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EFFECT OF MEDIUM, TEMPERATURE, LIGHT AND INORGANIC FERTILIZERS ON *IN VITRO* GROWTH AND SPORULATION OF *LASIODIPLODIA THEOBROMAE* ISOLATED FROM MANGO

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Abstract

In this study the effects of culture media, temperature, light and fertilizers on mycelial growth and pycnidial production by *Lasiodiplodia theobromae* were evaluated. Potato sucrose agar (PSA), Corn meal agar (CMDA) and Yeast extract manitol agar (YEMA) were most suitable for mycelial growth of the test fungus. Potato carrot agar (PCA) was not suitable for either mycelial growth or pycnidia production. The YEMA appeared to be the best medium for pycnidial formation. The fungus grew from 20 to 45ºC, with optimum growth at 30-40ºC with no growth below 15ºC. The maximum numbers of pycnidia were produced at 35-40ºC. Different light regimes had no impact on mycelium growth and pycnidia production. The increasing concentrations of urea and DAP reduced the mycelial growth and pycnidia production.

Introduction

Lasiodiplodia theobromae (Pat.) Griff. & Maubl. (Syn: *Botryodiplodia theobromae* Pat.) represents the asexual state of *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx. It has a worldwide distribution in tropical and subtropical regions and occurs on a very wide range of plants (Punithalingam, 1976). It attacks more than 280 species of plants in different parts of the world (Domsch, *et al.*, 1980; Sutton, 1980). In Pakistan, the fungus has been reported on more than 50 plant species (Ahmed, *et al.*, 1997). Since the late nineties the mango decline or dieback disease became one of the most severe problem in mango orchards of the Sindh province (Khanzada *et al*., 2004a, b). In most cases, the disease has been characterized by the exudation of gum, wilting, dieback, vascular browning and death of the whole tree (Narasimhudu & Reddy, 1992; Khanzada *et al*., 2004). The objective of this study was to evaluate the effects of culture media, fertilizers and light on mycelial growth and pycnidial production of *L. theobromae* isolated from mango.

Materials and Methods

Pathogen inoculum: Culture of *L. theobromae*, previously isolated from mango trees were obtained from Karachi University culture collection center (KUCC) and maintained on potato sucrose agar (PSA) at room temperature for further experiments.

Effect of culture media: Effect of different synthetic and semi-synthetuic media on the colony growth and sporulation of *L. theobromae* was evaluated. Agar media *viz.*, water agar, Czapek's Dox agar, potato sucrose agar (PSA), corn meal agar (CMA), corn meal dextrose agar (CMDA), potato carrot agar (PCA) and Yeast Extract Manitol Agar (YEMA) were poured in 90 mm diameter Petri plates. Five mm diameter agar plugs were

removed with a sterile cork borer from the edges of colonies and one such plug was placed in the center of each 90 mm Petri plate containing this edge media. Plates were then wrapped with Parafilm and incubated at 20ºC in the dark. There were three replicate plates of each medium. The colony diameter in each plate was measured at 24h interval along two axes perpendicular to one another. The two measurements for each day were averaged and daily radial growth rates were calculated. After 15 days of inoculation, the number of pycnidia per plate was also noted.

Effect of fertilizers: Chemical fertilizers viz., Urea (46% nitrogen), Di-Ammonium Phosphate (DAP) (nitrogen, 15%, phosphorus, 15% and potash 15%) and NPK (nitrogen 10%, phosphorus 20%, potassium 20%) were used to evaluate their effect on the mycelial growth and pycnidial formation by *L. theobromae*. The fertilizers used with three concentrations were mixed in the PSA medium. After solidification of the medium a 5 mm disc from 5 days old culture of *L. theobromae* was placed in the center of the Petri plates. Colony growth and number of pycnidia were recorded as described above.

