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Cultural and morphological characteristics of *Lasiodiplodia theobromae* of *Dianella* in various carbon and nitrogen containing media

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Abstract
Dianella, that contains about 41 species including the blue or Tasmanian flax lily (*Dianella tasmanica*) and the variegated flax lily (*Dianella tasmanica variegata*), are grown for their attractive foliage and shiny, blue to purple berries. Taking into consideration the different points *i.e.* bio-aesthetic planning, indoor decoration, social and religious functions the demand for ornamental plants is increasing gradually. But they are attacked by different diseases. The various cultural and morphological characteristics of fungi *Lasiodiplodia theobromae* from *Dianella* were studied which were grown on different carbon and nitrogen containing media. Among the carbon containing media, the most effective medium for rapid growth was peptone salt agar medium followed and oat meal agar and Czepek's Dox agar media. In case of nitrogen sources, the radial growth was higher in case of medium containing aspartic acid and a combination of sodium nitrate and ammonium sulphate. The bioassay of three non-systemic viz. Blitox, Mancozeb and Chlorothalonil and one systemic fungicide Difenoconazole using six different concentrations along with control were examined. It was found that the fungus was completely inhibited in all the three fungicides except Difenoconazole.

Keywords: *Dianella*, *Lasiodiplodia theobromae*, Czepek's Dox agar, Peptone Salt Agar, Oat Meal Agar

Introduction
Dianella tasmanica was first described in 1858 by eminent English botanist and explorer Joseph Dalton Hooker. The genus name is derived from the Roman goddess Diana, with a diminutive suffix -ella. It is a strappy herbaceous plant which grows to 0.5-2 metres (1-7 ft) high and wide, with a thick spreading rhizome under the ground. The green linear keeled leaves have finely toothed margins, and may reach 1 m (40 in) in length and 1.5-4 cm wide. The small (1.5 cm diameter) blue flowers bloom in spring and summer (August to February), and are followed by small roughly oval or globular violet berries which range from about 1.2 cm (0.5 in) in diameter (Rodger *et al*., 1984) [3]. They are affected by different kinds of fungal, bacterial, viral and nematode diseases as reported several times from various parts of the world including India (Bannerjee, 2016; Katakam, 2016) [2, 6]. The members of Deuteromycetes including pycnidia producing pathogens (Form order Sphaeropsidales) and acervuli producing pathogens (Form Order Melanconiales) play a major role for destruction of plant parts rapidly by infecting different parts along with production of various ranges of symptoms on plant. Different media are used for various groups of fungi that influence the vegetative growth, colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature and light (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008) [10, 8, 9]. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics. Furthermore, findings for one species are not readily extrapolated to others, particularly for filamentous fungi, where significant morphological and physiological variations exist. Thus, there is the need of testing of different media. Carbon and nitrogen sources and their concentrations bear a significant effect on the type of cultural growth of fungi on the media. Modification of the basal concentrations of these nutrients effects the viability and enzyme production of fungi. Several workers have recognized the importance of spores as inoculum and studies have been conducted on the effects of various media components along with important physiological parameters that lead to maximum sporulation (Kim *et al*., 2005) [7]. So, the use of carbon and nitrogen in the medium needs to be emphasized. Biomass production of a fungus either in solid or liquid medium is an important parameter to judge its efficiency in the utilization of

nutrients from the medium i.e. the better the efficiency in utilization, the better will be the production of biomass. It is a good indicator to evaluate the suitability of a medium for growth and maintenance of the fungus, varies according to the types of fungi, species, sub-species or isolates of a fungus and could be a vital parameter for genus/ species/isolate level morphological differentiation. For that reason, recoding of biomass is being emphasized. After isolation, characterization and identification, the evaluation of fungicide sensitivity is a vital exercise in order to find out the efficacies of various fungicides and their doses which can be used for the management of diseases of the above mentioned ornamental plants and help in reduction of crop loss.

