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Effect of different culture media on the growth and cultural charcteristics of *Lasiodiplodia theobromae*, an incitant of mango stem end rot

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Abstract

Lasiodiplodia theobromae causing mango stem end rot is a devastating pathogen which render the mango fruits completely ineffective because it infect the fruit in both field and storage condition. Every living being requires food for its growth and reproduction and the fungi are not an exception. In the present study, potato dextrose agar medium significantly supported the maximum (78.49 mm) growth of *L. theobromae* followed by beetroot sucrose medium (76.21 mm), carrot sucrose agar (71.95 mm) and bean juice agar (70.72 mm). Among the isolates tested, LT_6 from Villupuram had the highest mean mycelial growth of (90.00 mm) followed by LT_5 from Palakodu (88.37 mm) and LT_3 from Oothangarai (61.07 mm). Among the liquid media tested, potato dextrose broth recorded the highest mean mycelial dry weight (267.11 mg /100 ml) followed by beetroot sucrose broth (256.19 mg/100ml) and isolate LT_6 recorded the highest mean mycelial dry weight (268.22mg/100ml), whereas the least mean mycelial dry weight (132.44 mg/100ml) was found in LT_2. In general, all the seven isolates grow well on PDA and produced sporulation. Of these, isolate LT_6 recorded maximum mycelial growth (90.00 mm) and took 17 days for pycnidial production. The colony colour and shape of conidia in all the isolates were varies slightly and conidial colour varied from brown, golden brown and dark brown. The colony colour of all the isolates varied from greyish white to greyish black. Hence, each and every pathogen requires various culture media for their growth and development.

Keywords: Mango, *Lasiodiplodia theobromae,* mycelial growth, mycelial dry weight, culture characters

Introduction

Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruits known as ''King of fruits'' (Hayes, 1953) [13]. Being highly perishable, mango fruits have to be marketed immediately after harvest. Postharvest losses particularly due to fungal invasions are much more significant for highly perishable fresh fruits. Fresh mangoes are vulnerable to attack by different microorganisms because of their high moisture content, vital heat produced due to accelerated rate of respiration, high temperature developed in transport containers and godowns. The postharvest losses of mango are up to 17– 36%, which may rise up to 100% if proper management strategies are not in place, along with conditions favor the disease development.

Mango is prone to several bacterial and fungal postharvest diseases. Among the several postharvest diseases, the stem end rot caused by *Lasiodiplodia theobromae* Pat. is the major pre/postharvest diseases (Johnson *et al.,* 1992). Because they develop during ripening from latent infections that have occurred in the field. This pathogen is an important opportunistic pathogen with worldwide distribution in tropical and subtropical regions causing different types of diseases in many plant species. It has a wide host range estimated to be more than 280 plant species (Sutton, 1980; Khanzada *et al.*, 2006 and Domsch *et al.*, 2007) [29, 15, 10] although with varied pathological effects on its hosts. *L. theobromae* causes shoot blight, die-back, twig blight, cankers, etc., mainly in woody plants (Mohali *et al.,* 2005) [21]. Stem-end rot disease can render the mango fruits completely ineffective as it destroys the developed or developing fruits in field and storage condition.

Symptom appears as water soaked diffuse lesions emerging from the pedicel adjoining end of the fruit and quickly become dark coloured. Infected fruit may split open and get collapse which results in exuding out of straw-coloured fluid from the stem-end portion or from splits in the side of the fruit. Under favorable condition, steel-grey mycelium may cover the surface of fruit (Prakash, 2004) [24]. Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from the substrate upon which they grow. In order to culture the fungi artificially, it is necessary to supplement the medium, those essential nutrients needed for their growth, development and other metabolic processes. The present study was aimed on the use of various culture media on the mycelia growth and cultural characteristics of *L. theobromae.*

Materials and Methods

Isolation of pathogen

Mango fruits showing typical symptoms of stem end rot were collected from different places *viz*., Thiruvennainallur, Chidambaram, Oothangarai, Attur, Palakodu, Villupuram and Sirkazhi. Isolation of stem end rot pathogen *i.e., Lasiodiplodia theobromae* was made by tissue segment method (Rangaswami, 1958) [25]. The fruits showing typical symptom were collected and infected tissue bits were separated and cut into small pieces by using a sterilized scalpel. Then the separated tissue bits were surface sterilized with 1 per cent sodium hypochlorite solution for 1 min. and subsequently washed three times with sterile distilled water. Then they were transferred into a sterile Petri dish containing Potato Dextrose Agar (PDA) medium (Ainsworth, 1961) [4] amended with streptomycin. The plates were then incubated at room temperature (28±2 °C) for 3-4 days. The emerging colonies were sub cultured on to PDA slants. Single hyphal tip method was followed for making pure culture and maintained on PDA slants (Aneja, 2003) [5]. The pathogen was identified based on their cultural and morphological characters.

