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Cultural and morphological characterisation of *Colletotrichum fragrans* of *dracaena* using various nutrient sources along with fungicide sensitivity tests

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Abstract
Dracaena, is an ornamental plant which helps in air purification. The foliage is damaged by innumerable diseases. Various carbon containing media *viz.* Potato dextrose agar, Peptone salt agar, Czepek's Dox Agar, dextrose in Czepek's Dox Agar medium was replaced by the same amount of sucrose and lactose and Oat meal agar was used to study the radial growth of *Colletotrichum fragrans* from *Dracaena*. Among these six carbon media, the most effective medium for its rapid growth was Peptone salt agar medium. Also, the morphological characters differed with change in media constituents, wherein Oat meal agar medium was found to be effective for the production of acervuli, conidia, setae of higher dimensions. Bioassay was conducted using four fungicides *viz.* Blitox, Mancozeb, Chlorothalonil and Difenoconazole with six different concentrations, Difenoconazole with EC50 value 299.2µ/ml gave best results. Also, *in-vivo* studies confirmed that Difenoconazole could inhibit the diseased lesion most effectively than others.

Keywords: *Dracaena, Colletotrichum fragrans*, Czepek's dox agar, peptone salt agar, oat meal agar

Introduction
Commonly known as red-edge *Dracaena* or Madagascar dragon tree, it is an evergreen tree with stiff purplish-red leaves and slim, curving stalks for trunks. It suffers from many fungal diseases but the pathogens producing pycnidia which belongs to order Sphaeropsidales and pathogens producing acervuli which belongs to order Melanconiales take part in causing damage to plant parts hastily fabricating a variety of symptoms on plant. Particular media with definite pH and temperature are utilised for different categories of fungi so that it influences the radial growth, colony morphology and sporulation (Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008) [2, 3]. Thus proper media should be utilised while seeking to recognize a fungus in culture, in view of the fact that mycelial growth and sporulation on artificial media are critical biological features. Carbon source and its concentration plays a major role on the kind of growth of fungi on the media. As a result of which the inclusion of carbon in the culture media needs to be stressed. Biomass production of a fungus either in solid or liquid medium is a vital factor to determine its effectiveness in nutrient uptake from the growth media. So, it can be considered as a fine scale to analyse the appropriateness of a medium for the development of a fungus, which differs among the category of fungi in species/sub-species level. On this ground, keeping record of biomass is crucial. After studying the fungus carefully, it is important to identify the fungicides and its concentrations which are responsible for limiting its growth. Keeping all these points in mind, following objectives have been framed up:

1. Determination of suitable medium/ media containing various carbon source (s) for growth and sporulation of the fungi.
2. Account on morphometric data on various carbon containing media.
3. Determination of *in vitro* sensitivities (EC50) of the fungi towards four selective fungicides.
4. Management of anthracnose disease of *Dracaena fragrance victoriae* under net house condition with four selective fungicides.

Materials and Methods

Location of experiments

The experiments on the management of anthracnose and tip blight disease were performed on Dracaena (*Dracaena fragrans victoriae* L) under the net house of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal. Experimental studies like characterization of cultural and morphological parameters and determinations of fungicide sensitivities of four collected fungi were conducted under laboratory condition of the Department of Plant Pathology of the University.

Types of media used

For studying radial growth, biomass and sporulation the following media were used:

Potato Dextrose Agar (PDA), Peptone salt agar (PSA), Czepek's Dox Agar (CDA), dextrose in Czepek's Dox Agar medium was replaced by the same amount of sucrose (CDASWS) and lactose (CDASWL) and Oat meal agar (OMA), whereas potato dextrose broth (PDB), Czapek's Dox broth (CDB) and peptone salt broth (PSB) were used for studying biomass production.

Fungicide sensitivity analysis

The fungus *Colletotrichum fragrans* was tested using four different fungicides having six different concentrations *in-vitro* as proposed by Shervelle, 1979 [4]. A total of three non-systemic fungicides *viz.* Blitox, Mancozeb and Chlorothalonil and one systemic fungicide Difenoconazole were used as four treatments with three replications in the fungicide bioassay experiment. Degree of inhibition of mycelial growth by each fungicide was calculated by recording the percent reduction in mean mycelial radial growth over that of control (Vincent, 1947) [5]. Effective concentration for 50% growth inhibition (EC50) 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) [1]. Per cent inhibition was measured with the formula, which is given below -

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

Results and Discussion

Studies on cultural characteristics, colony characteristics, microscopic characters, fungicidal analysis *in-vitro* and *in-vivo*

Studies on radial growth of the fungi grown on different carbon sources

Results of the experiment represented in the Table - I revealed that radial growth of *Colletotrichum fragrans*, anthracnose pathogen of *Dracaena fragrans victoriae*, on different growth medium differed significantly upto 192 hrs except the same recorded at 24 hrs. Of incubation. In all the media, the radial growth increased gradually from 24 hrs to 192 hrs but covered the whole plate within 216 hrs. When the radial growth of fungus was examined after 192 hrs of incubation, it was found *at par* in PDA, CDA, OMA media whereas it differed significantly from the other three media.

