ^{a}	47.50 ^{d}
C16	26.35 ^{f}	42.60 ^{e}	53.00 ^{c}	63.25 ^{b}	89.00 ^{a}	90.00 ^{a}	49.00 ^{d}
Mean	23.52	43.89	56.29	70.81	87.69	89.41	46.22

*Mean of three replications

In a row, means followed by a common letter is not significantly different at the 5 % level by DMRT

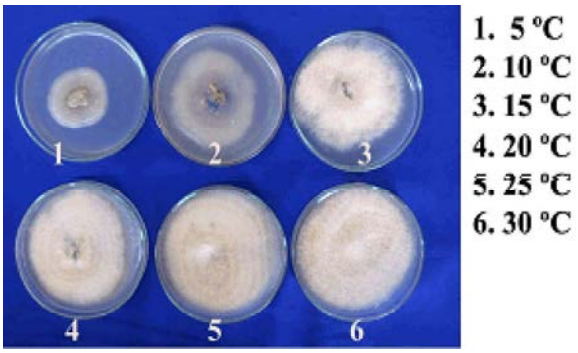

Fig. 5: Effect of different temperature on growth of *C. musae*

The results revealed that a highest mean radial mycelial growth of 89.41 mm was observed at 30°C which was followed by 25°C (87.69 mm) and 20°C (70.81 mm). Mean radial mycelial growth was found to be the lowest at 5°C as it recorded mean mycelial growth of 23.52 mm. Mean radial mycelial growth of 46.22 mm was observed at 35°C temperature. From the results it is understood that, the temperature below 30°C and above 35°C is indirectly proportional to the radial growth of *C. musae* [Table 6; Figure 5].

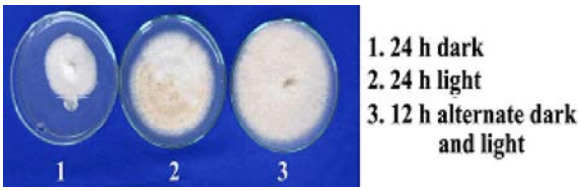

Fig. 6: Effect of light intensity on growth of *C. musae*

Effect of Light Intensity on the Mycelial Growth: Effect of light intensity on the mycelial growth of different isolates of *C. musae* was studied. The results showed that all the isolates grew well when they were exposed with alternate cycles of 12 h dark and 12 h light with mean mycelial growth of 87.32 mm followed by 24 h light exposure (56.20 mm) and the lowest growth of all isolates was found when exposed to 24 h dark (37.58 mm) [Table 7; Figure 6].

Growth Phase Study: The growth phase study for virulent isolate of *C. musae* (C12) was carried out. The results revealed that the mycelial growth of *C. musae* started to increase from second day after inoculation and reached the maximum at 13th day (1190.28 mg) and started decreasing from 14th day (978.00 mg) onwards and the least growth of 691.30 mg was noticed on 16th day after inoculation [Table 8].

Table 7: Effect of light intensities on mycelial growth of C. *musae* isolates

	Mycelial growth (mm)*		
Isolate	24 h light	24 h dark	12 h alternate dark and light
C1	57.25 b	36.00 c	87.45 a
C2	58.40 b	38.20 c	87.00 a
C3	66.35 b	32.14 c	88.41 a
C4	54.31 b	36.21 c	84.14 a
C5	58.30 b	39.64 c	87.64 a
C6	58.41 b	37.74 c	88.11 a
C7	54.31 b	38.45 c	85.41 a
C8	56.31 b	40.21 c	87.45 a
C9	57.21 b	35.45 c	87.64 a
C10	52.00 b	38.65 c	85.34 a
C11	54.00 b	38.26 c	87.24 a
C12	53.00 b	37.98 c	88.31 a
C13	54.99 b	38.17 c	88.46 a
C14	53.41 b	36.74 c	88.79 a
C15	53.00 b	39.45 c	86.34 a
C16	58.00 b	38.00 c	89.41 a
Mean	56.20	37.58	87.32

*Mean of three replications

In a row, means followed by a common letter is not significantly different at the 5 % level by DMRT