Cultural characters

Growth characters of *L. theobromae* isolates on different solid media

In order to compare the growth of *L. theobromae* on different solid media *viz*., Bean juice agar, Beetroot sucrose agar, Carrot sucrose agar, Corn meal agar, Czapek dox agar, Host extract agar, Peas sucrose agar, Potato dextrose agar and Rose Bengal agar medium. Sterilized warm medium amended with streptomycin was poured into sterilized Petri dishes (9 cm) at the rate of 15 ml and allowed to solidify. The isolates of the pathogen was inoculated at the centre of the plate by placing a five day old nine mm culture disc. The plates were incubated at room temperature (28 ± 2°C) for five days and three replications were maintained and radial growth of the mycelium was measured.

Growth characters of *L. theobromae* isolates on different liquid media

Bean juice broth, Beetroot sucrose broth, Carrot sucrose broth, Corn meal broth, Czapek dox broth, Host extract broth, Peas sucrose broth, Potato dextrose broth and Rose Bengal broth were prepared without adding agar. From the prepared broth, 100 ml was distributed in 250 ml Erlenmeyer flasks and autoclaved at 121 °C at 15psi for 15 min and then cooled. The flasks were separately inoculated with a five day old culture of nine mm disc of the pathogen. Five days after incubation, the mycelia mat was filtered through a pre weighed what man No.1 filter paper, dried in hot air oven at 100°C until constant weight was obtained. The mycelial dry weight was obtained by subtracting the weight of the filter paper.

Cultural and pycnidial characters of *L. theobromae*

From the five day old culture plates, nine mm culture disc of the pathogen was cut by using a sterilized cork-borer and placed at the center of the each sterile Petri dish containing 15 ml of previously sterilized and solidified PDA medium. The plates were incubated at room temperature (28 ± 2°C) for fifteen days. The growth and morphological characters of the isolates *viz*., conidia shape, colour, septation, pycnidial production were observed under microscope (magnification 45x) by using ocular and stage micrometer. In addition to this, colony growth, colony colour, mycelial type, pycnidial pattern, and days taken for pycnidial production were also observed.

Statistical analysis

The data on the effect of the treatments on the growth of pathogen and disease incidence were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease incidence was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984) [11]. The package used for analysis was IRRISTAT version 92 developed by the Biometrics Unit of the International Rice Research Institute, The Philippines.

Results and Discussion

Effect of different solid and liquid media on growth of *L. theobromae* isolates

In order to culture fungus in the laboratory, it is necessary to furnish all essential elements and compounds in the medium for their growth and other life processes. All media are not equally good for all fungi, nor there an universal substrate or artificial media upon which all fungi can grow. So, different media including both synthetic and non-synthetic media were tried for *L. theobromae* in the present investigation**.**