Table 1: Radial growth of *Colletotrichum fragrans* from *Dracaena fragrans victorae* at 24 hours interval on media with different carbon sources

Growth media	Radial growth (cm) after 24 hrs	Radial growth (cm) after 48 hrs	Radial growth (cm)after 72 hrs	Radial growth (cm) after 96 hrs	Radial growth (cm)after 120 hrs	Radial growth (cm) after 144 hrs	Radial growth (cm) after 168 hrs	Radial growth (cm) after 192 hrs	Radial growth (cm) after 216 hrs
PDA	1.0	2.1	3.2	4.3	5.1	6.5	7.1	8.1	9
CDASWS	1.1	2.3	3.6	4.6	5.6	6.7	7.4	8.3	9
CDASWL	1.2	2.5	3.3	4.6	5.3	6.3	7.6	8.3	9
CDA	1.1	2.4	3.4	4.3	5.1	6.1	7.6	8.1	9
PSA	1.1	3.6	4.0	4.6	5.3	6.2	7.4	8.4	9
OMA	1.1	3.4	3.6	4.5	5.3	6.2	7.1	8.1	9
SEm±	NS	0.06	0.06	0.07	0.08	0.07	0.05	0.06	-
$CD_{0.05}$	-	0.17	0.17	0.21	0.24	0.19	0.16	0.18	-

[1]PDA = Potato dextrose agar, CDASWS=Czepek Dox agar supplemented with sucrose, CDASWL= Czepek Dox agar supplemented with lactose, CDA= Czepek's Dox agar, PSA=peptone salt agar, OMA=oat meal agar

Studies on biomass production of the fungi grown on different carbon sources

The biomass production by the fungi was also studied after 144 hrs of incubation (Table: II). *Colletotrichum fragrans* displayed maximum biomass in PDA medium suceeded by CDASWL and CDA media.

Table 2: Biomass produced by the fungus on media with different carbon sources (after 144 hrs)

Growth media	*Colletotrichum fragrans*
PDA	0.5
CZASWS	0.3
CZASWL	0.4
CZA	0.4
PSA	0.2
SEm±	0.01
$CD_{0.05}$	0.04

[2]PDA = Potato dextrose agar, CDASWS=Czepek Dox agar supplemented with sucrose, CDASWL Czepek Dox agar supplemented with lactose, CDA= Czepek Dox agar, PSA=peptone salt agar, OMA=oat meal agar

Studies on colony morphology of the fungi grown on different carbon sources: The mycelia of *Colletotrichum fragrans* (Plate Ia-1f) was moderately thick to thick, cottony in all the media *viz.* PDA, CDASWS, CDA and CDASWL with clear variation whereas it formed acervuli in the media containing PSA and OMA.

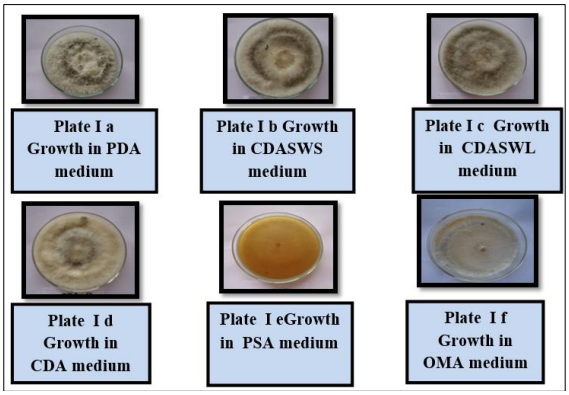

Plate 1: Growth of *Colletotrichum fragrans* in different carbon containing media

