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Influence of culture media, temperature and light/darkness on the mycelial growth of *Lasiodiplodia theobromae* (Pat.)

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ABSTRACT

In this study, the effects of culture media, temperature and light and darkness on mycelial growth of L. theobromae were evaluated. Results of potato dextrose agar (PDA) and potato dextrose agar stem exudates (PDASE) were found suitable for mycelial growth of the L. theobromae. The fungus grew from 20-40oC with optimum growth observed at 25-35oC on both media. On the 10th day, mycelial growth at 25-35oC was (15.6mm ± 0.02 - 30.6mm ± 0.05; 18.4mm ± 0.28 - 32.5mm ± 0.10) while mycelial growth recorded with PDASE was (18.2mm ± 0.23 – 31.0mm ± 0.25; 22.8mm ± 0.02 – 38.2mm ± 0.40). There were no significant effects of light and darkness on the mycelial difference (P≤ 0.05) on the growth of L. theobromae. It is recommended from this research work that amended potato dextrose agar (PDA) with stem exudates promoted the growth of test fungus L. theobromae and hence should be used as culture medium for fungi at 35oC.

INTRODUCTION

Lasiodiplodia theobromae (Pat.) is fungal pathogen of great economic importance. *L*. *theobromae* is an opportunistic plant pathogen that causes different types of plant diseases with worldwide distribution within tropical and subtropical regions (Faber *et al.,* 2007). Its host range estimated to be more than 280 plant species (Domsch *et al.,* 2007; Khanzada *et al.* 2006; Sutton, 1980) however, pathological effects varies among plants hosts.

In the tropics, *B. theobromae* is an economically important fungus known to cause major losses to mango, cocoa, banana and yam farmers (Rieger, 2006; Amuse *et al.,* 2003). The fungus is known to cause tuber rots in yam, root rot in cassava, collar rot in peanuts, crown rot in banana, Stem end rot in mango fruits, stem rot in pawpaw and leaf spot in citrus (Sangeetha *et al*. 2011; Rossel *et al.,* 2008; Khanzada *et al.,* 2004b; Jiskani, 2002; Arjunan *et al.,* 1999; Sangohote, 1988). *B. theobromae* is associated with die-back on mango (Khanzada *et al.,* 2004a,b) and pod rot of cocoa (Phillips, 2007).

Onyenka *et al.,* (2005) reported that the fungus is present in more than 70% of farms surveyed in Nigeria and it is linked to colossal yield losses around 80% of marco harvest. Jiskani (2002) and Sangchote (1988) respectively have identified *B. theobromae* to be a virulent fungus and a common isolate found on diseased mango fruits in Pakistan. French (2006) also reported that the pathogen infects and causes extensive damage to mango, cocoa, banana fruits and yam tubers. Rots caused by the fungus, particularly in the root and tuber crops often occur underground and so diagnosis of the disease is usually delayed or under repaired. Moreover, the wider host range (Crammer, 1979) and the host non-specificity (Mohali *et al.,* 2005) of *B. theobromae* makes control and management of the disease very difficult.

Regrettable there are limited information about the influence of culture media of *Lasiodiplodia theobromae*. The lack of information on host range of *L. theobromae* on the trees found in Aboretum of Forestry and Environment, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt has necessitated for this research. Therefore, the present study was undertaken to observe the influence of environmental factors on the mycelial growth of *Lasiodiplodia theobromae*.

This research is aimed at investigating the mycological studies on *Lasiodiplodia theobromae* the causal agent of gummosis infected African mahogany.

Specific objectives of this research were to:

(i) evaluate the effect of culture media on the mycelial growth of *Lasiodiplodia theobromae.*
(ii) determine effect of temperature on the mycelial growth of *Lasiodipodia theobromae*.
(iii) assess the effect of light and darkness on mycelial growth of *Lasiodiplodia theobromae.*

MATERIALS AND METHODS

Study Area

The study was carried out at the laboratory of Forestry and Environment (Pathology Unit) and Food Science and Technology, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria.

Effect of Culture Media on the Growth of *L. theobromae*

Effect of potato dextrose agar (PDA) and potato dextrose agar stem exudates (PDASE) media on the colony growth and sporulation of *Lasiodiploda theobromae* was evaluated. These media were poured into 9mm diameter Petri dishes and allowed to solidify. 5mm disc of the fungus was removed with a sterile cork borer from the edges of the fungus colony and placed in the centre of each 9mm Petri dish containing the media. The Petri dishes were then wrapped with aluminum foil and incubated at room temperature (28 ± 2^0C) in the dark for 5, 10 and 15 days respectively. There were five replicate Petri dishes of each medium. The colony diameter in each Petri dish was measured after 5, 10 and 15 days respectively along two axes perpendicular to one another. (Ukoima and Chukunda, 2016; Chukunda, 2014; Saleem and Nasir, 1991).

