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J. Mycopathol. Res. 61(4) : *503-517, 2023;*
ISSN 0971-3719

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J.Mycopathol.Res. 61(4) : 503-517, 2023, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.61.4.2023.503

Morphology- based Phenetic relationships among the pathogenic species of *Pestalotiopsis* associated with Mangroves of Indian Sundarbans

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Received : 22.08.2023 Accepted : 12.10.2023 Published : 25.12.2023

The Sundarbans are world's largest coastal mangrove ecosystem which houses several ethno medicinally important plants with diverse genetic resources. This diversity among the mangrove vegetation is under constant threat due to target of various fungal attacks. To this end, an effort was made to isolate, identify and understand the nature of relationships among one of the most notorious foliar pathogens of the mangroves- the pestalotioids. Eleven species of the genus *Pestalotiopsis* (Coelomycetes) were isolated from different mangrove plants of Indian Sundarbans. Among them four species (*P. disseminata, P. guepini, P. mangiferae, P. palmarum*) were recorded for the first time in this present investigation. Tests for pathogenicity confirms their parasitic relationship with their respective host plants. Multiple morphological characters of the fungal isolates were taken into account to understand the relationship among these *Pestalotiopsis* species. The phenetic analysis showed ample variation among the species. UPGMA based analysis suggested that *P. agallochae* and *P. heritierae* are closely related and *P. versicolor* is distantly related to all the studied pathogens. This study for the first time suggests that multiple morphological characters and virulence based approach can be helpful to characterize morphologically similar groups of fungal pathogens.

Keywords: Mangrove, *Pestalotiopsis*, Phenetic analysis, UPGMA

INTRODUCTION

Mangroves have long been a source of astonishment for the laymen and of interest for scientists. Mangrove plants are specialized woody plants growing in the swamps of tidal – coastal areas and river delta of tropical and subtropical parts of the world. The Mangrove Forest of Indian Sundarbans has a long and rich glory as eco-region and treasure house of genetic resources (Shara *et al.* 2023) throughout the historic past.

Mangroves provide innumerable direct and indirect benefits to human beings. The unique ecology, morphological characteristics, and traditional uses of mangrove plants have drawn the attention of researches over the years.Herbal drugs play an important role in health care programs especially in developing countries. Rural poor and marginalized people of Mangrove Forests of Sundarbans depend on mangrove plants for primary health problems. They harbour a wide array of novel natural products (Cadamuro *et al.* 2023; Cruz *et al.* 2023;) which are potential sources of future drugs against ancient and emergent diseases. It has been discovered that almost all plant species investigated by various researchers harbor one or more endophytes. They benefit their host by producing various secondary metabolites that can be employed in agriculture and medicine. (Younis *et al.* 2022) This treasure house of mangrove is now the target of various fungal attack which may create conservation problem in future and may affect the quality of drug. During the survey of foliicolous fungi of mangrove plants of Indian Sundarbans a number of *Pest altiopsis* Steyaert species were isolated from different mangrove hosts in different localities of Sundarbans (Pal, 2012; 2014; 2017; 2018). Species of *Pestaltiopsis* occur commonly as plant pathogens, and represent a fungal group known to produce a wide range of chemically novel, diverse metabolites. (Maharachchi-kumbura *et al.* 2014). *Pestalotiopsis* species are known to produce Taxol, an anti-cancer agent

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(Shukla *et al.* 2014; Lateef *et al.* 2018). Two new phthalide derivatives and four known phthalide compounds were purified from the culture of a mangrove endophytic fungus *Pestalotiopsis* sp. SAS4. Their chemical structures were established by analyses of 1D and 2D nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HR-MS) spectroscopic data. All of these compounds were evaluated *in vitro* for antibacterial, cytotoxicity, and resistance to hypoxic–ischemic injury activities. (Peng *et al.* 2022).

