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Report of Anthracnose Disease of Dumb Cane [*Dieffenbachia daguensis* Engl.] Caused by *Colletotrichum* sp. from West Bengal

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ABSTRACT

Dumb cane [Dieffenbachia daguensis Engl.] is an economically important house plant grown in the garden of Agri-Horticultural Society of India at Kolkata, West Bengal .At the study location, anthracnose disease caused devastating damage of the foliage. Symptoms appeared as large lesions with dark brown smooth to wavy margin and grey centres with fruiting bodies arranged in concentric manner over it. Acervuli produced were black, erumpent, 247.9 – 410.8 µ in size with black, 2 - 3 septate, 97. 0 – 169.5 x 3.0 – 5.0 µ sized setae. Conidia were hyaline, single celled, numerous, cylindrical with both ends rounded, 20.8 – 28.2 x 4.4 – 7.1µ in size. Acervuli productions were huge in the Peptone Salt Agar medium which was used as sporulation media in comparison to other media. On the medium, acervuli were 472.6 – 657.9 x 298.8 – 420.8 µ sized, light brown to black with 154.9 – 230.5 x 4.7 – 6.9 µ sized ,few to numerous, dark brown to black, 2 - 3 septate, unbranched, tapering /pointed tipped setae. Conidia were hyaline, single celled, smooth walled, eguttulate, short cylindrical to rod shaped, sometimes constriction was observed at the centre of conidia, measuring 19.9 – 25.6 x 4.2 – 6.2µ in size. At the time of conidial germination, the central region of the length of conidia became narrowed, formed dumble-shaped, one septate, ends tapered and germinated bipolarly. Pathogenicity test of the isolated fungus had been established under laboratory condition following detached leaf technique. On comparison of the isolated fungus with C. gloeosprioides and Colletotrichum capsici which were known to attack the host, it was found that acervuli and conidial characteristics of causal fungal pathogen of the present study differed from Colletotrichum gloeosporiodes due to the greater dimension and also from Colletotrichum capsici due to dissimilarity in spore shape and size. So, the causal fungus of presently described anthracnose disease of Dieffenbachia daguensis is being proposed as Colletotrichum sp. from West Bengal.

Key words: *anthracnose, Colletotrichum, Dumb cane, Dieffenbachia sp.*

INTRODUCTION

Dieffenbachia, also known commonly as Dumb cane, is a genus of about 55 species of tropical flowering plants in the family Araceae. It is native to the New World Tropics from Mexico and the West Indies south to Argentina. It is a perennial herbaceous plant with straight stem, simple and alternate leaves containing white spots and flecks, making it attractive as indoor foliage.

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Species in this genus are popular as houseplants because of their tolerance of shade. It has been reported from different parts of the world including India that *Dieffenbachia* is approximately attacked by 6 fungal, 4 bacterial and 5 viral diseases (Table-1).Among them anthracnose disease severely infects the foliage and rapidly destroys the whole plant, making the plant less marketable by reducing their aesthetic value.

Literature suggests that the genus *Dieffenbachia* suffers from anthracnose disease and is reported from different parts of the world. The incidence of anthracnose of *Dieffenbachia* caused by *C. gloeosporioides* was reported from India[26]. From the greenhouses of Tehran, the cause of leaf spots on *Dieffenbachia amoena* was identified as *Glomerella cingulate*[16]. *Glomerella cingulata* was reported as the causal agent of anthracnose from *D. picta* [*D. maculata*] in Argentina [9].In the greenhouses, *D. maculata* plant gained popularity, but its commercial production had come under threat from the fungus *C. gloeosporioides* (*Glomerella cingulata*). It was suggested that the appearance of *G. cingulata* on *D. maculata* was related to the introduction of cuttings from Poland and later the cultural and morphological characteristics, life cycle and host range of isolates were studied[12].

The anthracnose disease of *Dieffenbachia picta* was caused by *Colletotrichum capsici* and *Dieffenbachia sequine* Schott var. *variegata* Linn. was attacked by *Colletotrichum gloeosporioides*. Light to medium brown, circular to irregular spots appeared with yellowish haloes and narrow dark brown margins. In many cases adjacent spots coalesced forming large irregular patches. The affected portions became thin and papery[26]. Acervuli were numerous, black, dot-like, epiphyllous, erumpent, setose. Conidia were 1-celled, hyaline, 12 - 15µ. x 3.3 - 5µ. It was reported from Allahabad that the pathogen caused irregular marginal (sometimes also central) leaf spots, smoke-grey with a rim of olive-ochre, the infected areas dropped out in 10-15 days. In culture and on leaves inoculated with conidia, perithecia of *Glomerella cingulata* developed in 15-20 days and inoculation with ascospores produced both perithecia and conidia[13].