Materials and Methods

The experimental studies like characterization of cultural and morphological parameters using various carbon and nitrogen sources and determinations of fungicide sensitivities of the collected fungi was conducted under laboratory condition of the Department of Plant Pathology of the University. Synthetic, semi-synthetic and natural media were used for various laboratory studies and for the maintenance of plant pathogenic fungus. For studying radial growth, colony morphology and asexual fruit bodies (pycnidia) using various carbon sources like potato dextrose agar (PDA),Czapek's Dox agar (CDA) where dextrose was replaced by the same amount of sucrose(CDASWS) and lactose(CDASWL) per 1000ml of growth medium, oat meal agar (OMA), water agar (WA) and peptone salt agar (PSA) media were used. In the same line, CDA medium was used for nitrogen utilisation studies where sodium nitrate was replaced by same amount of $NH_4SO_4$and L-Asparagine or replaced with half of the amount of $NH_4SO_4$and $NaNO_3$ per 1000ml of growth medium whereas broth of all these media were used for studying biomass production. Micro-photograph of all the fungal structures in different carbon sources were taken with the help of Leica Binocular Microscope and or Karl Zeis Phase Contrast Microscope (under 10x,40x) and by using Canon Powers Shot A640 camera.Dimensions (e.g. length and breadth) of conidia, hyphae, acervulus, pycnidia of fungi were measured using Axio Vision (Rel. 4.8.) software.

Sensitivities of the fungus *Lasiodiplodia theobromae* to four different fungicides having five different concentrations were tested in-vitro following poisoned food technique proposed by Shervelle (1979) [12]. Different concentrations of fungicides were taken from stock solution with the help of sterilized micro tips which was then mixed with sterilized, molten PDA media before plating to obtain the desired concentrations of active ingredient. A total of three non-systemic viz. Blitox 50 WP, Indofil M-45 and chlorothalonil and one systemic fungicide Score 25 EC was used as four treatments in the fungicide bioassay experiment (Table-1). Radial growth of the various fungi on different concentration and control was recorded. Extent of inhibition of mycelia growth by each fungicide was calculated by estimating the percent reduction in mean mycelial radial growth over that of control (Vincent, 1947). Effective concentration for 50% growth inhibition (EC-50) by the fungicides for each fungus was determined by plotting the log values of the fungicide concentration against the probit values of percent inhibition on a log-probit scale(Horsefall,1956).A regression equation Y= a+ bx (Y =antilog of concentration of the fungicide, x= probit value of percent inhibition, b= regression co- efficient/ slope,a= intercepts) was worked out and the fitness of the equation was judged comparing the level of significance with the simple correlation coefficient (r) value at 5% or 1% level. Per cent inhibition was measured with the formula, which is given below

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control(C)-Radial growth in treatment (T) x 100}}{\text{Radial growth in control(C)}}$$

Table 1: Trade-chemical-, IUPAC- and manufacturer names along with the concentrations of the test fungicides

Trade name	Chemical name (formula)	IUPAC name	Manufacturer	Concentrations (µg/ml) used
Blitox 50 WP	Copperoxychloride ($Cl_2Cu_2H_3O_3$)	Dicopper dichloride trihydroxide.	Tata Rallis	0,10,25,50,100,200
Indofil M- 45	Mancozeb(Polymeric mixture of Mn and Zn)	Manganese ethylenebis (dithiocarbamate)	Indofil	0,10,25,50,100,200
Kavach 75 WP	Chlorothalonil ($C_8Cl_4N_2$)	2,4,5,6-Tetrachloroisophth--alonitrile	Kenvos Biotech Co., Ltd.	0,10,25,50,100,200
Score 25 EC	Difenoconazole ($C19H_{17}Cl_2N_3O_3$)	1-((2-(2-Chloro-4-(4chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole	Syngenta	0,10,25,50,100,200