The conidial morphology is an important character in the taxonomic study of fungi. This anamorphic and endophytic genus (Maharachchikumbura *et al.* 2011; Kathiresan and Bingham, 2001; Cannon and Simmons, 2002; Kumar and Hyde, 2004; Liu *et al.* 2007; Kamil *et al.,* 2012; Dele Cruz *et al.,* 2023) is responsible for leaf spot diseases of mangrove plants. *Pestalotiopsis* is a complex genus and consists of members difficult to classify at the species level (Kamil *et al.* 2012). At present, inter-specific delineation of this genus is based on morphology of the conidia (Maharachchi-kumbura *et al.* 2014), conidiogenesis (Maharach-chikumbura *et al.* 2014) and teleomorph association as well as the size of appendages and their number. *Pestalotiopsis* Steyaert was introduced to accommodate pestaloid species with 5 celled conidia (Senanayake *et al.* 2020). Maharachchikumbura *et al.* (2014) re-examined *Pestalotiopsis* at the morphological and molecular levels and introduced two new genera, *Neopestalotiopsis* and *Pseudopestalotiopsis*. Currently, these three genera placed in Sporocadaceae (Amphisphaeriales) (Wijayaw-ardene *et al.* 2018, Hyde *et al.* 2020). *Neopestalotiopsis* typified by *N. protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, is morphologically distinguished from other pestaloid genera by its varicolored median cells and indistinct conidiophores which are often reduced to conidiogenous cells.

Pestalotiopsis typified by *P. guepinii* (Desm.) Steyaert is easily distinguished from other pestaloid genera as its conidia have concolourous median cells (Maharachchikumbura *et al.* 2014). Species in both *Pestalotiopsis* and *Neopestalotiopsis* commonly occur as endophytes in leaves (Hu *et al.* 2007, Liu *et al.* 2010, Maharachchikumbura *et al.* 2012, Debbab *et al.* 2013, Chen *et al*. 2018, Norphanphoun *et al.* 2019), saprobes on dead leaves (Ariyawansa and Hyde, 2018, Tsai *et al*. 2018), bark and twigs (Senanayake *et al.* 2020) or human and animal pathogens (Monden *et al.* 2013). Some species found from soil, fabrics, wools and some are in the extreme environments (Senanayake *et al.,* 2020; Tejesvi *et al.,* 2007 to 2009). Some *Pestalotiopsis* species can degrade plastics (Russell *et al.* 2011). Pestaloid endophytes produce chemical compounds, which are used in therapeutic applications and agriculture (Aly *et al.* 2010; Xu *et al.* 2010, 2014). Therefore, investigation of novel pestaloid taxa and their chemical properties are of importance. In this investigation their conidial morphology was studied under light microscope with a view to determine the specific identifying character. The views of modern taxonomists still differ regarding the basic criteria used in delimiting the genera *Pestalotia* / *Monochaetia* / *Sematosporium* (Jeewon *et al.* 2002). Hence, it was considered worthwhile to study the conidial morphology of 11 species of the genus *Pestalotiopsis* and their pathogenicity tests on their respective host species and phenetic analysis on different species of *Pestalotiopsis*, which is expected to provide interesting biological information with those working in the field of fungal taxonomy.

MATERIALS AND METHODS

Isolation of fungi from infected plant parts

Fungi were isolated from infected plant parts following the method of Pal (2020). Briefly, both healthy and infected leaves were collected at random from living trees, shrubs and herbs of Indian Sundarbans forest (Bhagbatpur, Kultali, Bak-khali, Dhonchi, Chulkathi, Luthian Island, Chandanpiri) in different seasons placed in polythene bags and brought to the laboratory for examination. The organisms were isolated from infected leaves following the standard method (0.1% $HgCl_2$ and sterile distilled water), identified and maintained in Potato-dextrose-agar medium

at 25 ± 1°C. Dried infected plants were finally mounted on herbarium sheets.