Table 1: Disease spectrum of *Dieffenbachia*:

Disease	Causal organism	References
FUNGAL		
Anthracnose	*Colletotrichum gloeosporioides C.capsici*	India[26]; Tehran[16]; Argentina [9]; Poland [12] and Allahabad [13].
Phyllosticta leaf spot	*Phyllosticta dieffenbachiae*	*c. f* [26]
Root and stem rot	*Phytophthora nicotianae var. parasitica Phytophthora palmivora*	UK [19]; India [27] and Argentina and America [22]
Root rot	*Pythium splendens*	[29]
Cephalosporium leaf spot	*C. dieffenbachiae*	*c.f* [26]
Myrothecium leaf spot	*M. roridum*	Taiwan [10] ; [4]
BACTERIAL		
Burkholderia leaf spot	*Burkholderia gladioli*	Argentina [1]
Xanthomonas leaf spot	*Xanthomonas campestris pv. dieffenbachiae*	[5; 6 ; 18]
Pseudomonas leaf spot	*Pseudomonas marginalis pv. marginalis*	Assam [14] and Italy [25]
Soft stem rot and foliar spot	*Erwinia chrysanthemi*	Mexico [17, 8]; Syria [2]; Turkey [3]; Egypt [20]
Viruses	Tomato spotted wilt virus Dasheen mosaic virus Tobamovirus Arabis mosaic virus Konjac mosaic virus	- Poland [11] - Egypt [7] and South Africa [15]. - Brazil [23] - [24] - Andhra Pradesh [21]

MATERIALS AND METHODS

A detailed study on the disease along with its causal agent has been conducted during present investigation. The diseased leaf sample of *Dieffenbachia daguensis* Engl. grown inside and outside of the greenhouse of Agri-Horticultural society of India, No.1, Alipore Road, Kolkata, West Bengal (located at 22˚53'N latitude and 88°33' E longitude) were collected in brown paper packets and detailed *in situ* description of symptoms and necessary field photography of the diseased plant or its parts were taken. The severity of the foliage damage caused was assessed using the 0 - 6 scale (Table II). The percent damage caused was recorded by visual observation and scoring the plants in the greenhouse.

Table 2: Descriptions of 0 – 6 disease scoring scale with respective reaction categories

Scale	Description	Reaction categories
0	No infection or 0% infection	Immune
1	1-5% leaf area /length covered by disease	Highly resistant
2	6-10% leaf area /length covered by disease	Resistant
3	11-25% leaf area /length covered by disease	Moderately resistant
4	26-50% leaf area /length covered by disease	Moderately susceptible
5	51-75% leaf area /length covered by disease	Susceptible
6	76-100% leaf area /length covered by disease	Highly susceptible

Samples kept in brown paper packets were brought to the laboratory and examined for the presence of asexual fruit bodies, acervuli. Experimental studies like isolation, purification culture, micro-photography, identification, pathogenicity testing of the isolated pathogens *etc*. were conducted following standard protocol under laboratory condition of the University, B.C.K.V. The purification of the isolated pathogen was carried out on PDA (Potato Dextrose Agar) medium but the fungus failed to produce acervuli on the medium. Thus after further studies using different media combinations it was identified that PSA (Peptone Salt Agar medium) was the ideal medium for acervuli production and sporulation of the isolated pathogen. Series of slides were prepared from culture or infected parts for morpho-metric studies of fungal spores, spore bearing and other structures. Micro-photograph of all fungal structures were taken with help of Compound microscope or Karl Zeis Phase Contrast Microscope (under 10x, 20x, 40x &100 x) and by using Canon Powers Shot A640 camera. Dimensions (*e.g.* length and breadth) of conidia, acervuli and hyphae of fungi were measured using AxioVision (Rel. 4.8) software. For pathogenicity establishment detached healthy leaves after proper cleaning with sterile distilled water and absolute alcohol, were pin pricked and artificially inoculated with fungal mat while pin pricked uninoculated (only agar bit) leaves were used as control. These were covered with transparent polythene packets for 48 hours and observed regularly till symptom development. The pathogen was re-isolated from the inoculated diseased parts of leaf and compared with the fungal culture isolated initially from diseased leaf.

RESULTS AND DISCUSSION

Anthracnose is a common disease on *Dieffenbachia* and the extent of damage caused is very high. At the study location, the disease severity and sporulation of the pathogen basically starts from May and continues up to November to end of January. Affected leaf samples were collected from the

garden house during 2nd week of January, 2015 and last week of May, 2016. The disease severity was 75% based on 0 – 6 scale.