Result and Discussions

The cultural characteristics of the fungi on six different carbon sources were studied with a view to identify the best media for the radial growth and sporulation of the fungi. Out of six carbon sources viz. PDA, PSA, CDA, CDA supplemented with sucrose (CDASWS), CDA with lactose (CDASWL) and OMA used for the radial growth of the fungus, it was evident that the most effective medium for rapid growth of *Lasiodiplodia theobromae*, was PSA and OMA media whereas the growth of the fungus in all the other media were at par. with each other at 120 hrs of incubation and covered the entire plate at 240 hrs of incubation in all the media studied. Alam *et al.*, 2001, reported that highest mycelial growth and sporulation of B. theobromae was observed on PDA, The fungal growth rate was studied with an objective to find out the speed of growth of a particular fungus at a particular point of time. The growth rate was highest in case of CDASWS whereas the average mean of all the dates of observations were at par. in PDA, CDASWS, CDASWL and CDA media. The radial growth was evaluated using various nitrogen sources, both of organic and inorganic in origin with an aim to study about the suitable media for studies on mycelial characteristics. The radial growth was higher in case of medium containing aspartic acid and a combination of sodium nitrate and ammonium sulphate. The rate of radial growth shown by the fungus was fastest in

ammonium sulphate containing medium and it was found that the rate of growth in the control could be compared to the growth in other nitrogen containing media at the last dates of observations. The biomass production by the all the four fungus were also studied after 144 hrs of incubation. It exhibited highest biomass production in PDA medium followed by CDASWS and CDASWL media. The biomass production by the all the four fungus were also studied by using the various organic and inorganic nitrogen sources after 144 hrs of incubation and it exhibited highest biomass production in medium containing aspartic acid as its nitrogen source.

Colony morphological studies

Colony morphology, colour of the colony from the upper and lower sides of all the four fungus were also studied using the different carbon sources. The objective of the study was to identify the variation in different genus and species of the fungi. Colony of *Lasiodiplodia theobromae* produced very thin, uniform, thread like mycelia in all the media which was blackish in case of PDA,CDASWS, CDASWL and CDA media and whitish incase of PSA and OMA media. In all the nitrogen media tested, it produced thick, uniform, cottony mycelial growth (Plate 1A-E).

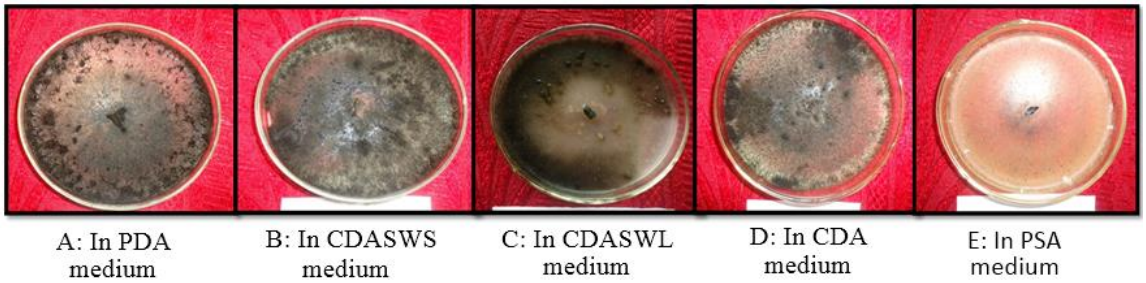

Plate 1: Colony characteristics in various carbon containing media

Microscopic characteristics studies

The microscopic characters relating to the dimensions of hyphae, pycnidia,setae and conidia were also recorded with a view to see whether there was any marked difference in the dimension of hyphae, pycnidia, conidia with regard to changes in media constituents. Significant difference was noted wherein it shows that PSA medium produces conidia, setae of higher dimensions (Plate-2a). The pycnidia produced were in the range of 460.3 – 582.7µ(av.500.45µ) (Plate-2b).Conidiophores were not clearly visible. Conidia developed from inner basal cells of the pycnidium. Conidia were double layered, hyaline and unicellular at initial stage but on maturity it became light to dark-brown colour with typical striate formation, equally 2-celled, oblong, bilamilate with the size of 14.5 -28.8µ(av. 26.9µ) x 8.0- 11.0 µ (av. 15.9µ). On OMA media, huge number of spores were produced (Plate-3a).The spore dimension were 22.9-35.7 µ (av.31.7 µ)x 8.0 – 11.0µ (avg.11.7 µ) and the pycnidia produced were 73.5-113.3µ (Plate-3b). On the CDA medium, conidia was hyaline with dimension of 9.4 – 16.8(av.7.2) x 2.3 – 3.1(av.2.8).So, this media was found superior to all the other media used.

