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IDENTIFICATION, ISOLATION, SCREENING OF SUITABLE MEDIUM AND TEMPERATURE AND *IN VITRO* MANAGEMENT OF ANTHRACNOSE-CAUSING PATHOGEN OF LUCKY BAMBOO GROWN IN WEST BENGAL INDIA

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ABSTRACT

Lucky bamboo (*Dracaena sanderiana*), is a popular ornamental plant prone to attack by anthracnose disease. Symptom's description of the disease as well as isolation, pathogenicity establishment, identification of the associated pathogen followed by identification of suitable growth medium and temperature along with the determination of EC_{50} value of the two fungicides were made. Based on conidial dimension and cluster analysis the causal pathogen was identified as *Gloeosporium leguminum*. Out of four growth media *viz.* Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Malt Extract Agar (MEA) and Czapek's Dox Agar (CDA) tested, the most suitable medium for mycelial growth was Malt Extract Agar (MEA). Among the three temperature regimes *viz.* 21°C, 24°C and 28°C examined, the temperature of 28°C was found optimum for its radial growth. The fungus produced whitish, cottony, fluffy mycelial growth in both CDA and OMA media whereas in PDA and MEA media greyish white mycelial growth at 21°C, 24°C and 28°C. For biomass production, Potato dextrose broth (PDB) was optimal at 24°C and 28°C. The fungicide Bavistin 50 WP (Carbendazim) outperformed Amistar (Azoxystrobin), by completely inhibiting mycelial growth at 10 ppm while same for the later was at 100 ppm with EC_{50} value of 6.03 ppm.

***Key words*:** lucky bamboo, anthracnose, media, temperature, fungicide.

Introduction

Lucky bamboo (*Dracaena sanderiana*), is a foliage plant native to Central Africa, widely utilized in ornamenting and charming the landscapes globally. Despite aesthetic appeal, several fungal diseases such as *Colletotrichum gloeosporioides*, *C. dracaenophilum*, *C. petchii* and *C. boninense* have been reported to affect lucky bamboo plants in Egypt, as well as other countries around the world (Bobev *et al*., 2008, Liu *et al*., 2014, Macedo and Barreto, and Morsy and Elshahawy, 2016) and Stem rot and leaf spots were caused by *Fusarium sp. and Alternaria alternata respectively* (Abbasi and Aliabadi, 2008 and Hilal *et al*., 2016). These diseases can significantly reduce the plant's vitality, hinder its growth, diminish its overall visual allure and commercial value. In the recent past, the lucky bamboo in West Bengal was encountered a severe threat of anthracnose type of disease. Neither the symptoms of the disease were studied earlier nor its causal agent was identified after its isolation. As the associated causal pathogen of this disease in fishtail plant was not isolated, obviously, identification of suitable medium and temperature for its growth and determination of EC_{50} to some common fungicides were not made. Based on abovementioned research gaps on this particular disease at the study location, especially in the eastern region and as a whole in India, the objectives of the present study were set as to study disease symptoms, isolate and identify the causal agent by morphological characters, screen the suitable cultural medium and temperature required for its optimum growth and find out its EC_{50} against the selected fungicides.

Materials and Methods

Collection of diseased samples and isolation of pathogen

Diseased leaf samples of lucky bamboo were collected from Pallishree Nursery, Arambag, Hooghly, West Bengal (22°53' N, 87°78' E), India and brought immediately to the Mycology Laboratory of the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India. The pathogen of the disease was isolated following the methodology used by Thilagam *et al*., (2018) with a little modification. The leaves displaying disease symptoms were cut into small pieces, surface sterilized with 0.1% $HgCl_2$ for 30-45 seconds, washed with 5-6 times with sterilized distilled water, blotted for the excess water, placed on the water agar medium in petri plates, incubated at 28±1°C until hyphal growth was observed; a small bit of hyphal growth was transferred onto the fresh PDA slant. The fungus was allowed to grow for 6-7 days in PDA slants. Full grown pure cultured slants were stored in refrigerator at 5°C for further experimental use.

Identification of the pathogen

Slides were prepared from diseased parts for morphometric studies. Microphotographs were taken using a Karl Zeis Phase Contrast Microscope. Conidia and conidiophores dimensions were measured with AxioVision software. The fungus was initially identified at the genus level (Sutton, 1981) and then the species level by comparing the dimensions of the conidia of this genus with morphometric characteristics of conidia other species following the nearest neighbour method of cluster analysis.