Table 8: Growth phase study with virulent isolate C12 of *C. musae*

Days interval	Dry mycelial weight (mg)*
1	53.00 p
2	89.00 o
3	160.08 n
4	243.85 m
5	290.24 l
6	370.00 k
7	480.00 j
8	563.32 i
9	748.20 g
10	930.42 e
11	985.60 c
12	1070.25 b
13	1190.28 a
14	978.00 d
15	838.25 f
16	691.30 h

*Mean of three replications

In a column, means followed by a common letter is not significantly different at the 5 % level by DMRT

DISCUSSION

Banana (*Musa* spp.) is the most important fruit crop grown in tropical and subtropical regions of India having a great socio-economic significance. Based on gross value, it is considered to be the fourth most important food crop in the world after rice, wheat and milk/milk products. It is not only known for its antiquity but also closely interwoven in our national heritage with its multifaceted uses. Hence, it is referred as Kalpatharu (a plant with virtues), Apple of Paradise and it also known as Adams Fig [29].

In the present study, the symptoms produced by the pathogen on artificial inoculation on the fruits were similar to the symptoms observed under natural infection. The symptom appeared as black, sunken lesions are distributed all over the outer part of the fruit. Under favorable moist conditions, the fungus starts developing acervuli, sometimes with concentric rings, sporulating with masses of pinkish conidia. Severely affected fruits become blackened and rot or sometimes shrivel and mummified.

The symptoms observed under artificial conditions agreed with same type of natural symptom. Similar symptom of anthracnose was also noticed on banana fruits with sunken lesions and covered with salmon-colored acervuli [5].

On ripening of banana fruits, sunken brown spots develop with orange acervuli [4]. The *Colletotrichum* species cause typical disease symptoms of anthracnose, which was characterized by sunken necrotic tissue where orange conidial masses are produced [30].

All the sixteen isolates showed hyaline and short conidiophores bearing single hyaline cylindrical conidia. The conidia measured 14.7 µm x 7.1 µm with a centrally placed oil globule. These characters agreed with the original descriptions given by Lemme and Sonoda [31] and Sutton [32]. Das Gupta [33] also reported the variation in the spore size (17.36-21.8 µm x 2.66-2.88 µm) among the isolates of *C. capsici* causing anthracnose of betelvine. The average size of the spores however, did not vary among the isolates. Chakrabarty *et al*. [34] reported that in *C. lindemuthianum* also the average size of the spores did not vary much among the isolates. Nandinidevi [10] reported that the conidiophores were hyaline and septate bearing ovoid to cylindrical conidia which were one celled with one or two oil globules, measuring 22.5 x 10 µm. Quimio and Quimio [35] found differences in the degree of virulence of eleven *C. gloeosporioides* isolates of mango and it was further reported that the conidial size was 12.0-17.0 x 3.5-6.0 µm. *C. gloeosporioides* isolates obtained from apple, peach, pecan and other hosts varied greatly in their growth, virulence and conidial size [36].

Every living being requires food for its growth and reproduction; fungi are not an exception to it. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life process. Neither all

media are equally good for all fungi nor there can be a universal substrate or artificial medium on which all fungi can grow well. So different media were tried for the growth of *C. musae.* Literature is with full of conflicting reports regarding the superiority of one medium over the other for the growth and sporulation of *C. musae.* In the present study, PDA followed by Oat meal agar medium supported the maximum growth of *C. musae.* This was in conformity with the findings of Nandinidevi [10] where in among the seven media tested, PDA recorded the maximum mean colony diameter followed by anthurium leaf extract and corn meal agar. The present findings is in similarity with the report by Amarjit singh *et al*. [9] who observed the maximum growth of *C. gloeosporioides* of Guava on PDA medium. Likewise, Jeyalakshmi and Seetharaman [37] and Patil and Moniz [8] reported that PDA was the best suited one for the growth and sporulation of *C. capsici*. But the maximum growth of *C. capsici* was observed in Richard's broth followed by potato dextrose broth [38].