Studies on microscopic characters

On the PSA medium, acervuli produced by *Colletotrichum fragrans* (Table - III) was huge in number. The hyphae were septate, hyaline and narrow in width when young but coloured and broader in width when matured. The hyphal dimension varied from 8.2 – 14.6 µ x (av. 9.8µ) and 4.8-8.6µ (av.7.8µ).The acervuli (Plate II a) were 664.8 - 1176.2µ (av. 982.8µ) x 121.3 - 445.6µ (av. 311.6µ) in size. The size and shape of acervuli progressively increased from centre of the Petri-plate to the periphery. Setae were plentiful, dark brown to black, 2 - 3 septate, tapering /pointed, 67.3 – 98.8µ (av. 78.6µ) x 4.8-8.6µ(av. 3.6µ).Conidia (Plate II b,c) were hyaline, single celled, sometimes guttulated, both ends rounded measuring 17.6 - 30.2µ(av. 24.0µ) x 5.2 – 7.3 µ(av. 6.3µ) in size. On the oat meal agar medium, the hyphal dimensions were 12.8 – 32.3µ (av.18.7µ) and 8.3 - 12.6µ (av.9.9µ). The acervuli were 886.5 – 904.7µ (av.865.4µ) and 223.7 -305.5µ (av.225.6µ). Setae were abundant, dark brown to black, 2 - 3 septate, which measured 75.7 – 101.3µ (av.88.6µ) x 23.9 – 45.68µ (avg.32.8). Conidia were hyaline, single celled, smooth sometimes guttulated, both ends rounded measuring 21.8 – 32.3µ(av.28.7µ) x 8.3 - 12.6 (av.9.9µ).

Table 3: Mycelial, acervuli, setae and conidial dimensions of *Colletotrichum fragrans* on two carbon sources

Growth media used	Fungal structures	Length(µ)	Breadth(µ)
PSA	Hyphae	8.2 – 14.6	4.8-8.6
	Acervulus	664.8 - 1176.2	121.3 - 445.6
	Setae	67.3 – 98.8	12.1 - 20.8
	Conidial size	17.6 – 30.2	5.2 – 8.5
OMA	Hyphae	12.8 - 20.5	6.8 - 9.9
	Acervulus	886.5 - 904.7	223.7 - 305.8
	Setae	75.6 - 101.4	23.8 - 45.8
	Conidial size	21.8 – 32.3	8.3 – 12.6

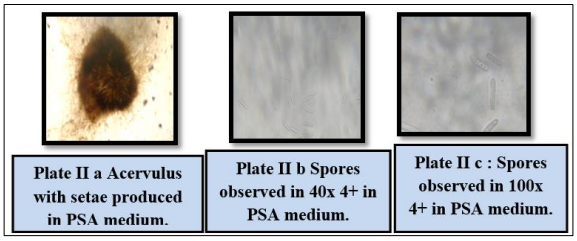

Plate 2: Conidia and acervuli produced by *Colletotrichum fragrans* in PSA medium

Studies on fungicide sensitivity *in-vitro*

The bioassay of 3(three) non-systemic fungicides *viz.* Blitox, Mancozeb and Chlorothalonil and one systemic fungicide Difenoconazole (Plate III a – III d) using six concentrations alongside control were examined. The percent inhibition of all the fungicides over control is shown (Table IV). *Colletotrichum fragrans* showed that difenoconazole with EC50 value 299. 2µ/ml is the most effective fungicide defeating the other fungicides. So, it can be concluded that Difenoconazole was the most suitable fungicide causing 50% growth inhibition of all the fungus studied.

Table 4: Percent inhibition of four fungicides over control of *Colletotrichum fragrans*

Fungicide used	0 ppm	10 ppm	25 ppm	50 ppm	100 ppm	200 ppm
Blitox	0	1.9	4.8	10.0	31.1	41.1
Difenoconazole	0	33.0	41.5	59.2	70.7	87.8
Mancozeb	0	5.5	10.0	28.9	38.1	40.0
Chlorothalonil	0	8.9	17.0	38.5	30.7	49.6