Effect of temperature: Effect of temperature on mycelial growth was evaluated on PSA. Inoculated plates were placed in plastic bags and incubated at 10, 15, 20, 25, 30, 35 and 40ºC in the dark. There were three replicate of each treatment. At each temperature the plates were arranged in a randomized complete block design. Colony diameters were measured as described above.

Effect of photo-periods: The effect of light on mycelial growth and pycnidial production was evaluated on PSA. Flourescent lamp and black carbon paper were used to maintain different photo-period *viz.*, continuous light, continuous dark, 24 hrs light and 24 hrs dark, 12 hrs light and 12 hrs dark, 16 hrs light and 8 hrs dark, 8 hrs light and 16 hrs dark. There were three replicate plates for each medium under each light regime. Observations were recorded as mentioned above.

Results and Discussion

Effect of culture media: The radial mycelial growth rates of *L. theobromae* were significantly affected by culture media (Fig. 1). In general, PSA, CMDA and YEMA were most favourable for fast radial growth of mycelium of *L. theobromae*. At 30ºC, colonies on these three media reached the edge of the plates after 5 days of inoculation. There was also a significant interaction between type of medium and on pycnidial production. The highest number of pycnidia per plate was formed on YEMA. On PSA, WA, CZA and CMDA moderate number of pycnidia (about half of those produced on YEMA) whereas; PCA and CMA either produced least number of pycnidia or not at all (Fig. 1). The radial mycelial growth and pycnidial production of *L. theobromae* was medium dependent. Our results were in close agreement with those of Alam *et al.,* (2001) who recorded highest mycelium growth of *B. theobromae* on PDA and maximum pycnidia on Czapek's Dox agar. Similarly Qureshi & Meah (1991) observed fastest liner growth of *B. theobromae* on Richard's agar, mango leaf extract agar and on PDA. They recorded highest number of pycnidia on mango leaf extract followed by PDA. Alasoadura (1969) observed maximum stromata of *B. theobromae* on malt agar and oatmeal agar. The size and number of pycnidia varied greatly within the substrate and biggest produced in nutrient rich medium (Sabalpara *et al.*, 1991).