All the nine media tested were supported the growth of *L. theobromae* in different manner. Among the solid media tested, potato dextrose agar medium significantly supported the maximum (78.49 mm) growth followed by beetroot sucrose agar medium (76.21 mm), carrot sucrose agar (71.95 mm) and bean juice agar (70.72 mm), while peas sucrose agar medium supported the least (32.32 mm) growth. Among the isolates tested, LT_6 from Villupuram had the highest mean mycelial growth of (67.39 mm) followed by LT_5 from Palakodu (61.62 mm) and LT_3 from Oothangarai (61.07 mm). The minimum mean mycelial growth (45.58 mm) was recorded in LT_2 from Chidambaram. Among the liquid media tested, potato dextrose broth recorded the highest mean mycelial dry weight (267.11 mg /100 ml) followed by beetroot sucrose broth (256.19 mg/100ml), while pea sucrose broth had least mean mycelial dry weight (126.20 mg/100ml). Among the isolates LT$_6$ recorded the highest mean mycelial dry weight (268.22 mg/100ml), whereas the least mean mycelial dry weight (132.44 mg/100ml) was found in LT$_2$. Fungi secure food and energy from the substrate upon which they live in nature. Similarly, Luo *et al.*(2011) also reported that the potato dextrose agar supported the mycelial growth and pycnidial production of *L. theobromae.* Potato dextrose agar medium was identified to be the best medium for the growth and sporulation of *Botryodiplodia* sp. causing die-back of Jabon seedling (Achmad and Arshinta, 2014) [1]. Similar results were also obtained by Deepan and Ebenezer, 2017 [7]; Saha *et al.*, 2008 [26]. Djeugap Fovo *et al.*(2017) [9] observed that PDA was found to be most suitable for mycelial growth and sporulation of *L. theobromae*. Also, Dheepa *et al.* (2018) [8] reported that that potato dextrose agar encouraged the maximum mycelial growth as well as pycnidial production. Similarly, Khazanda *et al.* (2006) found that potato sucrose medium supports the growth and sporulation of *L. theobromae.* Latha *et al.*, 2013 [17] observed that potato dextrose agar supported the highest growth of *L. theobromae* causing collar and root rot of physic nut. Suresh *et al.*, 2017 [28] perceive that potato sucrose agar supported the maximum growth of *L. theobromae*, a causal agent of mango gummosis which is followed by potato dextrose agar. Gowri Sankar *et al.* (2016) [12] reported that potato dextrose broth (PDB) supported maximum mycelial dry weight of *L. theobromae.*

Cultural and Conidial characteristics of *L. theobromae* isolates

In general, all the seven isolates grow well on PDA and produced sporulation. Of these, isolate LT$_6$ recorded maximum mycelial growth (90.00 mm) and took 17 days for pycnidial production. This was followed by isolate LT$_5$ (88.37 mm; 17 days). The colony colour of these isolate varied from greyish white, grey and greyish black and the mycelial pattern varied from aggregate non fluffy type, medium and aggregate fluffy type.The studies of (Adeniyi *et al.* (2016) [2] and Sangeetha *et al.* (2012) [27] also revealed that the obverse view of colony colour were gray to dark gray with fluffy colony texture. The results obtained from the colony characters of *L. theobromae* are in agreement with the earlier reports (Ko*et al.*, 2004; Malik *et al.*, 2005; Lima *et al.*, 2013; Muthukumar and Udhayakumar, 2017; Chaudhuri *et al.*, 2017) [20, 18, 22, 6]. The conidial colour varied from brown, golden brown, dark brown and black. The conidial shape varied from ovoid, obovoid, elongated and ellipsoidal. (Muthukumar and Udhayakumar, 2017) [22] had observed that the pycnidia were globose in shape and pycnidia were concentrated at the centre, in periphery and also found scattered all over the plates and the number of days taken for pycnidial production varied from 21 to 30 days of incubation. Similar such variation with regard to the pycnidial production of *L. theobromae* were observed by earlier workers (Adhikary *et al.*, 2013; Norhayati *et al.*, 2016 and Deepan and Ebenezer, 2017) [3, 23, 7].