Microscopic Examination

For microscopic examination of infected leaf materials, both dissecting and compound microscopes were used to study the vegetative and reproductive structures of fungi. A small portion of the sporing tissue was first placed under a dissecting microscope, mounted in lactophenol, teased carefully, covered with a cover glass and observed under a microscope. Vertical sections (V.S.) of pycnidia or acervulus or uredinium were also examined under compound microscope. In most cases, cotton blue lactophenol (0.5g cotton blue in 100 ml lactophenol) was used for staining purpose. The slides were finally sealed with nail varnish, labelled and stored for further examination, if necessary.

Microscopic measurements of reproductive structures were made with an ocular and a stage micrometer. For spore measurements, a number of isolated spores were chosen for micrometric measurements. Camera lucida drawings were also made under necessary magnifications depicting almost all the details.

Identification of fungi

The fungi were identified with the help of different monographs, books, authentic papers and available stock cultures in the Department of Botany, University of Calcutta. The identity of each fungus was confirmed by the experts of the International Mycological Institute (IMI), Kew, Surrey, England.

Maintenance of stock cultures

Fungal cultures were finally stored under three different conditions. Two sets of cultures were maintained at 5^0C and 20^0C respectively. The third set of culture was preserved in sterilized liquid paraffin and kept at 25^0C. Subculture was accomplished at a regular interval of time. To avoid mite infestation in culture the technique described by Smith (1967) was followed. Sometime fungal cultures were also fumigated with 'Pyridine' for 24 hr. for killing mites. The above techniques were followed as a precautionary measure as and when necessary.

Pathogenecity test

Pathogenicity test was performed following Pal (2018).For pathogenicity tests, 10 healthy detached leaves of each host species were thoroughly washed with sterile distilled water, allowed to dry, placed on sterilized moist filter papers in disinfected plastic trays and inoculated with drops (0.02 ml/drop) of spore suspension of test fungus. The trays were covered with glass plates and sealed with petroleum jelly to prevent evaporation of water during incubation. Control set was maintained in each case with drops of sterile distilled water instead of spore suspension. Inoculated leaves were incubated for 72 hrs at room temperature (30-32°C) and in diffused light. The percentage of lesion production was calculated on the basis of total number of inoculum drops placed on leaves of test species. The organisms were reisolated from leaves and compared with stock cultures.

Assessment of mycelial growth on solid medium

For the preparation of inocula, desired fungus was grown in a Petri dish (100 mm diam.) containing 0.5% Dextrose Agar medium. Usually a block (4 mm diam.) of agar containing mycelia was cut out with the help of a sterilized cork borer from the advancing zone of the mycelial mat (4-day-old culture) and transferred to PDA medium in a Petri dish (100 mm diam.), incubated at 26-28^0 C and under diffused light. The growth characteristics were noted after 5 and 30 days of inoculation. Colour chart was used for description of colour of mycelial matsas described by Rayner (1970).

Construction of Phenetic Tree

To analyze the morphological data, the NTSYS-pc version 2.2 statistical package (Rohlf, 2009) was used. The statistical method took into account the presence or absence of each character state as differential features. The binary qualitative data matrix was then used to construct

similarity matrix using the Jaccard similarity coefficient (Jaccard, 1908). The similarity matrix was then used to construct dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA) and Sequential Agglomerative Hierarical Nested (SAHN) cluster analysis. Cophenetic matrix was derived from the dendrogram using the COPH (cophenetic values) program, and the goodness-of-fit of the clustering of the data matrices were calculated by comparing the original similarity matrix with the cophenetic value matrix using the Mantel matrix correspondence test (Mantel, 1967) in the MXCOMP program. Principal co-ordinate analysis (PCOORDA) was performed based on the similarity coefficient using DCENTER module to transform the symmetric similarity matrix to scalar product form and then EIGEN module was used to extract eigenvectors resulting into a three-dimensional plot showing the taxa in a three-dimensional space. This is a multivariate approach which is very informative regarding distances among major groups (Hauser and Crovello, 1982). This can complement the cluster analysis and identify patterns of association among taxa in a three-dimensional space.

