



Effect of different growth media on growth and sporulation of *Colletotrichum gloeosporioides* and *C.acutatum* causing leaf spot in Acid lime

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Abstract

The present study was carried out to investigate the effect of different growth media on growth and sporulation of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* causing leaf spot in acid lime. Generally the growth of the fungus is greatly influenced by nutritional factors such as carbon and nitrogen source, these factors are mainly important for growth and sporulation of fungi. Four different media viz., Potato Dextrose Agar, Corn Meal Agar, Oat Meal Agar, Water Agar were studied for growth and sporulation of pathogen. The pathogens showed great variation in colony colour and sporulation rate on different media. *Colletotrichum gloeosporioides* showed Colony colour varied from white to greyish and semi-transparent to transparent. Culture colour of *Colletotrichum acutatum* varied from whitish orange to greyish green. Among different media studied, Potato Dextrose Agar and Oat meal Agar gave better growth and sporulation of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*.

Keywords: *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, morphology, cultural characteristics, different growth media

Introduction

India is the largest producer of acid lime in the world, ranks fifth position among the major lime and lemon-producing countries in the world. Acid lime is cultivated in almost all the states. Among them, Andhra Pradesh, Maharashtra, Tamil Nadu, Karnataka, Gujarat, Bihar, and Himachal Pradesh are the major producing states. Sweet orange and acid lime are the two major commercial citrus fruits grown in Andhra Pradesh (AP). Lemons, Pummelos and Mandarins are cultivated in a limited area. In India Kagzi lime is the commonly grown acid lime variety, Balaji and Petluru selection-1 are the majorly cultivated varieties in AP. In India, the total area under cultivation of acid lime is 259.3 thousand hectares with a production of 2789.0 thousand tonnes. AP is the leading lime producing state in the country grown in an area of 52.53 m ha with a production of 840.55 MT. (Anonymous, 2017) [2].

Acid lime is grown in almost all the districts of AP and the largest area is in semi-arid regions of Nellore, YSR Kadapa, Prakasam, Guntur, West Godavari, East Godavari and Anantapur. In Nellore district, acid lime has grown into a quite popular and more remunerative crop. Nearly 50 per cent area and production come from Nellore district alone as compared to rest of all districts in AP. Nellore district become major area (21,128 ha.) of acid lime contributing to 50 per cent of total production (3,16,920 MT). (Mukunda Lakshmi *et al.*, 2016) [7].

Leaf spot disease is an emerging disease in Nellore district of AP. In order to know the occurrence of different leaf spot diseases and the major one in acid lime grown in Nellore district and which leaf spot disease is more dominant, the study has been conducted to identify the causal agent associated with symptomatic citrus leaf samples collected from the Nellore district of Andhra

Pradesh, to examine their morphological and cultural characteristics in different growth media.

Materials and Methods

Isolation of pathogen in different growth media

In isolation studies different media viz., potato dextrose agar medium, corn meal agar medium, water agar medium and oat meal agar medium were used. The specimens showing typical leaf spot symptoms were cut into small pieces and surface disinfected by immersing in 1% sodium hypochlorite solution for one minute and then rinsed thrice in sterilized water. The surface sterilized pieces were placed on filter paper to remove excess moisture. Then tiny bits of diseased leaf placed on to the different media in petri plates and incubated at 26±0 C in Biologically Oxygen Demand incubator (BOD). Periodically observed the plates for growth of pathogen in different media.

Identification of pathogen

The cultures obtained from the samples were compared with original characteristics of the fungus mentioned in the literature. The conidia of the fungus observed under binocular microscope. Then the pure culture slants were sent to National Centre of Fungal Taxonomy (NCFT), New Delhi for further confirmation of the pathogen. The microscopic images were taken by using image analyser (De winter Capture Pro4.6 exe).

Morphological and cultural characteristic of pathogens

The morphological characterises like mycelia and conidia characteristics were observed under microscope and cultural characteristics like growth pigmentation and coloured pattern of

the pathogens were observed, to know the variation in different growth media.

Maintenance of the culture of the fungus

The pure cultures on PDA slants were kept at 26°C for seven days. When maximum growth observed, such slants were preserved in refrigerator at 6 to 10°C. The cultures were sub cultured once in a month to maintain the fungus viability.

Results and Discussion

Identification of the pathogen

The isolated pathogen from different media was identified by comparing the colony characteristics with original characteristics of the fungus mentioned in the literature. The conidia of the fungus observed under binocular microscope. All isolates collected at different locations were cultured and slants were sent to National Centre of Fungal Taxonomy, New Delhi, for further confirmation and it was confirmed as *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*.