Table 1: Growth of *L. theobromae* isolates on different solid media *in vitro.*

S. no.	Isolates	Diameter of Mycelial growth (mm)*									
		Bean juice agar	Beetroot sucrose agar	Carrot sucrose agar	Corn meal agar	Czapek dox agar	Host extract agar	Peas sucrose agar	Potato dextrose agar	Rose Bengal agar	Mean
1.	LT$_1$	55.43^{e}	59.84^{e}	52.17^{e}	41.25^{d}	30.21^{c}	48.73^{f}	43.63^{a}	66.28^{d}	51.14^{cd}	49.85
2.	LT$_2$	78. 50^{ab}	85.88^{a}	81.25^{bc}	51.00^{b}	37.25^{b}	63.75^{bc}	28.75^{c}	88.37^{bc}	59.82^{b}	45.58
3.	LT$_3$	75.15^{b}	82.47^{bc}	79.82^{c}	49.31^{c}	31.36^{bc}	62.61^{c}	25.14^{cd}	82.87^{cd}	60.87^{ab}	61.07
4.	LT$_4$	69.57^{d}	72.84^{cd}	78.84^{bc}	53.21^{b}	43.18^{a}	63.53^{bc}	23.51^{d}	75.62^{c}	61.48^{a}	60.19
5.	LT$_5$	72.91^{c}	79.14^{c}	73.94^{d}	52.37^{b}	42.87^{a}	59.47^{d}	32.95^{c}	84.67^{b}	55.23^{c}	61.62
6.	LT$_6$	79.23^{a}	84.96^{a}	87.43^{a}	55.93^{a}	45.13^{a}	65.84^{a}	35.74^{b}	90.00^{a}	62.25^{a}	67.39
7.	LT$_7$	64.23^{de}	68.32^{d}	50.21^{e}	43.19^{d}	28.56^{d}	53.39^{e}	36.51^{b}	61.26^{e}	45.31^{d}	50.11
Mean		70.72	76.21	71.95	49.47	36.94	59.62	32.32	78.44	56.59	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs C.D (P = 0.05%).

Table 2: Growth of *L. theobromae* isolates on different liquid media *in vitro*.

S. no.	Isolates	Mycelial dry weight (mg)*									
		Bean juice broth	Beetroot sucrose broth	Carrot sucrose broth	Corn meal broth	Czapek dox broth	Host extract broth	Peas sucrose broth	Potato dextrose broth	Rose Bengal broth	Mean
1.	LT$_1$	175.89^{f}	215.77^{e}	231.51^{d}	157.86^{e}	111.45^{e}	173.36^{d}	109.48^{de}	197.83^{d}	203.31^{e}	175.16
2.	LT$_2$	123.49^{g}	158.95^{f}	132.83^{e}	120.43^{f}	109.76^{e}	156.00^{e}	98.62^{e}	156.00^{e}	135.84^{f}	132.44
3.	LT$_3$	253.83^{c}	261.94^{c}	283.12^{b}	232.23^{b}	148.92^{bc}	240.51^{b}	137.05^{b}	298.83^{b}	244.69^{c}	233.46
4.	LT$_4$	221.42^{d}	250.37^{d}	261.59^{bc}	207.73^{c}	135.42^{c}	215.38^{bc}	128.73^{c}	285.98^{bc}	230.29^{cd}	215.21
5.	LT$_5$	284.41^{d}	313.73^{ab}	291.75^{c}	239.43^{i}	157.61^{j}	282.95^{e}	132.83^{b}	315.94^{a}	251.04^{bc}	252.19
6.	LT$_6$	301.41^{d}	338.92^{ab}	305.61^{c}	245.81^{i}	171.49^{j}	294.71^{e}	156.00^{k}	340.75^{a}	259.29^{a}	268.22
7.	LT$_7$	205.73^{e}	253.65^{d}	251.42^{c}	194.84^{d}	124.92^{d}	203.82^{c}	120.71^{d}	274.42^{c}	221.32^{d}	205.65
Mean		223.74	256.19	251.12	199.76	137.08	223.82	126.20	267.11	220.83	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs C.D (P = 0.05%).

Table 3: Cultural characteristics of *L. theobromae* isolates on PDA.

S. No.	Isolates	Mean Colony diameter (mm)	Colour of the mycelium	Colony type	Pycnidial pattern	Days for pycnidial production	Shape and colour of conidia
1.	LT_1	66.28^f	Greyish black	Aggregate and not fluffy	Centre	17	Ovoid and brown
2.	LT_2	61.26^g	Greyish white	Aggregate and fluffy	Periphery	14	Elongate and golden brown
3.	LT_3	84.67^c	Grey	Aggregate and fluffy	Scattered	15	Elongate and dark brown
4.	LT_4	82.87^d	Grey	Medium	Periphery	16	Ellipsoid and black
5.	LT_5	88.37^b	Greyish black	Medium	Centre	17	Elongate and golden brown
6.	LT_6	90.00^a	Grey	Aggregate and fluffy	Scattered	17	Obovoid and brown
7.	LT_7	75.62^e	Greyish black	Aggregate and medium fluffy	Periphery	14	Ovoid and dark brown

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs C.D (P = 0.05%).

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