In this study, the isolates C1, C3, C8 and C13 produced white coloured colonies on water agar; blackish white colour on oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish white on Martin's Rose Bengal. The isolates C2, C5, C6, C9, C10, C11, C12, C14, C15 and C16 produced pink coloured colonies on water agar, oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish slight pink colour on MRB and the isolates C4 and C7 produced orange coloured colonies on water agar, oat meal agar, Richards, PDA, Czapek Dox agar and Walksman's agar; reddish orange on MRB. Similar results were obtained by Manjunath [12], the isolate C1 of *C. gloeosporioides* produced black coloured colonies on water agar, white colour on Richards, oat meal agar, PDA, host leaf extract and Walksman's agar, blackish white colonies on nutrient agar, greyish white on Czapek Dox agar and dark white on MRB and reddish white on King's B agar. This is in agreement with the results of Anand [38], where in he noticed variation in *C. capsici* colony of different isolates on PDA. The present investigation revealed that the colony characters and growth of *C. musae* varied on different media. This might be due to the variation in the nutritional requirement of the fungus. There was a wide variation in the colony characters *viz.,* colour, topography, pigmentation, zonation, sporulation and mycelial growth of different isolates even in the same media. In the present study, isolates were characterized by blackish white, light pink and dark orange coloured colonies. Isolates with smooth and irregular margins were recorded. All the isolates varied with respect to sporulation, pigmentation, margin and topography. Similar observations were made by Denobys and Baudry [39], Kuramae *et al*. [40] and Manjunath [12].

Fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This brings importance of hydrogen ion concentration for better fungal growth. In the present study, maximum mycelial growth was observed at pH 7 which is in agreement with the results of Nandinidevi [10] and she reported maximum growth of *C. gloeosporioides* was at pH 7 followed by pH 6. However, this is similar to the work of Gina [41] who identified pH 7 as the optimum for the mycelial growth. At reduced pH, cell membrane becomes saturated with the hydrogen ions which limit the passage of cations. The reverse could be obtained when medium are alkaline and accumulated hydroxyl ions preventing the passage of essential anions. In addition, the enzyme activity is also conditioned by reaction of the medium, as a result the reduced growth of both fungi is observed at both the extremities [42].

Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. The present study revealed that good growth of fungus was observed at 25-30°C. Nandinidevi [10] reported maximum growth of *C. gloeosporioides* at 25°C followed by 30°C. The temperature of 25°C was reported to be the optimum for the growth of *C. gloeosporioides* on mango, almond and avocado [16-18]. Prabakar [43] reported that the mycelial growth was maximum at 25°C followed by 30°C and temperature below 20°C and above 35°C were inhibitory to the growth. These reports supported the results of the present study.

Light has profound effect on the mycelial growth of *C. musae*. Preliminary studies conducted in the present study revealed that maximum mycelial growth was observed when it was exposed to alternate cycles of light and darkness. This was followed by continuous light and continuous darkness. This result agreed with findings of Kamanna [44] where in he found that alternate cycle of 12 h light and 12 h dark yielded maximum growth of *C. gloeosporioides* when compared to the continuous exposure of light or dark. Similar results were recorded by Alexander *et al*. [45] in *C. gloeosporioides.*

Determination of optimum growth period is essential to study the physiology of fungi. Maximum dry mycelial weight was recorded on nineth day after inoculation on potato dextrose broth. Afterwards growth of mycelium was declined with increase in the number of days of incubation. This may be due to the autolysis of the

fungus and exhaustion of nutrients in the medium as opined by Lilly and Barnett [46] who also pointed out, the growth of fungi follow a definite pattern which depends on species, environment and nutrition. Mycelial growth started to increased from second day after inoculation and reached maximum at 13th day after inoculation and thereafter growth started to decline. Similar results were also observed by Sudhakar [21], who reported that vegetative growth of *C. gloeosporioides* isolated from stylosanthes reached maximum on 14th day after inoculation and after that autolysis was noticed. Yashodha *et al.* [20] reported that the maximum growth of *C. gloeosporioides*, causing anthracnose of arecanut on ten days after inoculation.