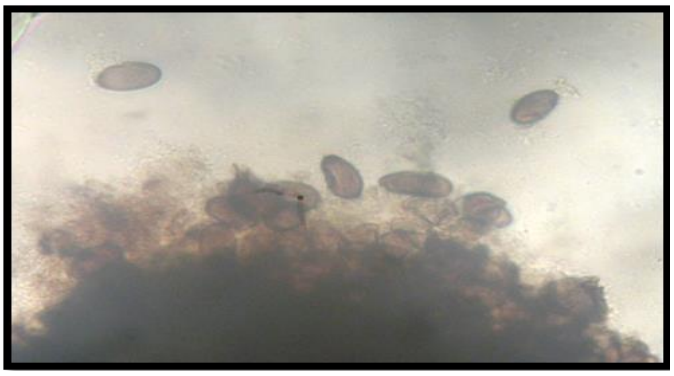

Plate 2a: Spore produced in PSA medium

Plate 2b: Pycnidia produced in PSA medium

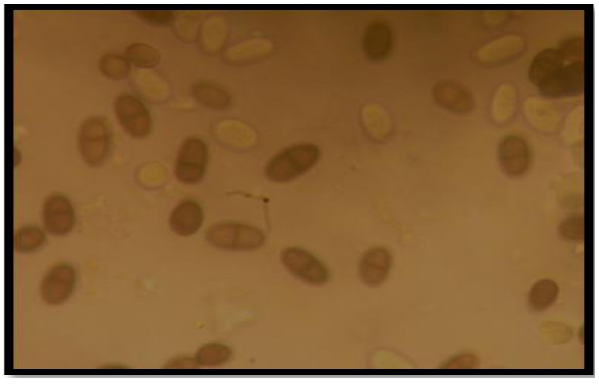

Plate 3a: Spore produced in OMA medium

Plate 3b: Pycnidia produced in OMA media

Fungicide sensitivity tests

The bioassay of three non-systemic viz. Blitox, Mancozeb and Chlorothalonil and one systemic fungicide Difenoconazole using six different concentrations along with control were examined (Plate 4a-d). When the bioassay was conducted against *Lasiodiplodia theobromae*, it was found that mycelial growth was produced only incase of Difenoconzole with EC 50 value of 243.2µ/ml whereas the plates where the other three fungicides i.e Blitox, Mancozeb and Chlorothalonil was able to completely restrict the fungal growth.