Symptoms of the anthracnose disease of *Dieffenbachia daguensis*

Infection began on the lower leaves as small, brown, circular to oval necrotic spots on the leaf lamina. Spots were generally surrounded by yellow halo. They gradually increased in size producing large lesions with dark brown smooth to wavy margin and grey centres. Spots appeared from margin or could start from any part of the leaf blade. Diseased tissues were torn off easily. Black dot- like, circular, acervuli were arranged in concentric fashion (sometimes scattered) on central greyish portion of the spots.

Pathogenicity establishment:

Pathogenicity was established by inoculating detached leaf under laboratory condition**.** The inoculated leaf produced same symptoms as produced under field condition. The pathogen was re-isolated from the inoculated diseased parts of leaf and compared with the fungal culture isolated initially from diseased leaf.

Table 3: Cultural characteristics of the fungus observed on various growth media

S.No.	Medium	Cultural characteristics
1.	PDA + Peptone	Huge productions of acervuli with huge number of spores with orange yellow coloration on the disc was observed whereas the mycelia developed from the disc on to the medium were fluffy greyish white producing initially hyaline, slightly convex, water droplets, turning light yellowish to light brown/ black acervuli on mycelia touching with the medium .
2.	PDA medium	More cottony mycelial growth was observed than the PDA+ Peptone medium. Formation of acervuli and sporulation was not observed on PDA medium other than at the point of inoculation. The aerial hyphae were hyaline to pale white whereas those at the bottom of the plate were blackish in color.
3.	Peptone Salt Agar medium	Huge productions of acervuli from the point of inoculation towards the periphery of the mycelial growth. The hyphae were hyaline. The acervuli were initially hyaline later turned to orange - yellow and a few gradually turned to black.
4.	NaCl and $CaCl_2$ agar medium	Very thin mycelial growth was observed which remained appressed to the medium. Acervuli were mainly confined to region near to the disc and sparsely observed beyond that region. Acervuli produced on the inoculated disc had huge sporulation and less prominent setae whereas those produced at the junction of PDA disc and medium produced less sporulation but with very prominent setae.

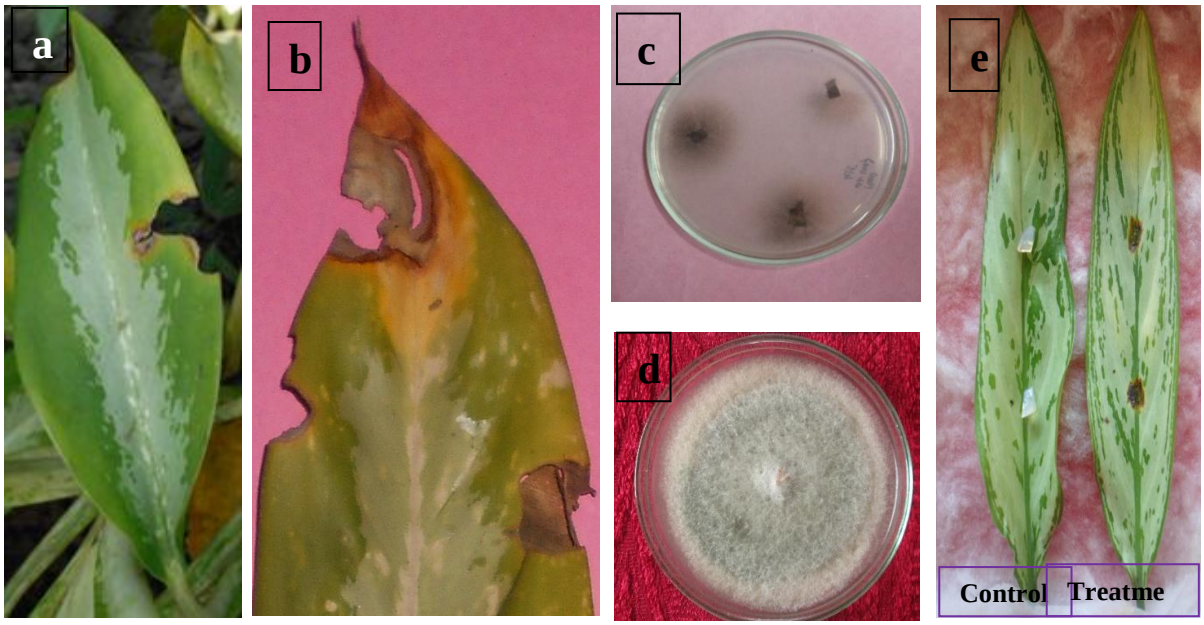

Plate 1a, b: Anthracnose symptom on leaves of *Dieffenbachia daguensis*

Plate 1c, d, e: Isolation, Purification and Pathogenicity establishment of *Colletotrichum* sp. of *Dieffenbachia* under laboratory condition.

