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CULTURAL AND PHYSIOLOGICAL CHARACTERIZATION OF *Colletotrichum musae*, THE CAUSAL AGENT OF BANANA ANTHRACNOSE

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ABSTRACT: *Colletotrichum musae* is the causal agent of anthracnose in banana fruits; infection by this fungal pathogen results in severe post-harvest losses. Hence various aspects of the disease and the causal agent such as morphological, cultural and physiological characters were investigated in present study. *C. musae* produced salmon pink coloured colonies with white margins on PDA, Conidia were hyaline, aseptate, broadly elliptical or cylindrical with rounded ends. Among the ten solid media tested, it produced maximum growth (89.83 mm) and excellent sporulation on oat meal agar after seven days of inoculation. Maximum dry mycelial weight of *C. musae* (340 mg) was recorded in Richards's broth among the ten liquid media tested. Temperature had shown significant effect on growth and sporulation, maximum radial growth of *C.musae* was recorded at 25°C (89.83 mm) and 30°C (89.17 mm), where as optimum pH range for growth fell in the range from 6.0 pH (398 mg) to 7.0 pH (383 mg) where the maximum dry mycelial weight was recorded.

Key words: *Colletotrichum musae*, Banana, anthracnose

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INTRODUCTION

Banana is one of the most popular fruits in India and many tropical countries and important dessert fruit in India eaten fresh. Major economic part of the banana plant is the fruit, suffers from many postharvest diseases. These diseases have had considerable influence on different aspects of cultivation, nutritive value, harvesting, transit and transhipment, storage of fruits. It is estimated that 20 to 25 per cent of harvested fruits are decayed by pathogens during postharvest handling even in developed countries (Zhu and Ma, 2007). However, in developing countries, post harvest losses are often more severe due to inadequate storage and transportation facilities (Rashad *et al.*, 2011). Anthracnose of banana is the most important disease encountering tremendous loss in the market. Fungus isolated from the anthracnose lesions on bananas was identified as *C. musae* based on conidial morphology in their study by many researches (Zakaria *et al.*, 2009; Ashwini *et al.*, 2015). Various aspects of the disease and the causal agent *C. musae* such as morphological, cultural and physiological characters were investigated in present study.

MATERIAL AND METHODS

The present investigations on various aspects pertaining to banana anthracnose were carried out during 2015-16. Laboratory studies on isolation and identification of pathogens, morphological, cultural and physiological characterization of postharvest disease of banana were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka.

Morphological studies

To determine the morphological characters, conidia were observed under microscope. The average size of the spore like breadth and length were thus obtained. Microphotographs were taken to show the typical spore morphology of the pathogen. Spores were thoroughly mixed with sterile water and incubated at 27± 2°C in cavity slides for 24 h and observed for spore germination (Kim *et al.*, 2008).

Cultural studies

Growth phase

Growth phase study was carried out in potato dextrose for *C. musae* Thirty ml of broth was added in each of the 100 ml conical flasks and sterilised at 1.1 kg/cm^2 pressure for 15 min. These flasks were inoculated with five mm disc from actively growing fungal culture and incubated at 27±1°C. Culture was filtered through Whatman No. 42 filter paper to record dry mycelial weight at two days interval. Number of days required for maximum growth was recorded and used in further studies.

Growth characters on solid media

Growth characters of *C. musae* were studied on ten solid media *viz.*, potato dextrose agar, potato carrot agar, cornmeal agar, host extract agar, oat meal agar, malt extract agar, V-8 juice agar, Richards's agar, Czapek's agar and Sabouraud's agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petriplates, such Petriplates were inoculated with five mm disc cut from periphery of actively growing culture and incubated at 27±1°C. Colony diameter was recorded when the fungus covered complete Petriplate in any one of the media. The data on radial growth was analysed statistically. Colony colour, texture, surface elevation and sporulation were also recorded.

Sporulation

For recording sporulation three uniform bits were cut from 10 day old culture plate with the help of corkborer representing periphery, center and middle portions of culture plate. These culture bits were transferred to a sterilised test tube containing 2 ml of sterile distilled water and shaken thoroughly to dislodge the spores in water. Thereafter, 20 µl of the spore suspention was transferred to a clean glass slide and mounted for microscopic observation. Number of spores per each microscopic field (400X) were recorded likewise 10 microscopic fields were examined to obtain mean sporulation per microscopic field. The intensity of sporulation was grouped into five classes *viz.*, excellent (++++), good (+++), average (++), poor (+) and no sporulation (-). Similar procedure was followed for all the media under study and compared.

