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RESEARCH NOTE

Cultural and morphological characteristics of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing anthracnose of cowpea

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Abstract: Cowpea anthracnose caused by *C. gloeosporioides* is an important disease that causes considerable economic losses to the farmers. The cultural studies of the pathogen on different solid media revealed that potato dextrose agar can be recommended to obtain good growth of the pathogen as it recorded maximum radial growth which is followed by oat meal agar, potato carrot agar and Sabouraud dextrose agar. The colour of the colony varied from white to different shades of greyish white on different media tested. Among the different media tested, potato dextrose agar showed cottony texture of growth, aerial type of mycelium and irregular margins. Excellent sporulation was observed in potato dextrose agar.

Key words: *Colletotrichum gloeosporioides*, Cowpea, Cultural studies, Mycelium, Potato dextrose agar

Introduction

Cowpea (*Vigna ungiculata* L. Walp.) is an important annual herbaceous legume crop, belonging to family Fabaceae/ Papilionaceae. It was originated in Central Africa. It is popularly known as "vegetable meat"as it is highly nutritious, with high protein (23-24%), carbohydrate (60.3%), minerals and vitamins and also rich source of iron and calcium (Modi and Tiwari, 2020). Cowpea is used in the form of dry grain, also as vegetable beside used as pot herb.Cowpea pod husks obtained after threshing are also used to feed livestock.

Globally, cowpea covers an area of 3.4 million hectares with a production of 18.29 million tonnes and productivity of 637 kg/ha (Anon., 2017), occupying Asia, Africa, parts of Southern Europe, USA and Southern America. In Asia, cowpea growing countries include India, Sri Lanka, Bangladesh, Myanmar, China, Korea, Thailand, Indonesia, Nepal, Pakistan, Malaysia and Philippines. In India, it occupies an area of about 39 lakh hectares with a production of 22 lakh tonnes and productivity of 600-750 kg/ha, which covers states like Andhra Pradesh, Karnataka, Madhya Pradesh, Gujarat, Haryana, Maharashtra, Tamil Nadu, Rajasthan and Uttar Pradesh. In Karnataka, it is grown in an area of about 0.88 lakh hectares with a production of 0.42 lakh tonnes with a productivity of 420 kg/ha (Anon., 2019).

Cowpea is susceptible to a number of biotic stresses *viz.*, fungal, bacterial, viral and nematode diseases at different growth stages. The major diseases are anthracnose (*Colletotrichum* spp.), brown blotch (*Colletotrichum capsici* Syd. and *C. truncatum*), charcoal rot (*Macrophomina phaseolina* (Tassi.) Goid.), Sclerotium rot (*Sclerotium rolfsii*Sacc.), Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum* (E. F.Smith) Snyder & Hansen), Cercospora leaf spot (*Cercospora canescens* and *C. cruenta*), brown rust/ leaf rust (*Uromyces appendiculatus* F. Strauss/ *U. vignae* Barclay), powdery mildew (*Erysiphe polygoni* DC), cowpea yellow mosaic, southern bean mosaic, bacterial blight (*Xanthomonas campestris* pv. *vignicola*) (Singh *et al.*, 1997).

Among the fungal diseases, anthracnose caused by *Colletotrichum* spp. is the major disease that causes a heavy yield loss in southern India. The disease is known to cause yield loss ranging between 35 to 80 per cent (Kumar and Narain, 2005).

The cowpea anthracnose is caused by various *Colletotrichum* spp., majorly *C. lindemuthianum* (Sacc. and Magn.) Scrib., *C. gloeosporioides* (Penz.) Penz. and Sacc., *C. dematium* (Pers.) Grove and *C. destructivum* O'Gara, which vary in their cultural and morphological characteristics. In case of *C. lindemuthianum,* it produces hyaline, falcate and fusiform conidia having a size of 10.5- 15.5 × 3.5- 4.5 µm and abundant black coloured acervuli (Rathava, 2017). *C. destructivum* produce straight, cylindrical conidia with obtuse apices (Sun and Zhang, 2009).