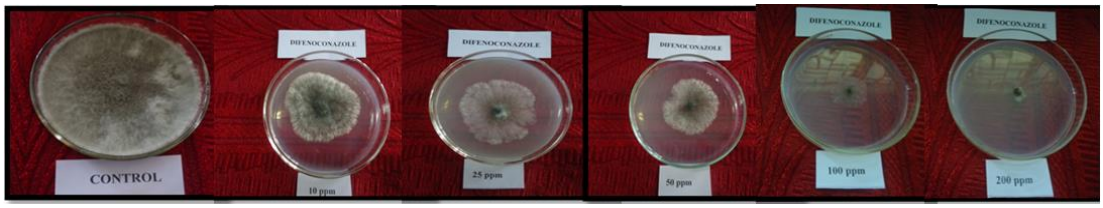

Plate 4a: Difenoconazole

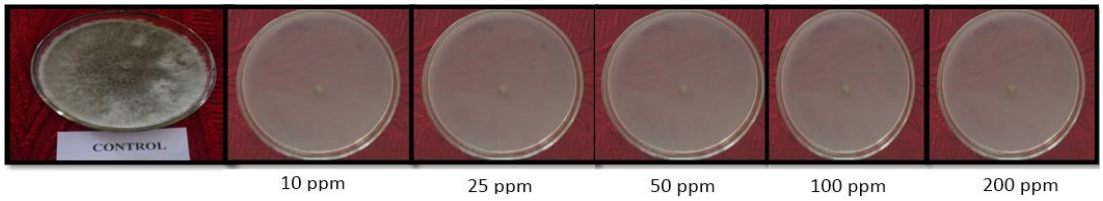

Plate 4b: Blitox

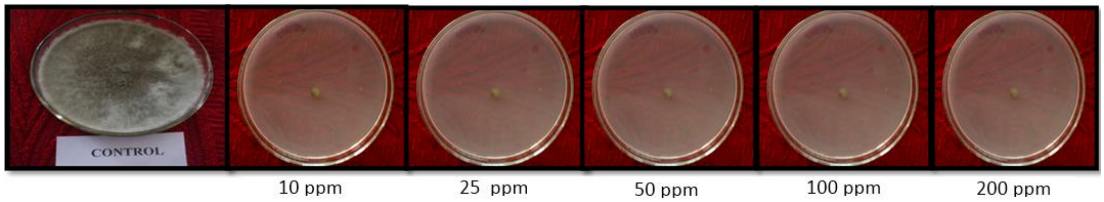

Plate 4c: Mancozeb

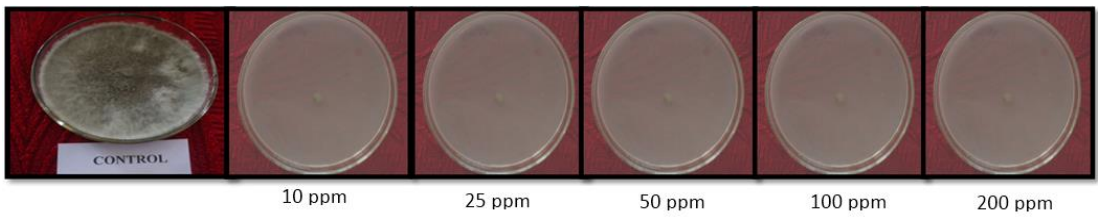

Plate 4d: Chlorothalonil

Plate 4: Effect of various fungicides on *Lasiodiplodia theobromae*

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References

1. Alam MS, Begum F, Sarkar MA, Islam MR, Alam MS. Effect of temperature, light and media on growth,sporulation, formation of pigments and pycnidia of Botryodiplodia theobromae. Pakistan Journal of Biological Sciences. 2001; 4(10):1224-1227.
2. Bannerjee A. Studies on pycnidia producing fungi of some ornamental plants grown under protected condition. M.Sc. thesis. Bidhan Chandra krishi Viswavidyalaya. 2016, 52.
3. Rodger E, Jones W, Blake DL, Trevor. Encyclopaedia of Australian Plants Suitable for Cultivation. Port Melbourne: Lothian Press, 1984, 260.
4. Hooker JD. The botany of the Antarctic voyage of H.M.

discovery ships Erebus and Terror. III. *Flora Tasmaniae*. 1858; 2(6):57.
5. Horsefall JG. Principles of fungicidal action. Chronica Botanica Co., Altham, Mass, USA, 1956.
6. Katakam M. Studies on acervulus producing fungi associated with selected greenhouse plants. M.Sc. thesis. Bidhan Chandra krishi Viswavidyalaya, 2016, 40.
7. Kim YK, Xiao CL, Rogers JD. Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia. 2005*;* 97:25-32.
8. Kuhn DM, Ghonnoum MA. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. Clinical Microbiology Review. 2003; 16(1):144-172.
9. Kumara KLW, Rawal RD. Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L). Tropical Agricultural Research and Extension. 2008; 11:7-12.
10. Northolt MD, Bullerman LB. Prevention of mold growth and toxin, 1982.
11. Palarpawar MY, Ghurde VR. Influence of different nitrogen sources on growth and sporulation of *Colletotrichum capsici* and *Colletotrichum curcumae*. Journal of Mycology and Plant Pathology. 1997; 27(2):227-228.
12. Shervelle EG. Plant disease control. AVI Publishing Company, Inc. West.Post. Connecticut, 1979.