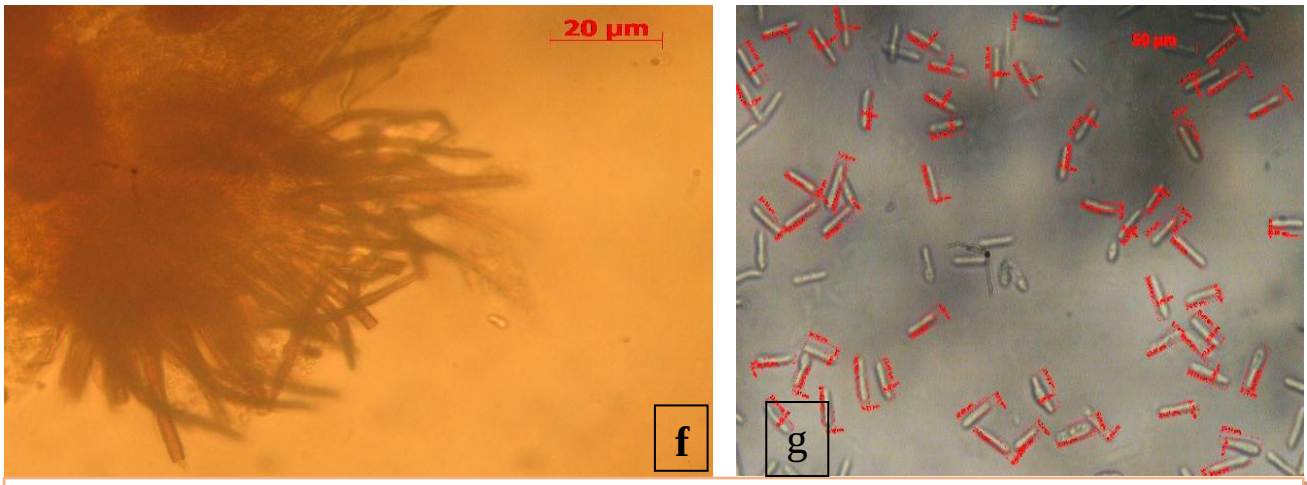

Plate 1f, g: Microscopic view of avervulus bearing setae and conidia produced on *Dieffenbachia*

Plate 2 a: Cultural characteristics of *Colletotrichum* sp. of *Dieffenbachia* on different media.

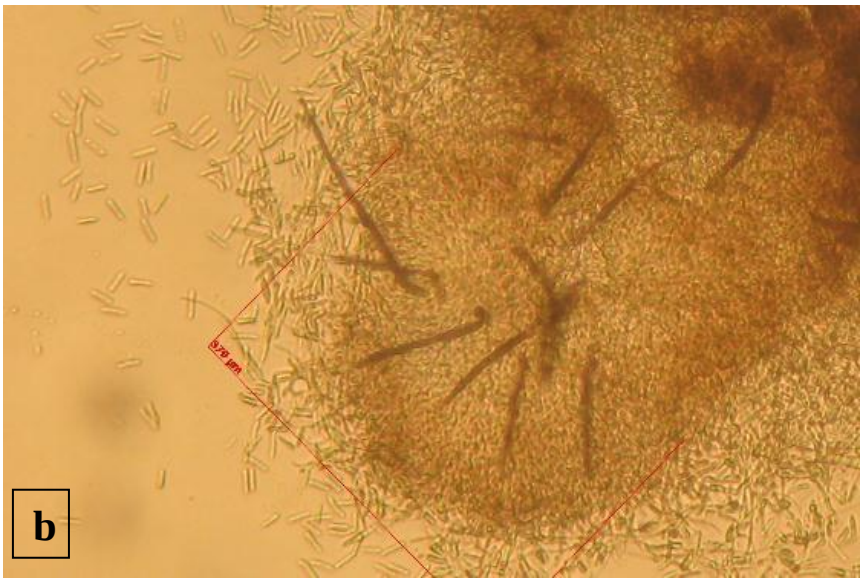

Plate 2 b: Microscopic view of avervulus bearing setae and conidia produced on PSA medium