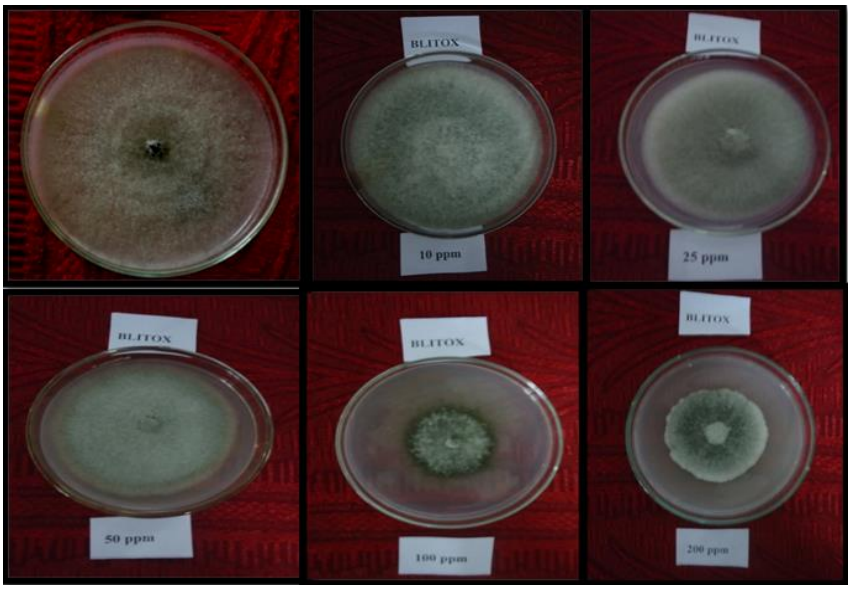

Plate 3(a): Blitox

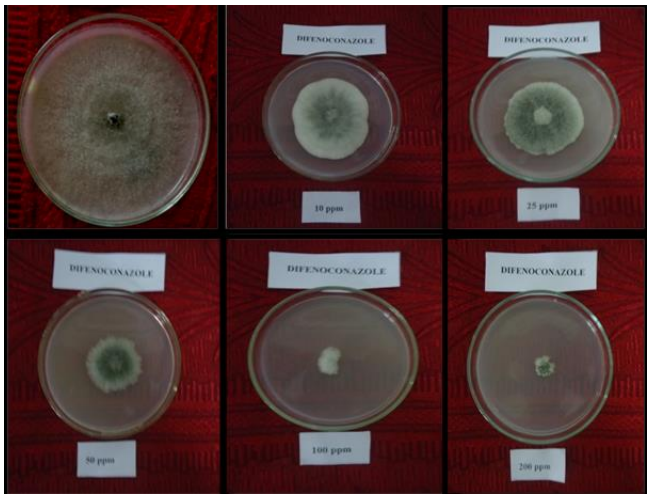

Plate 3(b): Difenoconazole

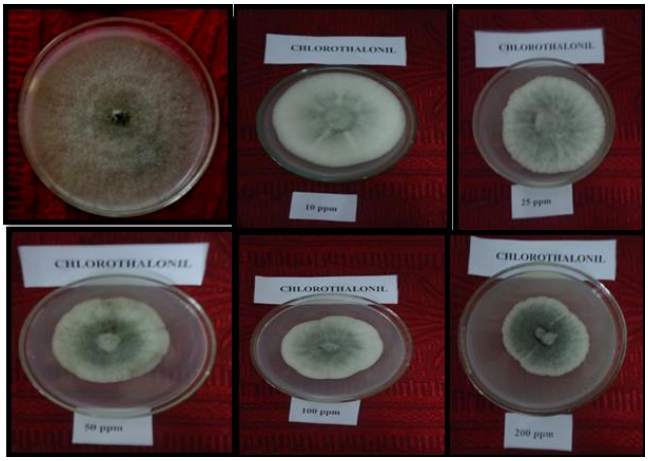

Plate 3(c): Chlorothalonil

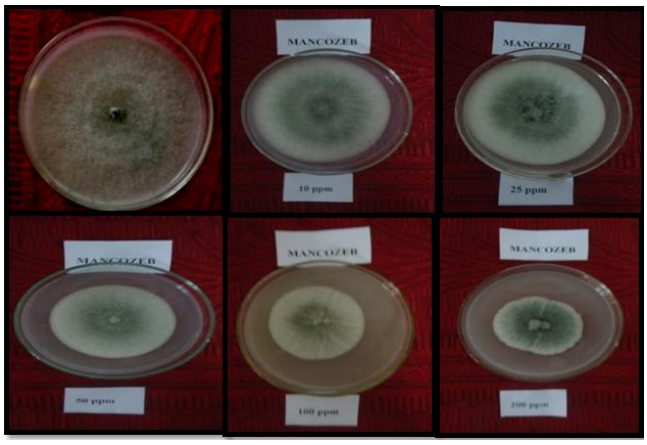

Plate 3(d): Mancozeb

Plate 3: Effect of different fungicides on *Colletotrichum fragrans*

References

1. Horsefall JG. Principles of fungicidal action. Chronica Botanica Co., Altham, Mass, USA 1956.
2. Kuhn DM, Ghonnoum MA. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. Clinical Microbiology Review 2003;16(1):144-172.
3. 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 (*Carica papaya* L). Tropical Agricultural Research and Extension 2008;11:7-12.
4. Shervelle EG. Plant disease control. AVI Publishing Company, Inc. West. Post. Connecticut 1979.
5. Vincent JM. Distribution of fungal hyphae in the presence of certain inhibitors. Nature 1947;159:850.