Effect of different culture media and temperatures on the radial growth and colony morphology

Four different culture media *viz*. Potato dextrose agar (PDA), Malt extract agar (MEA), Oat meal Agar (OMA) and Czapek's Dox agar (CDA) and three different temperatures *viz*. 21°C, 24°C, and 28°C were assessed for their effect on the radial growth. For this purpose, 20 ml of each of the sterilized media was poured into sterilized petri plates (90 mm dia) and allowed them to solidify. Each plate was inoculated at the centre with 5 mm diameter fungal mycelial disc, kept in incubators, ran at above mentioned three temperatures, for 10 days depending on growth behavior of fungus. The radial growth and colony morphology were recorded from the front and back side of petri plates. Radial growth data were subjected to 2 factors analysis.

Biomass production of fungi

Four growth media *viz*. Potato dextrose broth (PDB), Oat meal broth (OMB), malt extract broth (MEB) and Czapek's dox broth (CDB) and three temperatures viz. 21°C, 24°C, and 28°C were considered with three replications. 40 ml broth of above mentioned four media were taken into 250 ml conical flasks and inoculated with a 5 mm mycelial disc taken from active growing tip region of 6 days old culture. Both the flasks and plates were incubated at above mentioned three temperatures. The conical containing broths were incubated for 6 days. After 6 days the mycelial mat formed by fungi on 4 growth media were collected and filtered separately through previous dried and pre-weighted Whatman No. 1 filter paper. Filter containing the fungal mass was then kept in drier for drying at 65°C -70°C for 96 hours to a constant weight. Weighing was repeated till constant weight was obtained. 2 factor analysis was done for fungal dry biomass production.

Fungicide sensitivity analysis

In vitro sensitivity of seven concentrations *viz*. 0, 10, 25, 50, 100, 200, 400, 800 µl/ml of two fungicides *viz.* Amistar (Saxena *et al*., 2016) and Bavistin 50 WP (Ushakiran *et al*., 2006, Arora *et al*., 2020, Morsy and Elshahawy, 2016) were tested following the poisoned food technique. 20 ml of the fungicide poisoned PDA medium was poured into each sterilized Petri plate. Control plates were maintained by the addition of same volume of PDA medium in the Petri plates without the addition of any fungicide. Then 5 mm mycelial discs were cut out from the periphery of the actively growing cultures with the help of sterilized cork borer and placed at the centre of the Petri plates. Plates were incubated at 28±2°C temperature in BOD till the fungal growth covers the entire Petri plate used as control (Golakiya *et al*., 2020). Each treatment was replicated three times with suitable control. Radial growth was recorded and percent reduction in mycelial growth over control was worked out. The EC_{50} for each fungicide was determined by plotting the log value of concentrations against the probit values of percent inhibition (Ren *et al*., 2020). A regression equation (Y = a + bx) was derived and examined for fitness. Each treatment was replicated three times, and significance levels were compared with the simple correlation coefficient (r) at 5% or 1%. Percent inhibition of radial growth was calculated using the formula:

$$\text{Percent inhibition} = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control (C)}} \times 100$$

Results and Discussion

Symptoms of the disease

The symptoms are observed on leaves, leaf sheath

Table 1: Dimensions of conidia of Gloeosporium sp. obtained from lucky bamboo grown as pure culture on PDA medium.

Pathogen name	Conidial length (µm)	Conidial width (µm)	Length width ratio
Gloeosporium sp.	8.75-13.97	3.19-4.81	2.39-4.34

as well as on the stem. The symptoms progress downward, resulting in the drying followed by a demarcation of a dark brown line which is descended by yellow halo in the healthy tissue (Fig. 1). As the disease progresses, whole leaf dries up and reaches to the leaf sheath. Infections on the stems begin as a small depressed spot or observed as a continuation of disease from the leaf sheath. The affected portion of the stem gets dried and the lesion area increases gradually. Minute dot like structures often observed on the dry tissue of the stem (Fig. 1).

Isolation and pathogenicity establishment of pathogen

The pathogenicity of the isolated fungal pathogen was established by inoculating the detached leaf under laboratory conditions. The inoculated leaf produced similar symptoms (Fig. 2) as observed in the nursery. The fungal pathogen was re-isolated in purified form from the inoculated diseased leaf and compared it with the initially isolated fungal culture. Both the isolated pathogens were same. Thus, the pathogenicity of the isolated fungal pathogen was established (Azad and Shamsi, 2011).