RESULTS AND DISCUSSION

In course of this study, 11 species of *Pestalotiopsis* were isolated from infected leaves of 11 hosts of mangrove plant species of Sundarbans from different areas (Table1). Among them 4 species (*P. disseminata, P. guepini, P. mangiferae, P. palmarum*) were recorded for the first time on mangroves – *Aegiceras corniculatum, Xylocarpus granatum, Derris scandens, Phoenix paludosa* respectively of Indian Sundarbans. Photographs of infected leaves of *Excoecariaagallocha*, *Sonneratia apetala*, *Aegicerascorniculatum, Bruguiera gymnorrhiza, Sonneratia caseolaris, Ceriops decandra, Heritiera fomes,* and *Bruguiera cylindrica* are shown in Fig. 1. Hand drawings of some of the *Pestalotiopsis* species and infected leaves of their respective host plants are shown in Figs. 2-4. Microphotographs of conidia of some *Pestalotiopsis*species are shown in Fig. 5.

The spectrum of fungal diversity in Sundarbans appears to be very wide and hence frequent exploration is needed. It is not unreasonable to assume that diversity in parasitic fungi is related to parasitism since increase in diversity leads to enhanced parasitism. It is of common occurrence in the field that a fungal parasite having different strains may attack a wide range of host species. Genetic diversity in all types of organisms is increasing with time due to natural and/or unnatural causes. Besides, evolutionary changes also take place in both hosts and parasites which is probably necessary for balancing the changes in resistance of the host and virulence of the pathogen.

Pestalotiopsis is a species-rich asexual genus with appendage bearing conidia in the Amphisphaeriacae (Maharachchikumbura *et al.* 2014; Lee *et al.* 2006), and is widely distributed throughout tropical and temperate regions. *Pestalotiopsis* species are common phytopathogens that cause a variety of diseases, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and various post-harvest diseases. (Maharachchikumbura *et al.* 2012; Zhang *et al.* 2013).

The data in Table 2 makes a comparison of different *Pestalotiopsis* species based on their morphological characteristics. Highest diameter of mycelial mat was observed in *P. mangiferae*. Majority of the species showed felty texture. The species of *Pestalotiopsis* are usually limited on the basis of form, size and colour of the conidia, and the number and length of the apical appendages. Their cultural characteristics are remarkably homogeneous, but problem arises in the identification of cultures because their conidia mature slowly (Maharachchikumbura *et al.* 2011). Besides, spore morphology varies significantly on different media (Maharachchikumbura *et al.* 2011). However, 11 species of *Pestalotiopsis* reported in this paper will add new information about morphology, cultural characteristics and their host relationships. *P. disseminata, P. guepini, P. mangiferae and P. palmarum* were isolated for the first time from the infected leaves of *A. corniculatum, X. granatum, D. scandens and P. paludosa. P. bruguierae* appears to be very close to *P. mangiferae*, whereas *P. moluccens* is close to *P. disseminata*. Both the species (*P. bruguierae*

Table 1: Different species of *Pestalotiopsis (*Fungal Class: Coelomycetes) isolated from infected leaves of different plant species from Sundarbans