REFERENCES

1. Wardlaw, C.W., 1961. Banana disease including plantains and abaca. (Ed. Longman) William clowns and Sons limited, London, pp: 38.
2. Jeffries, P., J.C. Dodd, M.J. Jeger and R.A. Plumbley, 1990. The biology and control of *Colletotrichum* species on tropical fruit crops. Jounal of Plant Pathology, 39: 343-366.
3. Meredith, D.S., 1971. Transport and storage diseases of bananas: Biology and control of Tropical. Agriculture (Trinidad), 48(1): 35-50.
4. Stover, R.H. and N.W. Simmonds, 1987. Diseases and disorders. In: Bananas, Longman, pp: 281-323.
5. Sutton, B.C. and J.M. Waterston, 1970. *Colletotrichum musae.* CMI Description of pathogenic fungi and bacteria. CMI. No., pp: 222.
6. Jinyoung Lim., Lim, T.H and B. Cha, 2002. Isolation and Identification of *Colletotrichum musae* from Imported Bananas. Department of Agricultural Biology, Chungbuk National University, Cheongju, Korea, pp: 361-363.
7. Photita, W., P.W. Taylor, R. Ford, K.D. Hyde and S. Lumyong, 2005. Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. Fungal Diversity, 18: 117-133.
8. Patil, B.K. and L. Moniz, 1973. Growth and sporulation of *Colletotrichum capsici* on different culture media. Research Journal of Mahatma Phule Agricultural University, 4: 62-66.
9. Amarjit singh, Verma, K.S. and Chander Mohan, 2006. Effect of different culture media on the growth and sporulation of *Colletotrichum gloeosporioides* causing guava anthracnose. Plant Disease Research, 21: 224.
10. Nandinidevi, S., 2008. Studies on the foliar diseases of anthurium (*Anthurium andreanum* lind. Ex andre) M.Sc. (Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, pp: 168.
11. Anand, T., R. Bhaskaran, T. Raguchander, G. Karthikeyan, M. Rajesh and G. Senthilraja, 2008. Production of cell wall degrading enzymes and toxins by *Colletotrichum capsici* and *Alternaria alternata* causing fruit rot of chillies. Journal of Plant Protection Research, 48(4): 437-451.
12. Manjunath, 2009. Morphological and Molecular characterization of *Alternaria alternata* and *Colletotrichum gloeosporioides* incitants of leaf blight and anthracnose diseaseas of Noni and their Management, M.Sc.(Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, pp: 222.
13. Munro, A.L.S., 1970. Measurement and control of pH values In: Methods of Microbiology, J.R. Norris and D.W. Ribbons, (Eds.). Academic Press, London, 2: 39-90.
14. Purkayastha, R.P. and M. Sen Gupta, 1975. Studies on *Colletotrichum gloeosporioides* inciting anthracnose of jute. Indian Phytopathology, 28: 454-458.
15. Swart, G.M., 1999. Comparative study of *Colletotrichum gloeosporioides* from Avocado and Mango. Ph.D Thesis, University of Pretoria, Pretoria, pp: 112.
16. Gonzalez, E., 2003. Characterization of isolates of *Glomerella cingulata* causal agent of Glomerella leaf spot and bitter rot of apples based on morphology and genetic, molecular and pathogenicity tests. Ph.D. Thesis, North Carolina State University, pp: 77.
17. Moriwaki, J., T. Sato and T. Tsukiboshi, 2003. Morphological and molecular characterization of *Colletotrichum boninense* sp. *nov*. from Japan. Mycoscience, 44: 47-53.
18. Adaskaveg, J.E. and R.J. Hartin, 1997. Characterisation of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology, 87: 979-987.
19. Mishra, A. and B.S. Siradhana, 1980. Sorghum anthracnose on the growth and sporulation of causal fungus at different light exposures. Philippine Agricultural Scientist, 63: 67-70.
20. Yashodha, H., R.K. Hegde and K. Srikant, 1993. Physiological studies on *Colletotrichum gloeosporioides* (Penz) Penz and Sacc. causing anthracnose of arecanut. Karnataka Journal of Agricultural Sciences, 6: 411-412.