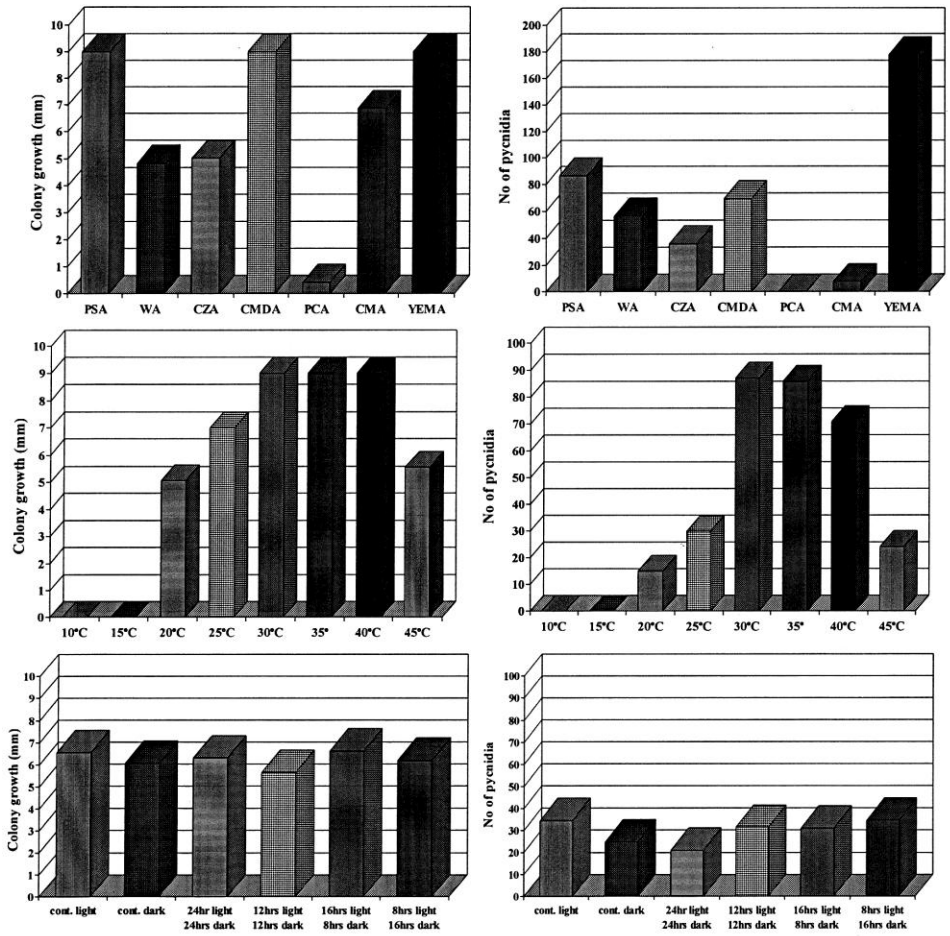

Fig. 1. Effect of culture media, temperature and light on the growth of *B. theobromae*.

Effect of temperature: The mycelial growth of *L. theobromae* showed, a variable trend in response to changes in temperature on PSA medium (Fig. 1). There was very little or no growth at low temperatures i.e. 10 and 15ºC., However mycelial growth increased as temperature increased up to 40ºC and then decreased rapidly with further increase temperature. Optimum growth occurred at 30-40ºC. At 10ºC mycelial growth was not observed after 15 d of incubation, but the fungus resumed growth when the plates were moved to 30ºC. The pycnidia formation also showed same trends as mycelium growth with respect to temperature change. Highest number of pycnidia was recorded at 30-35ºC followed by 40ºC. At low temperatures *L. theobromae* failed to sporulate. After 35ºC an increment in temperature also reduced the pycnidial formation on the culture media. Our results are in close agreement of those reported by Quroshi & Meah (1991) and Alam *et al.*, (2001) who reported 29ºC and 25-30ºC temperatures optimum for the colony growth and sporulation of *L. theobromae*.

Effect of photo period: There was no significant interaction between light regimes and radial mycelial growth of *L. theobromae*. Similarly pycnidial production was also not affected by different light periods. The average colony growth and number of pycnidia per plate were more or less same in different photo periods. Similarly, Alam *et al.,* (2001) observed that growth of *B. theobromae* was not significantly affected by different light conditions on PDA however; sporulation was highest in continuous light.

Effect of fertilizers: Fertilizers significantly affected radial mycelia growth of the test fungus (Table 1). Interaction between fertilizer or fertilizer's concentration for radial growth was significant. The highest colony growth was recorded at 10 ppm DAP followed by 10 ppm Urea. However, at all concentrations NPK fertilizer produced same impact on both mycelial growth and pycnidial formation of *L. theobromae*. In general, an increase or decrease in the fertilizers concentration resulted in the increase or decreasing colony growth and number of pycnidia per plate. Similarly, Sharma & Bhutani (1990) observed that soil mycoflora population showed an increase with Nitrogen application. Microbial biomass showed a significant correlation with soil organic matter and mean hyphal diameters decreased with decreasing soil organic matter (Schnurer *et al.,* 1985).

Table 1. Effect of fertilizers on the colony growth and pycnidial formation of *Lasiodiplodia theobromae*.

Fertilizers	Concentration (ppm)					
	10,000	**1000**	**100**	**10**	**1**	**Control**
	Colony diameter after 2 days (mm)					
Urea	1.43	1.98	4.09	6.51	2.31	4.3
NPK	4	3.5	4	5.5	4.75	
DAP	0.58	0.6	4.8	8.28	5.23	
	No. of pycnidia after 14 days					
Urea	-	23	70	84	13	79
NPK	76	74	79	81	76	
DAP	-	-	54	95	49	

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