Fungus	Plant Species (Host)	Vernacular Name of the Plant	Plant Family	Characteristics of Leaf Spots	Place of Collection	Fungal Holotype	Fungal Isotype
Pestalotiopsis a gallochae Pal & Purkayantha	*Excoecaria agallocha* L.	Gewa	Euphorbiaceae	Leaf spots amphigenous, distinct, small to medium, circular to irregular, sometimes coalescing, grayish centre with a dark brown border, rarely with a faint yellowish halo around the spots at the margin, discrete, sometimes concentrated towards the margin, frequency high, present in both young and mature leaves, 1 –5 mm diam. (Fig. 1 A).	Jammu Island, 24 PGS(S), W.B., India.	CUPH 6B	IMI 348454
Pestalotiopsis A petalae Purkayastha et Pal	*Sonneratia apetala* Buch.-Ham.	Tak-keora	Sonneratiaceae	Leaf spots amphigenous, distinct, small to large circular to irregular, usually delimited by a slightly raised dark brown border and silvery grey to yellowish brown centre, usually coalescing forming large patches; immature spots with faint yellowish halo; mature spots with numerous, blackish acervuli usually on upper surface, sometimes on both surfaces, often replaced by shot holes, discrete, occasionally concentrated towards the ma rgin and at the apical region of the lamina, frequency moderate, 0.5 -30 mm wide. (Fig. 1 D).	Kultali, 24 PGS(S), W.B., India.	CUPH 235A	IMI 352586
Pestalotiopsis brug uierae Purkayantha et Pal	*Bruguiera gymnorr hiza* (L.) Lamk.	Kankra	Rhizophoraceae	Leaf spots epiphyllous, distinct, very small, sometimes coalescing forming large spots, more or less spherical, brown to grey with dark brown border, margin regular, discrete, 1.5 - 6.0 mm wide, frequency moderate	Bak-khali, 24-Parganas, West Bengal, India	CUPH 230 (Holotype),	IMI 352580 (Isotype).
Pestalotiopsis case olaris Purkayastha & Pal	*Sonneratia caseola ris* (L.) Engl.	Chak-kewra	Sonneratiaceae	Leaf spots amphigenous, distinct, large, regular to irregular, usually sharply delimited by a dark brown border, centre usually grey to brown with yellowish halo, frequency low, more on older leaves, 1 -8 mm long and 1 -5 mm wide.	Dhonchi, 24 -Parganas, West Bengal, India	CUPH 333 D (Holotype)	IMI 356251 (Isotype)
Pestalotiopsis disse minata (Thüm) Stey	*Aegiceras cornicul atum* (L.) Blanco	Khalsi	Myrsinaceae	Leaf spots amphigenous, distinct, small, coalescing forming large spots or patches, circular to irregular, dark tan, numerous blackish acervuli on slightly depressed necrotic areas, discrete, sometimes on mid-veins, often concentrated towards the apical region of the lamina, frequency high, more on older leaves, 1.5 -12.0 mm wide	Chulkati, 24-Parganas, West Bengal, India ,	CUPH 225 (Holotype)	IMI 352585 (Isotype).
Pestalotiopsis guepini (Desmazieres) Stey.	*Xylocarpus granatum* Koen.	Dhundhul	Meliaceae	Leaf spots amphigenous, distinct, small, sometimes coalescing, regular to irregular, grey to brown, with numerous black acervuli on necrotic areas, frequency low, more on older leaves, 1-15 mm wide.	Dhonchi, 24 -Parganas, West Bengal, India ,.	CUPH 495 (Holotype)	

(contd. part table 1)