Culture medium serves as the source of nutrition and energy for the pathogen. However, there is no artificial medium upon which all fungi may grow and reproduce in the laboratory. Furthermore, temperature and light were critical factors in illness development. As a result, studies on several media were carried out in order to find the suitable medium for growth.

Material and methods

Collection and isolation of the pathogen

Anthracnose infected leaves and stems were collected from experiment field at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad and were observed under microscope for confirmation of the fungus. After confirmation of fungal structures and spores, the sample was used for isolation of fungus under *in vitro* using standard tissue isolation method. The infected tissue was cut into small bits of 1-2 mm size and surface sterilized in 1 per cent sodium hypochlorite solution for one minute and washed repeatedly thrice in sterile distilled water to remove the traces of sodium hypochlorite. These sections were placed on sterile potato dextrose agar (PDA) under aseptic conditions maintained inside the laminar air flow chamber. The plates were sealed, labelled, and then incubated at a temperature

of 27±1ºC for seven days to obtain good fungal growth (Tuite, 1969). The pathogen was identified by observing under light microscope for various morphological characters. Single spore isolation method was followed for obtaining pure culture of the concerned micro-organism (Choi *et al.*, 1999).

Cultural studies

The morphological characteristics of the pathogen were studied on seven different solid media *viz.*, Czapek's Dox agar, Potato dextrose agar, Potato carrot agar, Oat meal agar, V8 juice agar, Sabouraud dextrose agar and Richard's synthetic agar.

15 ml of each media was poured in Petri dishes. The plates were inoculated at the centre with fungal discs of 5 mm diameter using a cork borer and incubated at 27±1ºC until full growth appears. Three replications were maintained. Cultural and morphological characters, *viz*., colony diameter, colour of colony, texture of colony and sporulation were recorded by observing under compound microscope (10 x magnification).

Results and discussion

Isolation and identification of the pathogen

The pure culture of *C. gloeosporioides* was initially white in colour and later turned to greyish white with concentric rings on the lower surface of the Petri plate producing dot like acervuli. Mycelium was coloured, septate and branched. Conidia were hyaline, single celled, cylindrical in shape with characteristic oil globules. Length of conidia varied from 11.29-19.44 µm and width ranging between 3.93-6.95 µm (Plate 1).

Cultural Studies

The following characters were observed for *Colletotrichum gloeosporioides* on different media tested (Plate 2 and Table 1).

Radial growth

Among the seven different media tested for growth of *C. gloeosporioides*, potato dextrose agar (90.00 mm) resulted maximum radial growth of mycelia and was found significantly superior over all other media tested, which was followed by oat

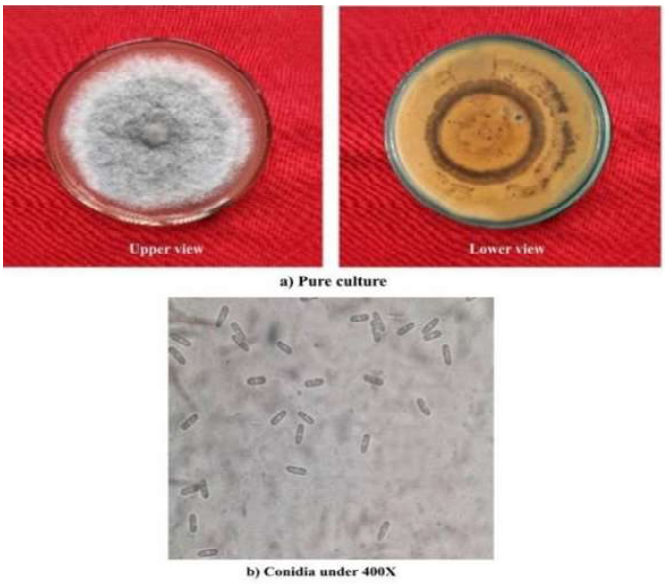

Plate 1. Morphological Characteristics *of Collectotrichum gloeosporioides*

Plate 2. Cultural Characteristics of *Collectotrichum gloeosporioides on* different media

Table 1. The sporulation was graded as follows:

Score	Grade	Description (conidia / microscopic field)10x
+++	Excellent	>50
++	Good	25-50
+	Less	<25