Morphological characteristics of the *Colletotrichum gloeosporioides* & *C. acutatum*

The morphological characteristics of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* were presented in table (1&2). *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* were morphologically similar with slight variation in conidial form and presence/ absence of setae.

Mycelium and Acervuli

The fungus in the present study produced, septate, irregularly branched mycelium. Initially profuse white mycelium produced which gradually turned grey. Initially acervuli appeared light orange in colour and later changed to dark brown in colour, produced within 4-7 days. The acervuli (including setae) measured $190.39 \mu\text{m}$ (120.24 to $344.05 \mu\text{m}$) \times $144.76 \mu\text{m}$ (57.87 to $180.04 \mu\text{m}$) in length and width. The base of the acervuli was

dark brown to black. These findings were quite close to those recorded by Sagar, (2009)^[9], Suvarna *et al.* (2014)^[11], Joshi *et al.* (2015)^[5]. Acervuli produced from *Colletotrichum acutatum* were brown to blackish in colour, on average $191.3 \mu\text{m}$ in length and $131.2 \mu\text{m}$ width.

Conidia and setae

Conidiophores were single celled, hyaline and aseptate. The conidia were oblong or cylindrical or slightly dumbbell shaped, hyaline, aseptate with rounded ends, on average, they measured $11.46 \mu\text{m}$ (9.185 - $13.193 \mu\text{m}$) in length and $4.72 \mu\text{m}$ (4.34 – $6.84 \mu\text{m}$) in width. The setae were brown to blackish with pointed end. The setae measured $30.39 \mu\text{m}$ (21.85 – $42.55 \mu\text{m}$) in length and $4.18 \mu\text{m}$ (3.80 – $5.32 \mu\text{m}$) in width (fig.1). These findings were in agreement with the findings of earlier workers (Sudhakar, (2000)^[10]; Venkataravanappa, (2002)^[2]; Prashanth, (2007)^[8] and Joshi *et al.* (2015)^[5].

Colletotrichum acutatum produced hyaline, ellipsoid and fusiform conidia with slightly tapered on one end, with two oil globules. On average conidial size 12.5 - $14.6 \mu\text{m}$ length, 4.5 - $5.2 \mu\text{m}$ width. No setae were observed (fig.2). These findings were in agreement with Lewis *et al.* (2004)^[6]; Adaskaveg *et al.* (2000).

Cultural characteristics of *Colletotrichum gloeosporioides* & *C. acutatum* on different media.

Colony characteristics of *Colletotrichum gloeosporioides* on different media has been shown in table (3) and (fig 3). Colony colour varied from white to greyish and semi-transparent to transparent. Pigmentation also varied from whitish to greyish and whitish to orange. On potato dextrose agar media white to greyish white, fluffy and cottony growth observed. The findings of present study were in consonance with earlier reports of Gangadevi and Muthumary (2008)^[3] and Joshi *et al.* (2015)^[5]. On corn meal agar media, semi-transparent type of colony colour was observed. It grows moderately. On oat meal agar media, greyish white colony colour was observed. It grows at faster rate.

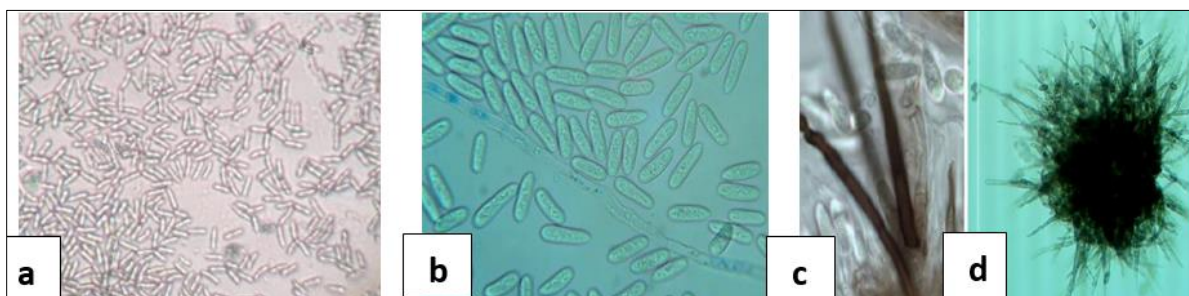


Fig 1: Morphological view of *C. gloeosporioides* a & b conidia c) acervuli d) setae