Pestalotiopsis Heritierae Purkayastha et Pal	*Heritiera fomes Buch.-Ham.*	Sundari	Sterculiaceae	Leaf spots amphigenous, small spots with yellowish halo, sometimes large patches at the margin or at the apex, brown to grey with a dark brown border, 1-6 mm wide or more, discrete, frequency low.	Bak-khali, 24-Parganas, West Bengal, India.	CUPH 149 A (Holotype)	IMI 353656 (Isotype)
Pestalotiopsis mangiferae (P. Henn .) Stey.	*Derris scandens Benth.*	Noyalata	Fabaceae	Leaf spots amphigenous, distinct, irregular, small, sometimes coalescing, usually sharply delimited by a dark, raised border, silvery grey to brown, frequency moderate, more on older leaves, 2 -40 mm wide.	Luthian Island, 24-Parganas, West Bengal, India ,	CUPH 159 (Holotype)	IMI 351338 (Isotype)
Pestalotiopsis moluccens Purkaysntha et Pal	*Xylocarpus moluccensis (L.) Roem.*	Pasur	Meliaceae	Leaf spots epiphyllous, distinct, small, more or less rounded, margin regular, greyish white centre with a dark brown border, numerous acervuli on necrotic areas, discrete, 2.5 - 5.0 mm wide, frequency low	Bak-khali, 24-Parganas, West Bengal, India	CUPH 229 (Holotype)	IMI 352578 (Isotype).
Pestalotiopsis palmarum (Cooke) Stey.	*Phoenix paludosa Roxb.*	Hental	Arecaceae	Leaf spots amphigenous, distinct, small, yellow-brown or whitish to grey with a dark brown border, oval to oblong, sometimes parallel to mid-vein, 1-12 mm long, coalescing, acervuli formed on lesions.	Bak-khali, 24-Parganas, West Bengal, India	CUPH 10 A (Holotype)	IMI 351333 (Isotype).
Pestalotiopsis versicolor (Speg.) Stey	*Ceriops decandra (Griff.) Dig Hou*	Garan	Rhizophoraceae	Leaf spots amphigenous, distinct, small to large, circular to irregular, sometimes coalescing forming large patches, reddish, dark brown to black, discrete, frequency moderate, more on older leaves, 0.5 – 30 mm wide	Chandanpiri, South 24 - Parganas, West Bengal, India ,	CUPH 400 (Holotype)	IMI 359184 (Isotype).

Table 2 : Comparison of characters of eleven species of *Pestalotiopsis*

Characters	Acervuli Shape size and peculiarity, if any	Condiophores	Conidia		Mycelia mat		
		Shape and peculiarity, if any/ Size range	Shape and peculiarity, if any/	Size (range)	Diameter (in 5 days)	Texture	Colour
P. agallochae	250–300 µm diam., globose to lenticular or ellipsoidal, dark brown to black, sub-epidermal; composed of thin -walled, hyaline, faintly brown, isodiametric, angular to irregular, pseudoparenchymatous cells. (Fig. 2A)	Cylindrical to lageniform 20.4 – 27.2 X 3.4 – 6.8 µm	Fusiform, Straight or slightly curved, apical cell with 2 – 3, apical setulae (rarely 1) (Fig. 5 A -D)	20.4 – 27.2 X 3.4– 6.8 µm (Fig. 2 C).	65.66 mm	Felty	White to rosy buff
P. apetalae	epiphyllous, rarely amphigenous , scattered, erumpent, ellipsoidal to globose, 250 -300 µm diam., released black spore masses at maturity through ruptured epideris, composed of thin - walled, hyaline to faintly brown, isodiametric, angular to irregular pseudoparenchymatic cells. (Fig. 2D)	Cylindrical to obovoid 15.6 – 21.4 X 3.9 – 5.8 µm (Fig. 2 F).	Fusiform, Straight or slightly curved, apical cell with 3 (occasionally 2) apical setulae	15.6 – 21.4 X 3.9 – 5.8 µm (Fig. 2 F).	58.66 mm	Felty to Subfelty	White
P. bruguierae	globose to ellipsoidal, black, upto 300 µm diam., released black spore masses at maturity. (Fig. 2G)	Cylindrical 15.6 – 29.0 X 5.6 – 7.8 µm (Fig. 2 I).	Fusiform, Straight or rarely curved, apical cell with 2 – 3 apical setulae	15.6 – 29.0 X 5.6 – 7.8 µm (Fig. 2 I).	55.0 mm	Felty	White
P. caseolaris	acervuli epiphyllous, rarely amphigenous, subepidermal, numerous, solitary to gregarious, globose, lenticular or ellipsoidal, 250 -400 µm diam., black, ruptured with maturity, releasing spore masses. (Fig. 2J)	Cylindrical to obpyriform 10.2 – 22.1 X 3.4 – 6.8 µm (Fig. 2 L).	Fusiform, Straight or rarely curved, apical cell with 2 apical setulae (Fig. 5 F)	10.2 – 22.1 X 3.4 – 6.8 µm (Fig. 2 L).	70.0 mm	Felty to Subfelty	White

(Contd. Part table 2)