Growth in liquid media

The composition and preparations of different liquid media used were the same as that of solid media except that the agar agar was not added. Thirty ml of the medium was added to each of 100 ml flask and were sterilized at 1.1 kg/cm^2 pressure for 15 min. Inoculum disc of five mm size from actively growing cultures of both the fungi in study was transferred to all flasks and incubated at 27±1°C for 10 days. The mycelial mat was harvested, dried and weighed as described in growth phase study.

Physiological studies

Effect of temperature

Potato dextrose broth was used in this experiment to compare growth of fungi in terms of dry mycelial weight. Conical flasks of 100 ml capacity and each containing 30 ml of liquid medium were inoculated with 5 mm culture disc and incubated at different temperature levels *viz.*, 15, 20, 25, 30, 35, 40 and 45°C. The dry mycelial weight at each temperature level was recorded after incubating for ten days. Linear growth of the fungi as well as sporulation were studied on potato dextrose agar and compared at different levels of temperature. Results were analysed statistically.

Effect of pH

pH of the potato dextrose broth was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). The reaction of the medium was adjusted to the desired pH by using di-hydrogen phophate citric acid buffer according to schedule of Vogel (1951). The pH of the medium was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. After sterilization there was slight change in pH, which was negligible. The cultures were inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 27±1°C for ten days. Dry mycelial weight was obtained as described earlier and results were analysed statistically.

Statistical analysis

The experiment was laid out in a completely randomized design (CRD) in three replications. The experiment was conducted at room temperature in the laboratory. Statistcal analysis was done as per the procedures given by Gomez and Gomez (1984). All comparisons of means were subjected to analysis of variance (ANOVA) and the significant differences among treatments were determined with a least significant difference (lsd) separation test. Arcsine or square root transformation was used wherever required to normalize variance.

EXPERIMENTAL RESULTS

Morphological studies

C. musae on potato dextrose agar produced salmon pink coloured colonies with white mycelial growth towards margins. Dark orange pigmentation on reverse side of the colony was also observed. Mycelium sparsely produced, hyphae septate and hyaline. Conidia were hyaline, aseptate, broadly elliptical or cylindrical with rounded ends. Setae were not observed in acervuli.

The average size of conidia was 11.43- 16.27 x 3.86-5.47 µm. Conidia germinated and produced appressoria which were dark brown coloured and irregularly lobed. Findings of present study are in agreement with the results of Lim *et al.* (2002); Photita *et al.* (2005) and Zakaria *et al.* (2009).

Cultural studies

Growth phase

Determination of optimum growth period is essential to study the physiology of fungi. In present investigations, efforts were made to study growth pattern of postharvest pathogens of banana (Table 1 & Fig 1). Maximum dry mycelial weight of both the fungi *C.musae* (342.67 mg). The growth declined significantly from then onwards. This might be possibly due to autolysis of the fungus and exhaustion of nutrients in the medium as opined by Lilly and Barnett (1951) who also pointed out that the growth of the fungus as in other organisms follow a definite pattern which depend on species, environmental and nutritional conditions. These results are in harmony with Hegde *et al.* (1993) who concluded that maximum dry mycelial weight of *C. gloeosporioides* was recorded on 10th day of inoculation.

Table 1: Growth phase of *Colletotrichum musae* in potato dextrose broth

Incubation period (days)	Dry mycelial weight (mg) *C. musae*
2	82.67
4	169.00
6	265.33
8	283.67
10	342.67
12	311.33
14	280.33
16	251.33
18	218.00
20	155.33
22	138.33
24	136.00
26	117.00
28	114.33
S.Em±	3.44
CD at 1%	13.44
CV%	2.91