21. Sudhakar, S., 2000. Biology and management of stylosanthes anthracnose caused by *Colletotrichum gloeosporioides* penz (penz) and sacc. M.Sc. (Ag.) Thesis, University of Agricultural Sciences, Dharwad, pp: 220.
22. Rangaswami, G., 1958. An agar block technique for isolating soil micro organisms with special reference to Pythiaceous fungi. Science and Culture, 24: 85.
23. Ainsworth, G.C., 1961. Dictionary of fungi. Commonwealth Mycological Institute, Kew Surrey, England, pp: 547.
24. Tuite, J., 1969. Plant Pathological Methods. Fungi and Bacteria. Publishing Co., Minneapolis, U.S.A., pp: 239.
25. Booth, C., 1971. Fungal culture media. In: Methods in Microbiology, C. Booth, (Ed.). Academic Press, New York, 4: 49-94.
26. Fahmy, T., 1923. The production by *Fusarium solani* of a toxic excretory substance capable of causing wilting of plants. Phytopathology, 13: 543-550.
27. Wastie, R.L., 1961. Factors affecting competitive saprophytic colonization of the agar plate by various root infecting fungi. Transactions of the British Mycological Society, 44: 145-159.
28. Kiryu, T., 1939. Studies on physiological properties of *Ceratocystis paradoxa*. Jaiwan province sugarcane experiments station Report, 6: 21-27.
29. Bose, T.K. and S.K. Mitra, 2001. Fruits: Tropical and Sub Tropical. Tata McGraw-Hill, New Delhi, 1: 227-318.
30. Bailey, J.A. and M.J. Jeger, 1992. *Colletotrichum*: Biology, Pathology and Control. CAB International. Wallingford, UK.
31. Lemme, J.M. and C.M. Sonoda, 1978. Occurrence of *Colletotrichum dematium* f. sp. *truncata* on *stylosanthes* spp. Plant Disease Reporter, 62(7): 641-644.
32. Sutton, B.C., 1980. The coelomycetes. Common Wealth Mycological Institute, Kew, pp: 78.
33. Das Gupta, B., 1986. Role of toxin secretion by *Colletotrichum capsici* on the expression of leaf spot symptoms in Betelvine. Journal of Plantation Crops, 14: 36-41.
34. Chakrabarty, P.K., B.M. Singh and K.R. Shyam, 1988. Physiological specialization in *Colletotrichum lindemuthianum*. Indian Journal of Mycology and Plant Pathology, 18: 308-310.
35. Quimo, T.H. and A.J. Quimo, 1975. Pathogenicity of Mango anthracnose. Philippine Agricultural Scientist, 58: 322-329.
36. Bernstein, B., E.I. Zehe, R.A. Dean and E. Shabi, 1995. Characteristics of *Colletotrichum* from peach, apple, pecan and other hosts. Plant Disease, 79: 478-482.
37. Jeyalakshmi, C. and K. Seetharaman, 1999. Studies on the variability of the isolates of *Colletotrichum capsici* (Syd.) Butler and Bisby causing chilli fruit rot. Crop Research, 17: 94-99.
38. Anand, T., 2002. Studies on fruit rot of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby and *Alternaria alternata* (Fr) Keissler. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, pp: 157.
39. Denobys, B. and A. Baudry, 1995. Species identification and pathogenicity strictly of France *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. Phytopathology, 85: 53-57.
40. Kuramae, E.E., C.R. Lopes, N.L. Souza and Machado, 1997. Morphological and molecular characterization of *Colletotrichum* spp. from citrus orchads affected by post bloom fruit drop in Brazil. European Journal of Plant Pathology, 103: 323-329.
41. Gina, M.S., 1999. Comparative study of *Colletotrichum gloeosporioides* from Avocado and Mango. Ph.D. Thesis, University of Pretoria, Pretoria, pp: 112.
42. Bilgrami, K.S. and R.N. Verma, 1978. Physiology of fungi. Vikas Publishing House, New Delhi, pp: 438.
43. Prabakar, K., 1997. Anthracnose disease of mango fruits caused by *Colletotrichum gleosporioides.* Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore. India, pp: 196.
44. Kamanna, B.C., 1996. Epidemology and control of anthracnose diseases of coffee incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc.(Ag.) Thesis, University of Agricultural Sciences, Bangalore, pp: 190.
45. Alexander, F.S., A.C. Mello, M. Zamboni and P.B. Ivan, 2004. Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperatures and light regimes. Scientific Agriculture, 61(5): 542-544.
46. Lilly, V.G. and H.L. Barnett, 1951. Physiology of fungi. McGraw Hill Book Co. Inc., London, pp: 240.