Morphological descriptions and identification of the pathogen

Slides prepared from diseased parts as well as from pure culture were used for morphometric studies. The fungus produced typically short, rod shaped, oblong, hyaline aseptate conidia with both end rounded (Fig. 3). Conidia were 8.75 to 13.97 µm in length and width ranged from 3.19 to 4.81 µm. The length width ratio was found to range from 2.39 µm to 4.34 µm (Table 1).

The anthracnose-causing pathogen associated with lucky bamboo closely resembled *Gloeosporium* species based on conidial characteristics. Dimensions of spore of various *Gloeosporium* species were considered for hierarchical cluster analysis (Salotti *et al*., 2022) and using

Fig. 1: Anthracnose symptoms on leaves and stems of lucky bamboo.

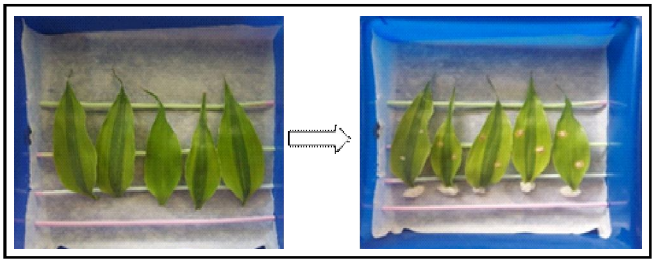

Fig. 2: Pathogenicity establishment of *Gloeosporium* sp. of lucky bamboo.

the nearest neighbor method, aligned the lucky bamboo isolate with *G. leguminum* whose conidia were hyaline, typically rod shaped, oblong. Comparison of dimensions place it within the range of various *Gloeosporium* species (Fig. 4), but single linkage hierarchical analysis pinpointed a close match with *G. leguminum*, with spore length and breadth at 10-15 µm × 4-6 µm. Thus, the lucky bamboo-infecting *Gloeosporium* sp. was identified morphologically similar as *G. leguminum*.

Effect of different culture media, temperature and culture media × temperatures on radial growth

The effect of 4 growth media *viz*. PDA, CDA, OMA and MEA and three different temperatures *viz*. 21°C,

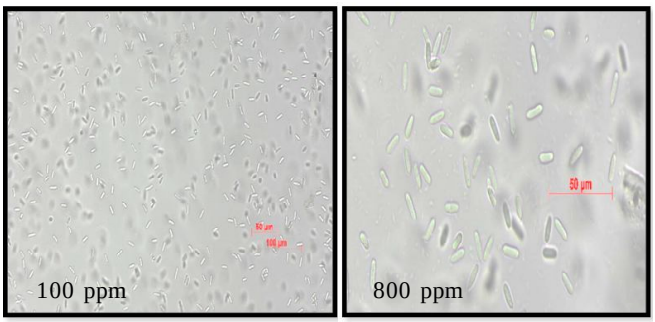

Fig. 3: Microscopic view of conidia produced by *Gloeosporium leguminum*.

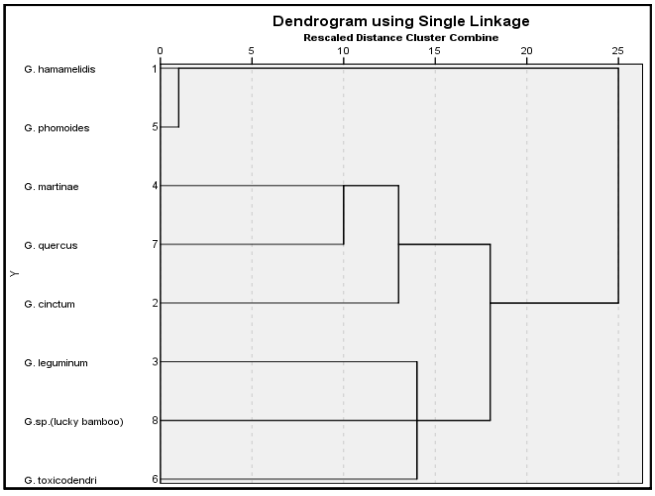

Fig. 4: Dendogram based on conidial characteristics of *Gloeosporium leguminum* obtained from lucky bamboo.