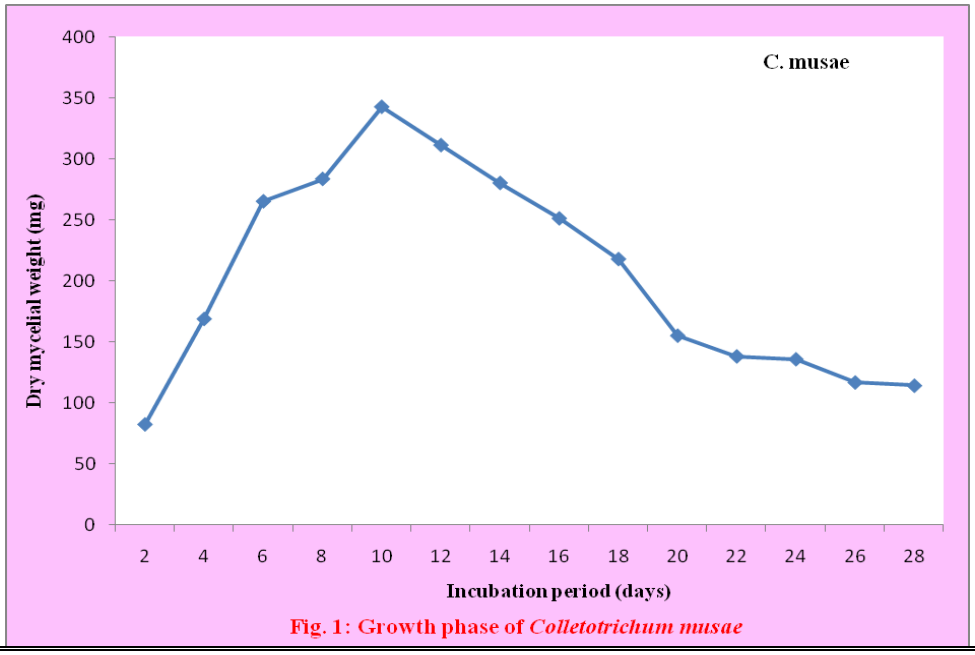

Fig. 1: Growth phase of *Colletotrichum musae*

Growth characters on solid media

Fungi secure food and energy from the substrate upon which they live in nature. Not all the media are equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow. So, different media were tried to study the variation in growth and cultural characteristics of *C. musae* (Table 2 & Fig 2).

Maximum linear growth of *C. musae* (89.83 mm) was recorded in oat meal agar after seven days of incubation which was on par with potato dextrose agar (89.42 mm), Richards's agar (87.38 mm) and host extract agar (87.33 mm). Colour of the colonies was also varied in different media however most of the times salmon pink colonies with white margin were observed. Cinnamon coloured colonies were produced in oat meal agar and potato carrot agar whereas host extract agar and Sabouraud's agar produced greyish white colonies. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation. Excellent sporulation of *C. musae* was recorded on oat meal agar, good sporulation in potato dextrose agar and potato carrot agar. Though good radial growth was recorded in host extract agar, sporulation was average. Media had significant effect on growth of pathogens which may be attributed to complex nature of media supporting good fungal growth. In similar studies carried out by Abd-Elsalam *et al.* (2010) and Thangamani *et al.* (2011), potato dextrose agar was reported to be the best medium for *C. musae.* In another study, Priyadarshanie and Vengadaramana (2015) found that potato dextrose agar and Carrot dextrose agar media supported significantly the maximum growth of all the six isolates of *C. musae*.

Table 2: Cultural characters of *Colletotrichum musae* in different solid media

Media	Colony characters				Sporulation
	Radial growth (mm)	Type of growth	Colony margin	Colony colour	
Corn meal agar	55.85	Submerged	Circular, rough	Light orange colony, margin light orange	+
Czapek's agar	73.00	Aerial	Irregular, rough	Salmon pink colony, margin white	++
Host extract agar	87.33	Aerial	Circular, rough	Grayish white colony, margin white	++
Malt extract agar	48.91	Merged	Circular, rough	Salmon pink colony, margin white	++
Oat meal agar	89.83	Merged	Circular, smooth	Cinnamon colony, margin white	++++
Potato carrot agar	83.55	Aerial	Circular, wavy	Cinnamon colony, margin white	+++
Potato dextrose agar	89.42	Aerial	Circular, rough	Salmon pink colony, margin white	+++
Richards's agar	87.38	Merged	Irregular, rough	Orange colony, margin white	++
Sabouraud's agar	81.00	Merged	Circular, wavy	Grayish white colony	++
V-8 juice agar	68.85	Aerial	Circular, wavy	Buff white colony, margin white	+
S.Em±	**1.31**				
CD at 1%	**5.28**				
CV%	**2.88**				