P. disseminata	acervuli mostly epiphyllous, rarely amphigenous, erumpent, globose to lenticular, dot -like, scattered, 50-400 µm diam., releasing black spore masses at maturity. (Fig. 3A)	Cylindrical to obovoid 10.0 – 15.0 X 1.0 – 3.5 µm (Fig. 3 C).	Fusiform to slightly clavate, straight, rarely curved, apical cell with 3 apical setulae	10.0 – 15.0 X 1.0 – 3.5 µm (Fig. 3 C).	47.33 mm	Felty to Subfelty	White
P. guepini	amphigenous, mostly hypophyllous, irregularly scattered, globose to lenticular, 100-250 µm diam., wall pseudoparenchymatic and ruptured the epidermis by a pore releasing black spore masses in cirrhi. (Fig. 3E)	Cylindrical to obpyriform 20.0 – 27.0 X 6.5 – 8.0 µm (Fig. 3 G).	Fusiform, Straight or rarely curved, apical cell with 2 – 5 (mostly 3) apical setulae	20.0 – 27.0 X 6.5 – 8.0 µm (Fig. 3 G).	59.33 mm	Felty	White
P. heritierae	aecervuli 250 -300 µm diam., amphigenous, scattered, subglobose to lenticular or ellipsoidal, sub -epidermal, pseudoparenchymatous, at maturity releasing black spore masses. (Fig. 3H)	Cylindrical 5.1 – 15.3 X 1.7 – 3.4 µm (Fig. 3 I).	Fusiform to straight or slightly curved, apical cell with 2 – 3 apical setulae	13.6 – 24.0 X 2.0 – 7.0 µm (Fig. 3 J).	65.00 mm	Felty	Rosy buff to whitish
P. mangiferae	acervuliamphigenous, scattered, globose to lenticular, sometimes ellipsoidal, sub - epidermal, composed of thin - walled, hyaline, angular to irregular pseudoparenchymatic cells; 150-300 µm diam. (Fig. 3L)	Cylindrical to lageniform 5.0 – 15.0 X 1.5 – 4.0 µm (Fig. 3 M).	Fusiform, Straight or slightly curved, apical cell with 3 apical setulae (occasionally 1, 2 or 4) (Fig. 5 E)	16.0 – 24.0 X 4.0 – 7.5 µm (Fig. 3 N).	79.66 mm	Felty	Faint Grey to Yellowish
P. moluccens	granular, 200 -390 µm diam., greenish black, released spore masses at maturity. (Fig. 4B)	Cylindrical to obovoid 9.5 – 14.0 X 1.0 – 4.0 µm (Fig. 4 C).	Straight or Fusiform, rarely curved, apical cell with 3 apical setulae	20.0 – 28.0 X 7.0 – 9.0 µm (Fig. 4 D).	50.66 mm	Felty	White
P. palmarum	acervuli epiphyllous, globose to ellipsoidal, subepidermal, 200-300 µm diam., composed of thin-walled, hyaline angular to irregular pseudoparenchymatic cells. (Fig. 4F)	Cylindrical to obovoid 5.1 – 17.0 X 1.7 – 4.2 µm (Fig. 4 G).	Fusiform, Straight or rarely curved, apical cell with 2 – 3 (rarely 4) apical setulae (Fig. 5 G)	13.6 – 20.4 X 4.7 – 6.8 µm (Fig. 4 H).	59.66 mm	Floccose to Felty	White
P. versicolor	acervuli mostly epiphyllous, globose, dark brown, 100 – 300 µm diam., subepidermal. (Fig. 4I)	Obovoid to obpyriform 10.2 – 15.3 X 1.3 – 5.1 µm (Fig. 4 J).	Fusiform, apical cell with 2 (rarely 3) aplicalsetule (Fig. 5 H)	25.5 – 37.4.0 X 6.8 – 5.5 µm (Fig. 4 K).	77.66 mm	Felty	White

and *P. moluccens*) differ from other known species of this genus, particularly in their growth characteristics, conidial dimensions, spore morphology and colour (Maharachchikumbura *et al.* 2014) (Table 2). *P. apetalae* appears to be very close to *P. mangiferae* (Henn.) Steyaert in number of apical and basal appendages and septation of conidia together with the conidiogenous cell. Both these species described here differ from other known species of the genus, particularly in growth characteristics and colour, morphology and dimensions of conidia. *P. bruguierae* was found to have the highest size variation of conidia.