Table 2: Effect of media*temperature on radial growth (cm) of *Gloeosporium leguminum* at different dates of observations.

	Day 1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9
Media	**T1 (21°C)**								
PDA	0.67	1.67	2.47	3.40	4.83	5.57	6.53	7.77	8.94
CDA	0.87	1.67	2.93	3.37	4.27	5.37	6.30	7.23	8.83
OMA	0.53	2.17	3.33	4.07	5.00	5.77	7.03	8.07	9.00
MEA	0.83	1.93	3.37	4.23	5.33	5.97	7.30	8.90	9.00
Temperature means irrespective of media	0.73 a	1.86 a	3.03 a	3.77 a	4.86 a	5.57 a	6.79 a	7.99 a	8.94 a
Media	**T2 (24°C)**								
PDA	1.13	2.17	3.53	3.90	5.07	6.03	7.03	8.03	9.00
CDA	1.03	2.07	3.13	3.70	4.73	5.70	6.77	7.57	8.91
OMA	1.13	2.37	3.30	4.10	5.63	6.33	7.43	8.73	9.00
MEA	1.07	2.43	3.40	4.67	5.80	6.70	7.87	8.97	9.00
Temperature mean irrespective of media	1.09 b	2.26 b	3.34 b	4.09 b	5.31 b	6.19 b	7.27 b	8.32 b	8.98 a
Media	**T2 (28°C)**								
PDA	2.43	3.47	4.37	5.23	6.17	6.97	8.03	8.77	9.00
CDA	2.13	3.07	4.20	4.87	5.87	6.73	7.23	8.13	9.00
OMA	2.43	3.67	4.57	5.77	6.53	7.27	8.50	8.97	9.00
MEA	2.63	3.73	4.73	5.87	6.77	7.90	8.87	9.00	9.00
Temperature means irrespective of media	2.41 c	3.48 c	4.47 c	5.43 c	6.33 c	7.22 c	8.16 c	8.72 c	9.00 a
Media mean irrespective of temperature									
PDA	1.41 a	2.43 b	3.46 a	4.18 b	5.35 b	6.19 b	7.20 b	8.19 b	9.00 b
CDA	1.34 a	2.27 a	3.42 a	3.98 a	4.95 a	5.93 a	6.77 a	7.64 a	8.92 a
OMA	1.37a	2.73 c	3.73 b	4.64 c	5.72 c	6.46 c	7.66 c	8.59 c	9.00 b
MEA	1.51 b	2.70 c	3.83 c	4.92 d	5.97 d	6.86 d	8.01 d	8.95 d	9.00 b
SEm±for temperature	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.03	NS
$CD_{.05}$ for temperature	0.04**	0.08**	0.07**	0.08**	0.06**	0.07**	0.07**	0.09**	-
SEm+- for media	0.02	0.02	0.03	0.04	0.02	0.03	0.03	0.04	NS
$CD_{.05}$ for media	0.05**	0.05**	0.08**	0.09**	0.07**	0.08**	0.08**	0.10**	-
SEm+- for temp*media	0.04	0.04	0.05	0.07	0.05	0.06	0.06	0.07	NS
$CD_{.05}$ for temp*media	0.09**	0.09**	0.13**	0.16**	0.12**	0.14**	0.14**	0.18**	-

PDA = Potato Dextrose Agar; OMA = Oat Meal Agar; MEA = Malt Extract Agar; CDA = Czapek's Dox Agar ; SEm = Standard error of mean; CD = Critical difference; ** = Significance at 0.01%; NS = Non-significant

24°C, and 28°C were assessed for their sole and interaction effects (Table 2) on the radial growth performance of *Gloeosporium leguminum* at 9 different dates. At 8th day of observation, out of the 4 media tested MEA medium appeared to be superior to OMA, PDA and CDA media (Fig. 5). Ivanová and Bernadovicová (2006) also found that MEA was the most suitable medium for *Gloeosporium tiliae*.

The radial growth of the fungus irrespective of media when examined, it was found significantly enhanced in all dates of observation (Table 2). Out of 3 temperatures tested, the radial growth was significantly highest at 28°C (Denoyes and Baudry, 1995; Goos and Tschirsch, 1962) and lowest at 21°C. However, the performance of the radial growth of the fungus at 24°C looked intermediate.