Sporulation	Conidia/ microscopic field (400X)
++++	> 50
+++	31-50
++	11-30
+	≤10

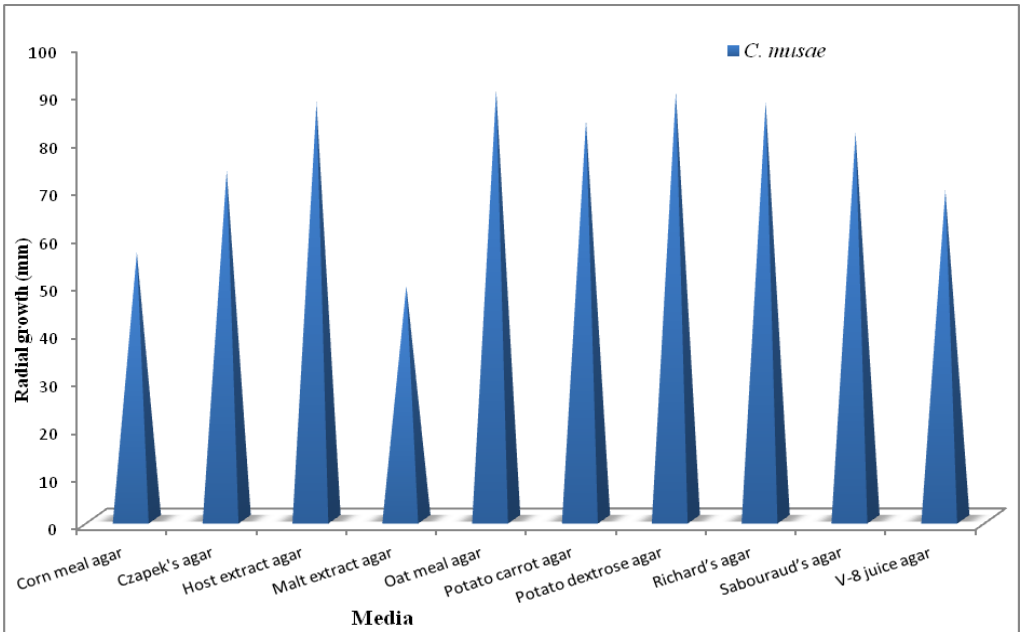

Fig. 2: Cultural characters of *Colletotrichum musae* in different solid media

Growth in liquid media

For isolation and characterization of fungi it is necessary to know their nutrient requirements. In the radial measurements, it is not possible to consider the amount of submerged mycelium. Hence, Cochrane (1958) has opined the determination of dry mycelial weight as the best method for precise work. Both the pathogens were grown in different liquid media and dry mycelial weight was recorded at 10th day after inoculation in present study (Table 3 & Fig 3). The results indicated that significantly highest dry mycelial weight of *C. musae* was recorded in Richards's broth (340 mg) which was on par with potato dextrose broth (333 mg). The next best medium was Sabouraud's broth (323 mg) followed by oat meal broth (268.33 mg). Though oat meal agar could support good radial growth and abundant sporulation, dry mycelial weight was not significant in oat meal broth. Fungal growth was rich cinnamon in colour in oat meal agar and potato dextrose agar and whitish orange in most of the other media. Similar results were obtained by Rani and Murthy (2004), who reportrd that Richards's broth was the best medium for *C. gloeosporioides.* In another study, Ashoka (2005) opined that growth of *C. gloeosporioides* was well supported by Richards's both and potato dextrose broth.

Table 3: Growth of *Colletotrichum musae* in different liquid media

Media	Dry mycelial weight (mg) of *C. musae*
Corn meal broth	42.33
Czapek's broth	160.67
Host extract broth	150.67
Malt extract broth	102.00
Oat meal broth	268.33
Potato carrot broth	250.67
Potato dextrose broth	333.00
Richards's broth	340.00
Sabouraud's broth	323.00
V-8 juice broth	203.67
S.Em±	3.61
CD at 1%	14.51
CV%	2.87