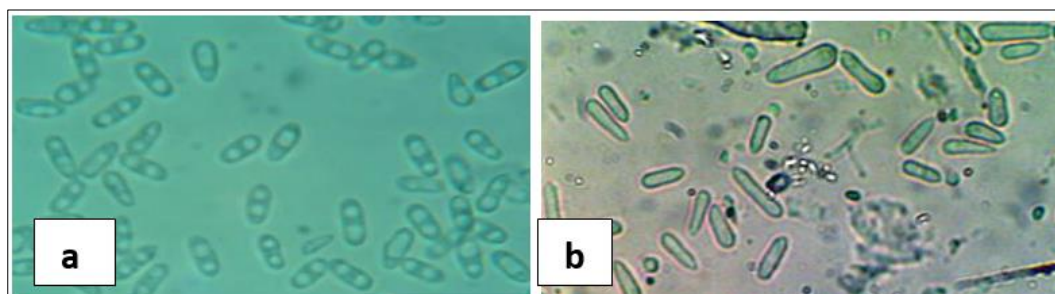


Fig 2: Morphological view of *Colletotrichum acutatum* a) fusiform shaped conidia b) acute shaped conidia

On water agar media, transparent colony colour was observed. Colony was fast growing. Excellent sporulation was observed on potato dextrose agar media and oat meal agar media when compared, less sporulation was observed on corn meal agar media. The present findings were comparative with that of Udaykumar and Usha Rani, (2010) [12]; Jayalaxmi *et al.* (2013) [4] and Joshi *et al.* (2015) [5]. They reported excellent sporulation of

Colletotrichum gloeosporioides was recorded on potato dextrose agar media and oat meal agar media.

Growth of *Colletotrichum acutatum*, on corn meal agar media was whitish orange in colour. On potato dextrose agar and oat meal agar initially white becoming orange then turning greenish grey as the cultures aged. White concentric circles on water agar (fig.4).

Table 1: Morphological characteristics of *Colletotrichum gloeosporioides*.

S.No	Morphological structures	Colour	Measurement (μm)	
			Length	Width
1	Mycelium	Greyish white		
2	Acervuli	Dark brown-black	120.24 to 344.05 μm	57.87 to 180.04 μm
3	Conidia	Hyaline	9.185-13.193 μm	4.34 – 6.84 μm
4	Setae	Brown-black	21.85 – 42.55 μm	3.80 – 5.32 μm

Table 2: Morphological characteristics of *Colletotrichum acutatum*

S.No	Morphological structures	Colour	Measurement (μm)	
			length	width
1.	Mycelium	Greyish white	-	-
2.	Conidia	Hyaline	12.5-14.6	4.5 – 5.2

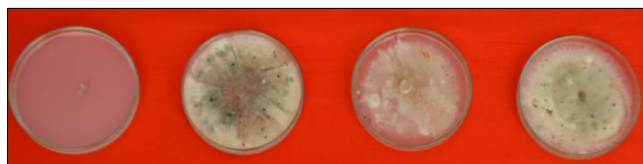


Fig 3: Colonial growth of *Colletotrichum gloeosporioides* on different growth media

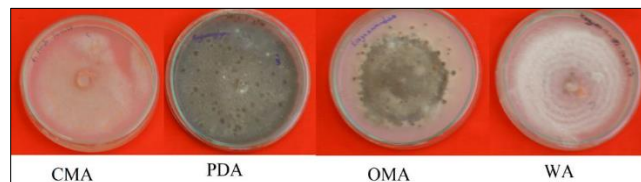


Fig 4: Colonial growth of *Colletotrichum acutatum* on different growth media

Table 3: Cultural characteristics of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* on different media

S.No	Media	<i>C.gloeosporioides</i>	<i>C. acutatum</i>	<i>C.gloeosporioides</i>	<i>C. acutatum</i>
		Growth Pigmentation		Colour Pattern	
1.	Oat meal agar	Greyish white	Cottony	Greyish- Black	Greyish – green
2.	Potato dextrose agar	Cottony, greyish white	Cottony	Greyish – Black	Greyish-green
3.	Corn meal agar	Semi -transparent	Semi transparent	Whitish –orange	Whitish – orange
4.	Water agar	Transparent	Semi transparent	Whitish	Whitish

Finally, the results indicates that for better growth and sporulation of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, potato dextrose agar media and oat meal agar media was best compared to corn meal agar media and water agar media. Cultural characteristics of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* on different media.

Conclusion

The culture of *Colletotrichum gloeosporioides* showed variation in colour and sporulation in different media (PDA, CMA, OMA and WA) tested. Colony colour varied from white to greyish and semi-transparent to transparent. Culture colour of *Colletotrichum acutatum* varied from whitish orange to greyish green. From the results obtained, for better growth and sporulation of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, potato dextrose agar media and oat meal agar media was best.

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