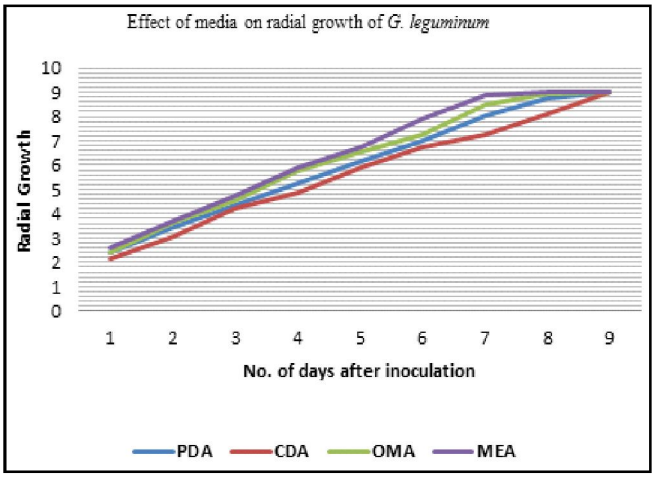

Fig. 5: Effect of media on radial growth of *G. leguminum* at 28°C temperatures.

Table 3: Effect of media on biomass production (g) of *Gloeosporium leguminum* irrespective of temperatures at 6 days after inoculation.

Growth media	Biomass (g)
Potato dextrose broth	0.322b
Czapek's Dox broth	0.251a
Oat meal broth	0.222a
Malt extract broth	0.250a
Standard error of mean±	**0.021**
Critical difference $_{.05}$	**0.063**

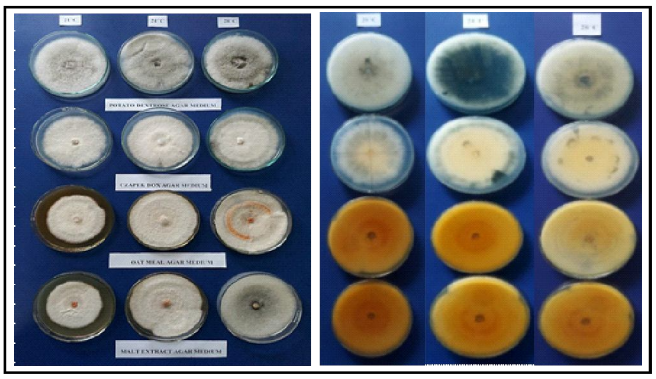

Fig. 6: Growth of *Gloeosporium leguminum* in different media at different 21°C, 24°C, 28°C respectively (Front side and Back side)

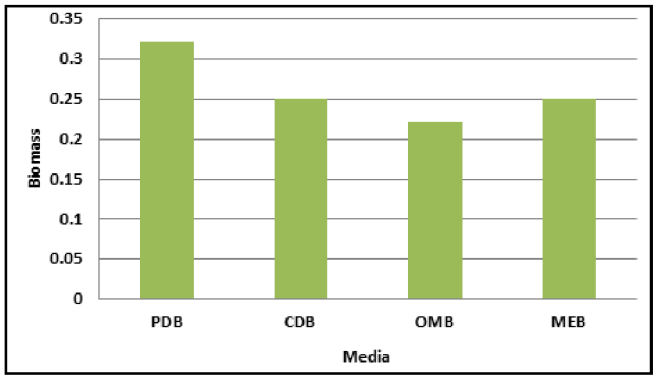

Fig. 7: Effect of media on biomass production (g) of *Gloeosporium leguminum* irrespective of temperatures at 6 days of inoculation.

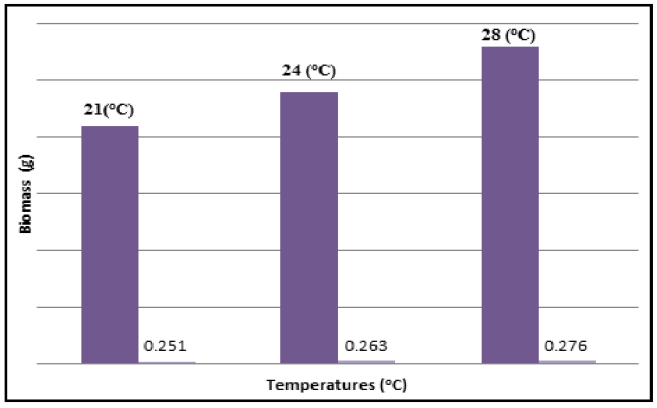

Fig. 8: Effect of temperatures (°C) on biomass production (g) of *Gloeosporium leguminum* irrespective of media at 6 days of inoculation.