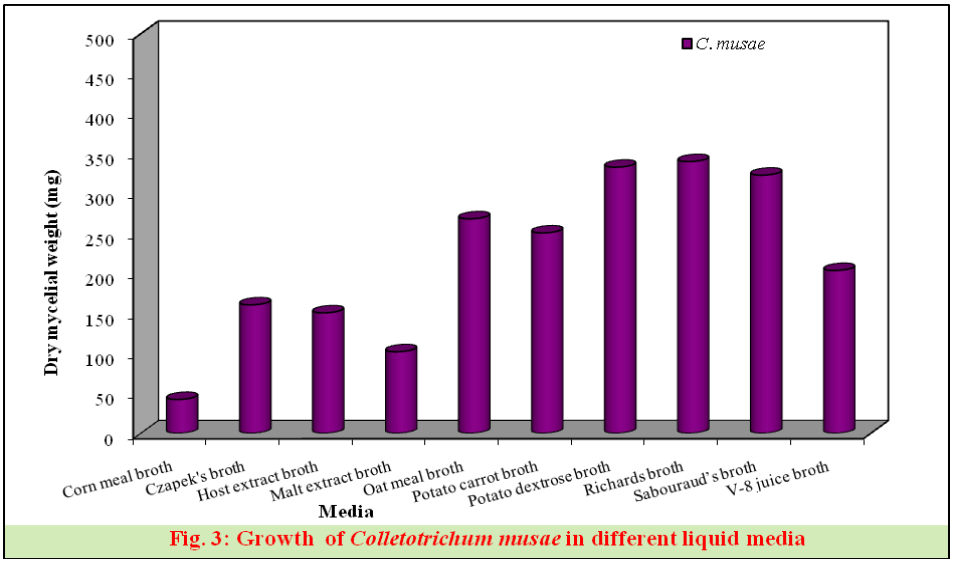

Fig. 3: Growth of *Colletotrichum musae* in different liquid media

Physiological studies

Effect of temperature

Temperature is the most important physical environmental factor for regulating vegetative and reproductive activity of the fungi. Effect of temperature on the growth of postharvest pathogens of banana was studied in present investigation, which had shown significant role in growth of *C.musae* (Table 4 & Fig 4). Maximum dry mycelial weight was recorded at 25^{0}C (342.20 mg) which was at par with 30^o C (337.33 mg) and were significantly superior to other temperature levels tested. Temperature influenced linear growth also; maximum radial growth of *C. musae* on PDA was recorded at 25^{o}C and 30^{o}C (89.83 mm), which was significantly superior to other treatments. No growth was observed in 40^o C and 45^{o}C. Temperature had played crucial role in sporulation of pathogen also. The sporulation was excellent at 25^o C and 20^o C and good at 30^o C and 35^o C. Results obtained in this study are in agreement with findings of Thangamani *et al.* (2011) and Rani and Thammaiah (2014) who reported that optimum temperature required for growth of *C. musae* was 25°C to 30°C.

Table 4: Effect of temperature on growth of *Colletotrichum musae*

Temperature (^{o}C)	Dry mycelial weight (mg)	Radial growth (mm)	Sporulation
15	110.67	26.64 (5.26)*	++
20	299.00	66.37 (8.21)	++++
25	342.20	89.83 (9.53)	++++
30	337.33	89.17 (9.53)	+++
35	266.00	35.67 (6.06)	+++
40	103.00	0.00 (1.00)	-
45	73.73	0.00 (1.00)	-
S.Em±	3.01	0.02	
CD at 1%	12.64	0.10	
CV%	2.38	0.68	

*Figures in the parenthesis indicate √X+1 transformed values

Sporulation	Conidia/ microscopic field (400X)
++++	>50
+++	31-50
++	11-30
+	≤10
-	No conidia