Effect of light and darkness on mycelial growth of fungus *L. theobromae*

To study the effect of light and darkness on mycelial growth of isolated fungus 5 mm culture discs were cut with the sterilized cork borer from advancing margin of the colonies of *L. theobromae* and inoculated on PDA and PDASE plates separately at 5 days interval for 15 days. Carbon paper was used to wrap the Petri dishes for darkness, while unwrapped Petri dishes were used for light exposure. All the Petri dishes were incubated at 28 ± 2 °C in five replicates under continuous light and darkness, (Kausar *et al*., 2009).

Effect of Temperature on the Growth of *Lasiodiplodia theobromae.*

Five millimeter culture disc of *L. theobromae* were cut with sterilized Cork borer from advancing margin colonies of the fungus and inoculated on PDA and PDASE plates separately. The effect of temperature on mycelial growth of *L. theobromae* was evaluated on potato dextrose agar (PDA) and potato dextrose agar stem exudates (PDASE). The inoculated plates were placed in an inoculating chamber and incubated at 15, 20, 25, 30, 35^{o}C in the dark. Each treatment was replicated three times. At each temperature the plates were arranged in a completely randomized design (CRD). Colony diameters were measured along two

axes perpendicular to one another. The measurement of the mycelial growth was calculated after 5, 10 and 15 days of inoculation (Ukoima and Chukunda, 2016).

Experimental Design and Statistical Analysis

The experiment was laid out in a Completely Randomized Design (CRD). The treatment were replicated three time. Data collected were analyzed by analysis of variance (ANOVA) using SPSS Genstat software as described by Steel and Torrie (1980). Duncan Multiple Range Test at probability of 5% (DMRT) to separate the means.

RESULTS

Effect of culture media on mycelia growth of *Lasiodiplodia theobromae*

The results on the effect of culture media on the mycelial growth of *L. theobromae* are shown in Table 1. The results indicated that potato dextrose agar (PDA) and Potato dextrose agar stem exudates (PDASE) significantly (P≤0.05) affected the growth of *Lasiodiplodia theobromae*. PDA and PDASE affected the growth of *L. theobromae* at different days. On the 5th days of incubation *L. theobromae* growth on both media was (15.6mm ± 0.01; 18.2mm ± 0.02). However, the highest growth was observed on the 10th day for both PDAE (28.2mm ± 0.02) followed by PDA (20.6mm ± 0.01).

Table 1: Effect of culture media on mycelia growth of *Lasiodiplodia theobromae* (Mean ± SD)

	Incubation period/mycelial growth (mm)/days		
Culture media	5	10	15
PDA	15.6 ± 0.01[b]	20.0 ± 0.01[b]	16.0 ± 0.03[b]
PDASE	18.2 ± 0.02[a]	28.3 ± 0.02[a]	20.5 ± 0.04[a]

Mean ± SD (n=4) DMRT (0.05)

Effect of different temperature in the mycelial growth of *Lasiodiplodia theobromae*

The results on the effect of different temperatures on *Lasiodiplodia theobromae* mycelial growth are presented in Table 2. The result showed that different temperature and culture media influenced the mycelial growth of *L. theobrommae.* The relative increase in fungus mycelial growth increased with the increase in temperature. It was observed that the temperature range of 25-35°C was optimum for mycelial growth in both media (15.6 ± 0.02mm – 30.6 ± 0.05mm; 18.4 ± 0.28mm – 32.5 ± 0.10mm). Potato dextrose agar (PDASE), had the highest mycelial growth within the temperature range of 25-35°C (18.2 ± 0.23mm –31.0 ± 0.25mm; 22.8 ± 0.02mm – 38.2 ± 0.40mm).