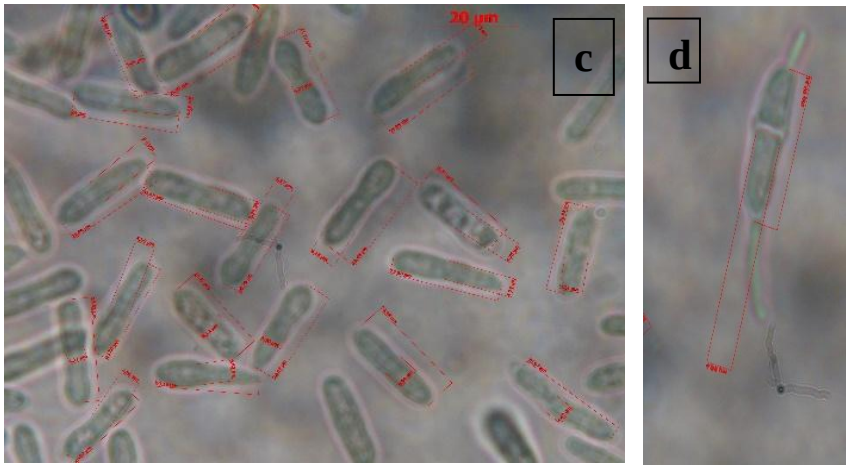

Plate 2 c, d: Microscopic view indicating conidial morphology and conidial germination(2 d)

Morpho-metrical descriptions of various structures of the pathogen obtained from Dumb cane and on PSM:

On the host, acervuli produced were black, numerous, arranged in concentric rings or scattered, erumpent, 247.9 – 410.8 µ (av. 334.8). Setae were numerous, black, 97.0 – 169.5 (av. 133.5) x 3.0 – 5.0 µ (av. 4.0), 2 - 3 septate, unbranched and tapered towards the tip. Conidia were hyaline, single celled, numerous, cylindrical with both ends rounded, 20.8 – 28.2 (av. 23.9) x 4.4 – 7.1µ (av. 5.9) in size.

Acervuli productions were huge in the peptone salt agar medium which was used as sporulation media in comparison to other media. The hyphae were hyaline, thin and septate. The hyphal width varied from 10.5 – 14.6 (av. 12.7) µ. The acervuli were 472.6 – 657.9 (av. 580.4) x 298.8 – 420.8 (av. 359) µ in size. The size of the acervuli gradually increased from centre of the Petri-plate to the periphery. Acervuli were light brown to black and dot like. Setae were a few to numerous, dark brown to black, 2 - 3 septate, unbranched, tapering /pointed, 154.9 – 230.5 (av. 192.9) x 4.7 – 6.9 (av. 5.8) µ. Conidia were hyaline, single celled, smooth walled, eguttulate, short cylindrical to rod shaped, sometimes constriction might be present at the centre of conidia, measuring 19.9 – 25.6 (av. 22.7) x 4.2 – 6.2 (av. 5.0)µ. At the time of conidial germination, the central region of the length of conidia became narrowed, formed dumble-shaped, one septate, ends tapered and germinated bipolarly.

DISCUSSION

Two species of *Colletotrichum, C. gloeosporioides* and *Colletotrichum capsici* were reported from *Dieffenbachia* sp. Acervuli of *Colletotrichum gloeosporioides* on *Dieffenbachia sequine* Schott var. *variegata* Linn were numerous, black, dot-like, epiphyllous, erumpent and setose. [26]Conidia were 1-celled, hyaline, 12 - 15µ. x 3.3 - 5µ . Colonies of *C. capsici* were recorded as dense whitish to dark grey aerial mycelium, reverse dark brown, conidial masses pale buff to salmon. Sclerotia were absent. Setae were abundant. Conidia were falcate, fusiform, apices acute, 18 – 23 x 3.5 – 4µ. Appressoria were abundant, medium brown, clavate to circular, edge usually entire, 9 -14 x 6.5 – 11.5 µ, often becoming complex and forming long closely branched chains[28].

When the acervuli and conidial characteristics of causal fungal pathogen of the present study were compared with both the above mentioned anthracnose causing fungal spp. it differed from *Colletotrichum gloeosporiodes* due to the greater dimension and also from *Colletotrichum capsici* due to dissimilarity in spore shape and size .Our anthracnose pathogen showed similarity in dimension with the *Colletotrichum trichellum* (14 -24 x 4 – 6 µ) but differed in shape which was falcate in shape. So, the causal fungus of presently described anthracnose disease of *Dieffenbachia daguensis* is being proposed as *Colletotrichum* sp. from West Bengal.

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