Table 4: Effect of temperatures (°C) on biomass production (g) of *Gloeosporium leguminum* irrespective of media at 6 days after inoculation.

Temperatures (°C)	Biomass (g)
21	0.251a
24	0.263a
28	0.276a
Standard error of mean±	**non-significant**
Critical difference $_{.05}$	-

At day 9, the tested fungus covered the entire diameter of the Petri plates at all the temperatures *viz*. 21°C, 24°C and 28°C. In the present experiment, out of 3 temperatures tested, the radial growth was significantly highest and lowest at 28°C and 21°C respectively.

When the interaction of the said 4 media and 3 temperatures on the radial growth performance of the fungus *Gloeosporium leguminum* was studied at different dates of observations, the significant differences were also noted in all dates of observation. At day 8, both 24°C and 28°C temperatures were better than 21°C for radial growth. MEA medium was the best medium at 21°C, 24°C and 28°C followed by OMA, PDA and CDA medium.

Growth characteristics or colony morphology of *Gloeosporium leguminum*

Gloeosporium leguminum from lucky bamboo when grown on four different media with three temperature regimes, the differences in colony morphology were observed in 21°C, 24°C and 28°C

Table 5: Effect of media x temperatures on biomass production (g) of *Gloeosporium leguminum* at 6 days after inoculation.

Growth media	Biomass (g)
21 °C	
Potato dextrose broth	0.310
Czapek's Dox broth	0.242
Oat meal broth	0.204
Malt extract broth	0.246
24 °C	
Potato dextrose broth	0.314
Czapek's Dox broth	0.253
Oat meal broth	0.241
Malt extract broth	0.254
28 °C	
Potato dextrose broth	0.332
Czapek's Dox broth	0.264
Oat meal broth	0.223
Malt extract broth	0.274
Standard error of mean±	**0.032**
Critical difference $_{0.5}$	**0.096**

Table 6: Percent mycelia growth inhibition of *Gloeosporium leguminum* by two fungicides over control.

Fungicides used	Percent growth inhibition over control at							
	0p	10p	25p	50p	100p	200p	400p	800p
Amistar (Azoxystrobin)	0	59.7	69.1	79.2	100	100	100	100
Bavistin 50 WP	0	100	100	100	100	100	100	100
p=ppm								

temperatures and media**.** The tested fungus produced whitish cottony fluffy growth in both CDA and OMA media whereas in PDA media the fungus produced light greyish colour mycelial growth and whitish with brick yellow centred growth in MEA medium at 21°C and 24°C. At 28°C the fungus produced whitish, cottony, fluffy mycelial growth in both CDA and OMA media whereas in PDA and MEA media greyish white mycelial growth without any ring (Fig. 6).

The colour and charcteristics of the colony at the back side, yellowish white cottony mycelial growth was observed in PDA, OMA and MEA media whereas in CDA media whitish thin cottony growth was observed at 21°C whereas, at 24°C and 28°C whitish thick mycelial growth was observed in CDA media whereas in both OMA and MEA media yellowish white thick mycelial growth with circular ring was observed (Akhter *et al*., 2009) and in PDA media blackish white mycelia was appeared (Fig. 6).

Effect of media, temperature and media temperature on biomass production of *Gloeosporium leguminum* irrespective of temperatures

The effect of 4 growth media viz. PDB, CDB, OMB and MEB on the biomass production of *Gloeosporium leguminum* irrespective of temperatures at 6 days (Fig. 7). Out of 4 media tested, the PDB medium was found better than other 3 media for the production of fungal dry biomass after 6 days of inoculation whereas CDB, OMB and MEB media were found at par in dry biomass production performance among themselves (Table 3).

The effect of 3 temperatures *viz.* 21°C, 24°C and 28°C on the biomass production of *Gloeosporium leguminum* irrespective of 4 growth media *viz.* PDB, CDB, OMB and MEB considered was studied and dry fungal biomass was recorded up to 6 days after inoculation (Fig. 8). It was evident that there is no significant effect of temperatures on the dry biomass production of tested fungus *Gloeosporium leguminum* (Table 4).