Table 2. Cultural and morphological characteristics of *Colletotrichum gloeosporioides* on different media

Media	Radial growth	Colour of of mycelium (mm)	Texture of colony	Growth colony	Margin nature	Sporulation
Czapek's Dox agar	49.33(44.62)*	Greyish white	Cottony	Aerial	Irregular	+
Potato dextrose agar	90.00(71.57)	Dark greyish white	Cottony	Aerial	Regular	+++
Potato carrot agar	71.33(57.63)	White	Cottony	Immersed	Regular	++
Oat meal agar	73.67(59.13)	Greyish white	Cottony	Aerial	Irregular	+
V8 juice agar	56.33(48.64)	Greyish white	Velvety	Immersed	Irregular	+
Sabouraud dextrose agar	70.67(57.21)	Greyish white	Cottony	Aerial	Irregular	+
Richard's synthetic agar	62.00(51.94)	Dark greyish white	Cottony	Aerial	Regular	++
S.Em.±	1.15					
C.D. (p=0.01)	4.86					

*Arcsine values

+++ : Excellent sporulation (>50 spores/10 x microscopic field)

++ : Good sporulation (50-25 spores/10 x microscopic field)

+ : Less sporulation (<25 spores/10 x microscopic field)

meal agar (73.67 mm), potato carrot agar (71.33 mm) and Sabouraud dextrose agar (70.67 mm) and are on par with each other. Poor radial growth was observed in Richard's synthetic agar (62.00 mm), V8 juice agar (56.33 mm) and least radial growth of mycelium was observed in Czapek's Dox agar (49.33 mm).

Colony colour

The colony colour of *C. gloeosporioides* varied from white to shades of greyish white. White coloured colony was noticed in potato carrot agar. Greyish white coloured colonies were observed in Czapek's Dox agar, oat meal agar, V8 juice agar and Sabouraud dextrose agar. Dark greyish white coloured colonies were observed in potato dextrose agar and Richard's synthetic agar.

Texture of the colony

Texture of *C. gloeosporioides* appeared cottony in Czapek's Dox agar, oat meal agar, potato dextrose agar, potato carrot agar, Sabouraud dextrose agar and Richard's synthetic agar. Whereas, in V8 juice agar, velvety texture was observed.

Growth nature

Aerial growth was observed in Czapek's Dox Agar, oat meal agar and Sabouraud dextrose agar. Whereas, potato dextrose agar, potato carrot agar, V8 juice agar and Richard's synthetic agar showed immersed growth.

Margin of the colony

Regular margin was observed in potato dextrose agar, potato carrot agar and Richard's synthetic agar. Whereas, Czapek's Dox Agar, oat meal aga, V8 juice agar and Sabouraud dextrose agar showed irregular margin.

Sporulation

The sporulation of *C. gloeosporioides* was excellent in potato dextrose agar. In potato carrot agar and Richard's synthetic agar, good sporulation was observed, whereas, in Czapek's Dox agar, oat meal agar, V8 juice agar and Sabouraud dextrose agar less sporulation was observed.

Among the different media tested for cultural studies of *Colletotrichum gloeosporioides*, good growth with excellent sporulation was observed in potato dextrose agar, followed by oat meal agar, potato carrot agar and Sabouraud dextrose agar. Hence potato dextrose medium can very well be used for obtaining maximum fungal growth as well as for the excellent sporulation of *C. gloeosporioides*.

The results were in agreement with Rajesha and Mantur (2014), Sardhara *et al.* (2016) and Mahadeo (2016), who observed maximum radial growth in PDA media out of different media tested. Chaudhari *et al.* (2017) also tested the effect of different media on growth and sporulation of *C. gloeosporioides* and observed that potato dextrose agar (74.00 mm) followed by oat meal agar (71.00 mm) and Czapek's Dox agar (68.00 mm) showed the maximum growth. Colony colour varied from dull white to grey in different media tested and also observed abundant sporulation in potato dextrose agar and oat meal agar.

Conclusion

Cultural studies conducted revealed that among the different media, potato dextrose agar was suitable, followed by oat meal agar, potato carrot agar and Sabouraud dextrose agar for the growth and excellent sporulation of the *C. gloeosporioides*.

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