Table 2 Effect of Different Temperature on the mycelial Growth of *Lasiodiplodia theobromae* (Mean ± SD)

Temperature (t°C)	Incubation period/mycelial growth (mm)/days			
	PDA		PDASE	
	5	10	5	10
20	12.0 ± 0.01[d]	14.6 ± 0.81[e]	15.7 ± 0.20[d]	18.8 ± 0.20[d]
25	15.6 ± 0.02[c]	18.4 ± 0.28[d]	18.2± 0.23[c]	22.8± 0.20[c]
30	20.5 ± 0.03[b]	24.0 ± 0.22[b]	22.5 ± 0.20[b]	26.6 ± 0.20[b]
35	30.6 ± 0.05[a]	32.5 ± 0.10[a]	31.0 ± 0.25[a]	38.2 ± 0.40[a]
40	21.5 ± 0.06[b]	23.5 ± 0.22[c]	22.6 ± 0.21[b]	25.7 ± 0.25[b]

Mean ± SD (n=4) * PDA = Potato dextrose agar, PDAE = Potato dextrose agar stem exudates, DRMT (p<0.05)

Effect of light and darkness on mycelial growth of *Lasiodiplodia theobromae* on potato dextrose agar (PDA) and potato dextrose agar stem exudates (PDASE) media incubated at room temperature (28 ± 2°C)

The result on the effect of light and darkness in *Lasiodiplodia theobromae* growth on stem bark tissues and leaves portions of *Khaya grandifiolia* are shown in Table 3. The result indicated that light and darkness significantly (p≤ 0.05) affected the growth of *L. theobromae* at different days. On the 5th day of incubation, *L. theobromae* under continuous darkness mycelial growth on PDA and PDASE was (13.5 ± 0.20mm – 18.2 ± 0.8mm). In continuous light, *L. theobromae* mycelial growth was (12.3 ± 0.02mm – 16.0

± 07mm). Generally, the highest growth was observed after 10 days for light and darkness on both media (16.8 ± 0.22mm – 28.1 ± 0.22mm) for continuous light while continuous darkness was (25 ± 0.30mm – 30.5 ± 0.31mm).

Table 3: Effect of light and darkness on mycelial Growth of *Lasiodiplodia theobromae* on PDA and PDASE media incubated at room temperature 28 ± 2°C (Mean ± SD)

Light/darkness	Incubation period/mycelial growth (mm)/days			
	PDA		PDASE	
	5	10	5	10
Continuous light	12.3 ± 0.02[b]	16.8 ± 0.22[b]	16.0 ± 0.70[b]	25.1 ± 0.22[a]
Continuous darkness	13.5 ± 0.20[a]	18 ± 0.30[a]	18.2 ± 0.81[a]	26.5 ± 0.31[a]

*** PDA = Potato dextrose agar, PDASE = Potato dextrose agar stem exudates, DRMT (p<0.05)**

DISCUSSION

The mycelia growth of *Lasiodiplodia theobromae* (Table 1) were significantly affected by the culture media of potato dextrose agar (PDA) and potato dextrose agar stem exudates (PDASE). The results indicated that there was significant interaction between type of medium and mycelia growth of *L. theobromae*. The present research findings agreed with the reports of Alam *et al.,* (2001) who recorded good mycelium growth of *Botryodiplodia theobromae* on potato dextrose agar (PDA) than on potato dextrose agar stem exudutes (PDASE). Similarly, Qureshi and Meah (1991), observed linear growth of *B. theobromae on* Richard agar solution, mango leaf extract agar on PDA. Alasoadura (1969) observed maximum stromata of *B. theobromae* on malt agar and oat meal agar. Sabalpara *et al.,* (1991) reported that nutrient rich medium supported the size and number of pycndia produced by *B. theobromae.* Saha *et al.,* (2008) and Jash *et al*., (2003) reported on the influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae*: in their findings addition of root extract increased sporulation and mycelial growth of *L. theobromae* which was in agreement to our findings. Several other researchers stated that PDA was the best media for mycelial growth (Xu *et al*., 1984).

Alam *et al*., (2001) reported that highest mycelial growth and sporulation of *B. theobromae* was recorded on PDA, which was in agreement to the present work. Several other researchers also stated that PDA was the best media for mycelial growth (Xu *et al*., 1984; Maheshwari *et al*., 1999). Kumar and Singh (2000) also stated that *L. theobromae* grew well in potato dextrose medium. Result of this study agrees with that of Karlatti and Hiremath (1989), who observed high mycelial growth of *Altermaria zinniae* on potato dextrose agar medium and recorded higher sporulation on leaf extract dextrose agar medium.

The mycelia growth of *lasiodiplodia theobromae* (Table 3) showed a variable trend in response to temperature change using potato dextrose agar (PDA) and potato dextrose stem exudates (PDASE) media used. mycelia growth increased as temperature increased from 20-35^{O}C and then decreased with further increase temperature. However, optimum mycelia growth of test fungus occurred at 25-35^{O}C. This results agreed with those reported by Quroshi and Meah (1991) and Alam *et al.,* (2001) who reported that 25-30^{O}C temperatures was optimum for the colony growth and sporulation of *lasiodiplodia theobromae*.