When the interaction of 4 media viz. PDB, CDB, OMB and MEB and 3 temperatures *viz.* 21°C, 24°C and 28°C on the fungal biomass production of *Gloeosporium leguminum* was studied at 6 days after inoculation, it was found significant (Table 5). PDB at 24°C and 28°C

Fig. 9: Growth of *G. leguminum* on 4 types of broth (PDB, CDB, MEB and OMB).

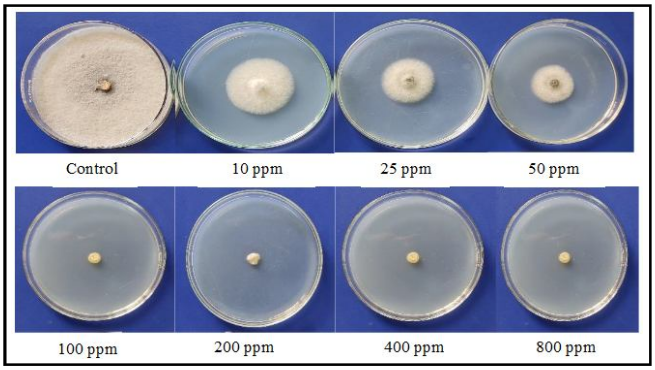

Fig. 10: Percent growth inhibition of *Gloeosporium leguminum* by 7 concentrations of Amistar.

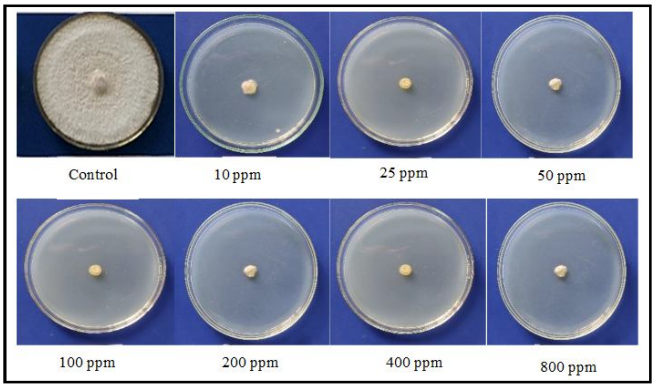

Fig. 11: Percent growth inhibition of *Gloeosporium leguminum* by 7 concentrations of Bavistin 50 WP.

Table 7: Regression and simple correlation of toxicity of *Gloeosporium leguminum* against two fungicides and determination of EC_{50}

Fungicides used	Chemical name	Regression equation (RE)	Simple correlation (r)	EC_{50} (ppm)
Amistar	Azoxystrobin	Y= 0.8665x + 4.32	1.0**	6.03
Bavistin 50 WP	Carbendazim	-	-	-

exhibited the higher value in fungal dry biomass production as compared to other media and temperatures examined (Fig. 9).

Fungicide sensitivity analysis

Amistar (Azoxystrobin) exhibited mycelial growth inhibition of the pathogen of 59.7%, 69.1%, 79.2 at concentrations of 10, 25, 50 ppm respectively, achieving complete inhibition at 100 ppm and above (Fig. 10). Conversely, Bavistin 50 WP (Carbendazim) was proved to be effective against the control in all concentrations (Fig. 11) (Table 6). Bavistin 50 WP proved superior to Amistar against the anthracnose-causing pathogen. The EC_{50} values against the pathogen were determined to be 6.03 ppm for Amistar but EC_{50} values of Carbendazim was not possible to work out due to 100% inhibition of fungus (Table 7).

Conclusions

The pathogen causing serious anthracnose disease on the leaves of lucky bamboo has been identified as *Gloeosporium leguminum* based on spore dimensions and cluster analysis. MEA medium proved to be the most favorable medium for its mycelial growth followed by OMA, PDA and CDA medium. The pathogen exhibited varying radial growth at different temperatures, with 28°C and 21°C promoting the highest and lowest growth respectively and Potato dextrose broth (PDB) was found better than CDB, OMB and MEB media for the production of fungal dry biomass after 6 days of inoculation. Bavistin 50 WP (Carbendazim) demonstrated superior to Amistar in antifungal activity compared where the EC_{50} values of Amistar was 6.03 ppm.

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