SHORT COMMUNICATION

Effect of culture media and different temperatures on mycelial growth and pycnidial production of *Lasiodiplodia theobromae* casual agent of mango gummosis

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Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruit of the tropical and subtropical world and is cultivated extensively as a commercial fruit crop in India. Mango gummosis incited by *Lasiodiplodia theobromae* (Pat.) Griffon and Moube [synonym: *Botryodiplodia theobromae*] is becoming a serious problem in India on many popular varieties of mango particularly during monsoon and post-monsoon periods. In this study, the effects of culture media and different temperatures on mycelial growth and pycnidial production of *L. theobromae* were evaluated. The radial growth of the mycelium was maximum (8.89cm and 8.83cm.) on Potato Sucrose medium (PSA) at 30 and 35^{O}C followed by Potato Dextrose Agar (8.46cm.) at 35^{O}C. Least mycelial growth (6.3cm) was observed in Malt Extract Agar at 25^{O}C. The maximum pycnidial production was observed on Oat Meal Agar followed by PDA at temperature above 30^{0}C. Least pycnidial production was observed on MEA at all the temperatures tested. The fungal growth on various media was categorized as circular with sparse aerial mycelium, circular with moderate aerial mycelium and circular with abundant mycelium. The colour of colony ranged from whitish grey to blackish grey. Pycnidia were separate or aggregated, dark brown, thick or thin-walled. Conidiophores were hyaline, cylindrical to sub-obpyriform, with oblong, straight and hyaline single celled conidia. Gradually the conidia became dark brown and produced one septum with longitudinal striations; the size of conidia measured 22-29×11–15 μm.

Key words : *Lasiodiplodia theobromae,* mango, morphological studies

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruit crop of India and is grown in an area of 25 lakh ha with an annual production of 1800.4 MT. In Andhra Pradesh, it is cultivated in an area of 4.89 lakh ha with a production of 4,404 thousand MT. Mango fruit is very popular among people due to its wide range of adaptability, high nutritive value, and richness in variety, delicious taste and excellent flavor. It is a rich source of vitamin A and C. The fruit is consumed raw or ripe. The production in mango is reduced by important fungal diseases like anthracnose, powdery mildew and die-back. Since late nineties, mango gummosis incited by *Lasiodiplodia theobromae* (Pat.) *Griffon & Moube [*Synonym: *Botryodiplodia theobromae]* is becoming serious in various mango growing areas of Andhra Pradesh. The disease is characterized by the presence of profuse oozing of gum on the surface of the affected wood and bark of the trunk and also on the larger branches but more common on the cracked branches. Under severe infection in susceptible varieties, droplets of gum trickle down on stem and bark turns dark brown

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with longitudinal cracks and the tree dries up because of cracking, rotting and girdling of stem. Severely infected mango trees also dies. Commonly mango trees live on average for average 80 to 100 years but when it is infect with gummosis the tree is killed . The objective of this study was to evaluate the effects of culture media and temperatures on mycelial growth and pycnidial production of *L. theobromae* isolated from mango.

MATERIALS AND METHODS

Isolation of the pathogen

Mango twigs infected with gummosis were collected from severely infected Navaneetham mango variety. Infected plant material was cut into 1 -2 cm long pieces containing disease portion along with healthy portion; surface sterilized with 0.1% Mercuric chloride for two minutes and were placed in Petri plates containing Potato Dextrose Agar at 25± 1^0C with 12 hrs alternate periods of light and darkness. After 3 days of incubation mycelial growth was observed along the disinfected diseased twigs. Hyphal tips from the advancing mycelia were transferred to the Potato dextrose agar slants. The isolated pathogen was identified as *Lasiodiplodia theobromae* based on its mycelial and conidial characters through standard mycological descriptions by CMI keys. and pathogenicity proved by using stem inoculation method.

Effect of culture media and temperature

Effect of different culture media on the colony growth and sporulation of *L. theobromae* was evaluated. Agar media *viz.,* Potato dextrose agar, Potato sucrose agar, Oat meal agar and Malt extract agar were poured in 90 mm diameter Petri plates. Five mm diameter agar discs were removed with a sterile cork borer from the edges of colonies and one such disc was placed in the center of each 90 mm Petri plate containing this edge media. Plates were then wrapped with parafilm and incubated at 25, 30 and 35ºC with three replicate plates of each medium. The colony diameter in each plate was measured at 24 h interval along two axes perpendicular to one another. The two measurements for each day were averaged and daily radial growth rates were calculated. Along with colony growth colony colour, type of growth and pycnidial production was recorded.

RESULTS AND DISCUSSION

Effect of different temperatures on the growth of L. theobromae on different solid media

The cultural characters of *L. theobromae* was studied on four different media *viz.,* Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Oat Meal Agar (OMA) and Malt Extract Agar (MEA) at three different temperatures (25ºC, 30ºC and 35ºC) in Table 1and Fig.1.