However, fungi grow on diverse habitants in nature and ark cosmopolitan in distribution requiring several specifics element for growth and reproduction. A wide range of media are used for isolation of different fungi and colony morphology (Kuhn and Ghannoun 2003; Kumara and Rawal, 2008). Observed that culture media influenced the growth and sporulation of some indian isolates of *Colletotrichum gloeosporides.* Similarly, Kuhn and Ghonnoum (2003) reported that a wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony, morphology, pigmentation and sporulation depending upon the composition of specific culture medium. Ray (2004) showed that lactose and glucose had similar effect on growth of *L. theobromae.* Jash *et al.,* (2003) also, observed that sucrose was the best carbon source for growth of *Altemaria zinnia* followed by starch and maltose, mannitol produced the least growth.

The results of the effects of light and darkness on fungal growth (Table 3) revealed that there was an increase in growth of *L. theobromae* in both light and darkness. Rewal and Grewal (1989) studied the effect of light on conidial germination of three strains of *Botrytis cinerea* infecting chickpea, and found that conidia of *B. theobromae* germinated best under continuous light and strain $B_{2,}$ *B. theobromae* of germinated well under light and darkness treatment. From the study, it implied that light and darkness are necessary for growth and sporulation of test fungi. This is in agreement with Ahmed (1985) who observed that light promoted the growth and sporulation of *Collectotrichum gloeosporoides.*

Similarly, Marshi *et al.,* (1959) reported that fungi exhibited varying response to light depending on the light intensity, quality and duration of exposure. Prota

(1992), Oladiran and Iwu (1993) and Pihet *et al*., (2009) reported that ultra violet (UV) radiation or sunlight affected the survival of fungal spores, sclerotia and pycndia. However, some fungi need light to sporulate whereas other fungi sporulate better in darkness. In their investigation, *Aspergillus ornatus* produced abundant conidia when grown in continuous light and virtually none when grown in dark while cleistothecia and ascospores are produced in the dark whereas neither is produced in continuous light (Schwemmin, 1960).

Hill (1976) further explained that light inhibits glucose uptake and phosphorylation which caused starvation and retards fungi growth and conidia formation. Conversely the growth of *Mycospharella pinodes, Aspergillus niger* increased when exposed to darkness.

On the contrary, Alam *et al.,* (2001) reported that light is not necessary for growth and sporulation of *B. theobromae,* but it enhances the growth and the number of conidia formation which is in partial agreement with the observation of Rewal and Grewal (1989). However, the increasing glucose in medium, may have cause the fungus to utilize it in a certain level and grow properly, and after that level, the fungal physiology does not permit the utilization of glucose for the growth of the pathogen. The fungus might utilize the glucose by different ways instead of growth and formed more pigmentation using more glucose (Teyegaga and Clerk 1972). According to Cochrane (1958), temperature range permitting reproduction is usually narrower than that permitting growth. Earlier, Leach (1979) had reported variations in optimum temperature requirements within the same species for light induced sporulation at continuous light and continuous darkness. Alam *et al.,* (2001) obtained more growth of *L. theobromae* under continuous light and less in continuous darkness. These findings agreed with the present research work where *L. theobromae* test fungi had a good growth performance for both light and darkness. However, Teygaga and Clerk (1972) earlier demonstrated the relationship between *Cercospora canescens* conidia longevity and storage humidity, and observed that in the dark there was longest survival of conidia at low humidity than those under light. Generally the spores stored in the darkness appeared to be more viable than those in light. This may be due to metabolic disruption by light or that light inhibited the spores of test fungi thus reducing their percentage conidial germination.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Our findings revealed that culture media differently influenced the growth, colony character and sporulation of the test fungi in the two test media employed in the present study, potato dextrose agar stem exudate (PDASE) was found to be most suitable for mycelial growth while potato dextrose agar PDA produced most visible colony morphology. It is concluded that instead of using single culture medium, a combination of two or more media will be more appropriate for routine cultural and morphological characterization.

Recommendations

Based on the present findings the following recommendations are made;

1. From the study potato dextrose agar stem exudates was found to be good medium of growth that supported the growth of *L. theobromae.*
2. It is revealed from the study that temperature, light and darkness significantly (P≤0.05) affected the mycelial growth of *L. theobromae.*

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