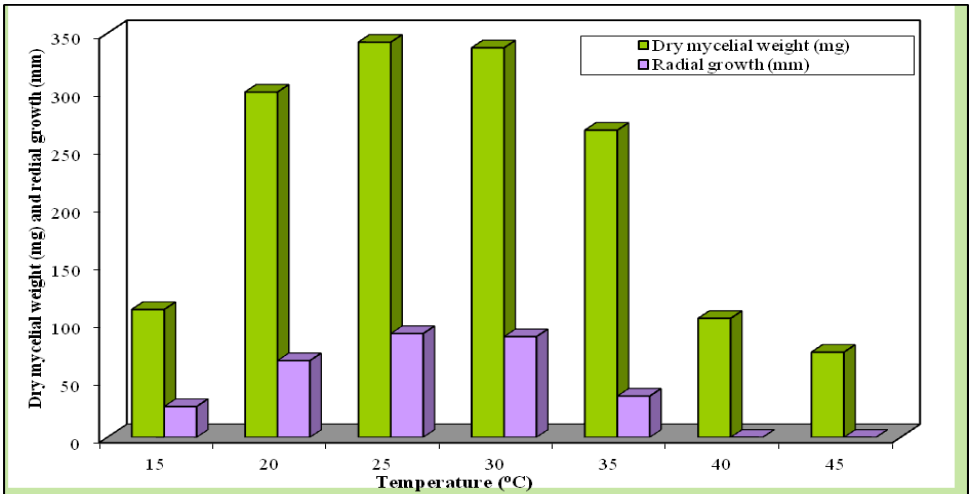

Fig. 4: Effect of temperature on growth of *Colletotrichum musae*

Effect of pH

Different fungal pathogens require a particular range of pH for their growth and development. It was felt necessary to study pH as it might be one of the factors influencing pathogenesis as discussed by De-Costa and Chandima (2014) who reported that, infection and colonization of *C. musae* depends on pH of banana fruit peal. Present study revealed that, both the pathogens grew in wide range of pH levels (Table 5 & Fig 5). However, maximum dry mycelial weight of *C. musae* was recorded at pH 6.0 (398 mg) which was on par with pH 7.0 (383 mg), both were significantly superior over rest of the treatments followed by pH 8.0 (377.33 mg). Fungal growth was submerged and varied greatly in colour from dark grey at lower pH to orange at higher levels of pH. Results obtained by Thangamani *et al.* (2011) and Rani and Thammaiaha (2014) are in agreement with the findings of present investigations. In contrast, Zaemey *et al.* (1994) concluded that optimum pH for germination and growth of *C. musae* varied between 4.0 and 5.0 depending on temperature.

Table 5: Effect of pH on growth of *Colletotrichum musae*

pH	Dry mycelial weight (mg) of C. musae
3	165.33 (++)*
4	188.00 (++)
5	317.00 (+++)
6	398.00 (++++)
7	383.00 (++++)
8	377.33 (+++)
9	285.87 (+++)
10	233.67 (++)
S.Em±	4.14
CD at 1%	17.13
CV%	2.44

*Sporulation	Conidia/ microscopic field (400X) (*C .musae*)
++++	>50
+++	31-50
++	11-30
+	≤10

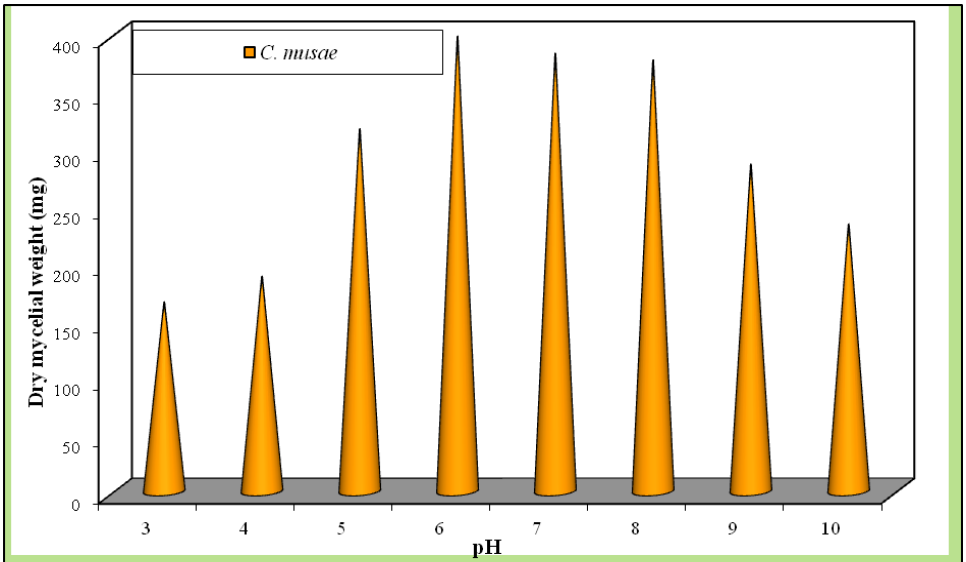

Fig. 5: Effect of pH on growth of *Colletotrichum musae*

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