L. theobromae varied in their growth rate on different media at different temperatures. The radial growth of the mycelium was maximum (8.89 cm

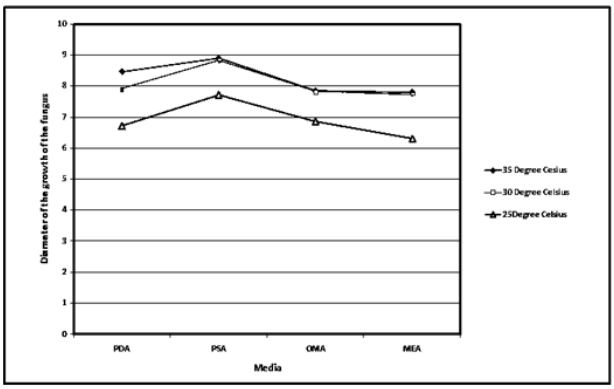

Fig. 1 : Effect of different temperatures and media on the growth of *L. theobromae* under *in vitro* condition

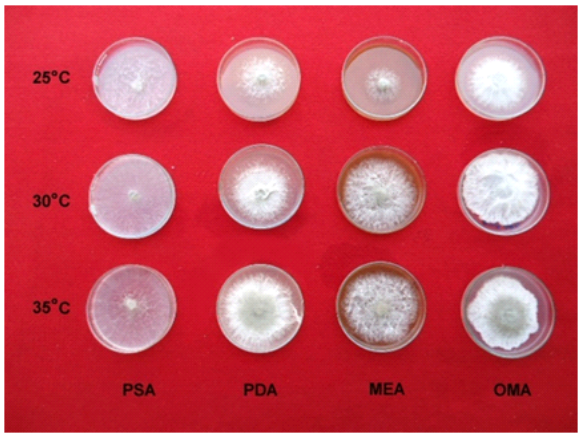

Fig. 2: Effect of different temperature on *Lasiodiplodia theobromae* on different solid media.

and 8.83 cm) on PSA medium at 30 and 35 ºC followed by PDA (8.46 cm) at 35ºC. Least mycelial growth was observed in MEA at 25ºC (Fig.2.). The results are in accordance with that of Saha *et al*. (2008) who reported that glucose and sucrose are the best carbon sources for the mycelial growth of *L. theobromae* and PDA was found to be one of the best medium for its growth.

Table 1 : Effect of different temperature on *Lasiodiplodia theobromae* on different solid media

Temprature (^{0}C)	Media	Diameter	Colony Colour	Type of Growth	Pycnidia Production
25	PDA	6.71	Whitish grey	CA	+
	PSA	7.71	Whitish grey	CS	+
	OMA	6.86	Whitish grey	CA	++
	MEA	6.3	Black grey	CA	+
30	PDA	7.91	Whitish grey	CA	+ +
	PSA	8.83	Whitish grey	CS	+
	OMA	7.82	Whitish grey	CA	+ + + +
	MEA	7.74	Black grey	CA	+
35	PDA	8.46	Whitish grey	CA	+ +
	PSA	8.89	Black grey	CS	+
	OMA	7.84	Black grey	CA	+ + + +
	MEA	7.79	Black grey	CA	+

	Temperature (T)	Media (M)	TxM
C.D. (0.05)	0.1067	0.1232	0.213
SE(m)	0.0375	0.0433	0.0433

PDA – Potato Dextrose Agar
PSA-Potato Sucrose Agar
OMA-Oat Meal Agar
MEA-Malt Extract Agar

CA-Circular with abundant aerial mycelium
CM-Circular with moderate aerial mycelium
CS-Circular with sparse aerial mycelium
IS-Irregular with sparse mycelium

\+ : sparsly
+ + : Moderate
+ + + : High

Similarly Fu *et al.* (2007) reported that PDA and PSA were most suitable for vegetative growth of *L. theobromae*. Several carbon sources including dextrose, sucrose and D-mannose were found to be utilized by the fungus for mycelium growth. Moreover, the growth on different media was found to be dependent on temperature of incubation. The temperature for mycelial growth of *L. theobromae* was found to be in the range of 4 to 36^{0}C (Saha *et al*. 2008) with optimum being 25-30 ^{0}C.

In the present investigation, a significant interaction was found between the type of medium and temperature of incubation on pycnidial production. The maximum pycnidial production was observed on OMA followed by PDA at temperature above 30^{0}C. The pycnidial production was more and rapid when incubated at high temperatures (30 and 35^{0}C) compared to low temperature (25^{0}C). Least pycnidial production was observed on MEA at all the temperatures tested. Pycnidia were separate or aggregated, dark brown, thick or thin-walled. Conidiophores were hyaline, cylindrical to sub-obpyriform, with oblong, straight and hyaline single celled conidia. Gradually the conidia became dark brown and produced one septum with longitudinal striations; the size of conidia measured 22-29×11–15 μm.

The fungal growth on various media was categorized as circular with sparse aerial mycelium, circular with moderate aerial mycelium and circular with abundant mycelium. The colour of colony ranged from whitish grey to blackish grey. Venugopal (2013) also categorized 373 isolates of *L. theobromae* isolated from rotting nuts of coconut into three major groups, *viz*., dark gray, greyish black and white type isolates. They observed that isolates of dark gray and greyish black group did not exhibit much variation in response to tem-

perature and showed highest growth at 30°C on PDA whereas the selected isolate of white colony group showed the highest growth at 25°C. Growth of selected isolates of all three groups was found lowest at 10°C.

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