Although several species of *Pestalotiopsis* were isolated and identified, it was not possible to differentiate among saprophytes, epiphytes and parasites in the foliar environment. Hence it was considered worthwhile to test the pathogenicities of the isolated fungi on their host species. From the pathogenicity test results (Table 3), the lowest and the highest percentages of growth of leisons are observed in *P. moluccens* and *P. apetalae*, respectively. *P. palmarum* has shown a percentage difference of 22.50 between 48 and 72 hours. It appears from the literature that previous workers have also reported some parasitic *Pestalotiopsis* from mangrove plants, such as *P. disseminata, P. guepini,* and *P. versicolor.* In the present investigation, *P. disseminata, P. guepini* and *P. versicolor* were isolated from *A. corniculatum, X. granatum* and *C. decandra* respectively and their pathogenicities were tested and confirmed.

Pestalotiopsis Steyaert is a difficult genus for the taxonomists. Steyaert (Maharachchikumbura *et*

Table 3: Pathogenicity tests of different species of *Pestalotiopsis* on detached leaves of living mangrove plants

Host	Pathogen	*% production of lesions		
		24 h	48 h	72 h
Excoecaria agallocha	*Pestalotiopsis agallochae*	50.00	55.00	55.00
Sonneratia apetala	*P. apetalae*	77.50	80.00	80.00
Bruguiera gymnorrhiza	*P. bruguierae*	0	60.00	70.00
Sonneratia caseolaris	*P. caseolaris*	0	25.00	27.50
Aegiceras corniculatum	*P. disseminata*	0	27.50	30.00
Xylocarpus granatum	*P. guepini*	0	5.00	25.00
Heritiera fomes	*P. heritierae*	0	20.00	20.00
Derris scandens	*P. mangiferae*	0	40.00	40.00
Xylocarpus moluccensis	*P.moluccens*	0	5.00	12.50
Phoenix paludosa	*P.palmarum*	0	0	22.50
Ceriops decandra	*P. versicolor*	0	20.00	20.00

*% based on the total number of spore suspension drops placed on leaves of each host species (4 drops /leaf; 10 leaves/host species); Temp. 30-32°C.

al. 2014) found it necessary to reassign many species described in *Pestalotia* to some other genera, after accepting a single species in *Pestalotia*. According to the author, *Pestalotiopsis* includes only species with 4-septate conidia. Sutton (Maharachchikumbura *et al.* 2014) also supported Steyaert's idea to a large extent by introducing additional criteria; the most important of these were wall structures of conidia. As suggested by Sutton, the cell numbers are important because they are consistent within a species under natural conditions. Besides, single layered conidial wall structures could also be used as one of the criteria. Nagraj (Maharachchikumbura *et al.* 2014) preferred to adopt a broader concept for the genus *Pestalotiopsis* and included 3-septate forms also. However, a large no. of taxa in *Pestalotia,* which should be placed in *Pestalotiopsis,* remains in *Pestalotia*. In the present investigation 11 species of *Pestalotiopsis* have been studied. All the species are 5 celled, single layered wall structures and with 2-3 apical appendages. The characteristics observed are inconformity with the description of previous workers. However, none of the species described here are 3 celled.

Phenetic analysis

The UPGMA based phenetic tree consists of two subclades (Fig. 6A). The first subclade consists of *P. agallochae*, *P. heritierae* and *P. mangiferae*. The second subclade consists of the other eight species. In the first subclade, *P. agallochae*, *P. heritierae* are closer to each other than *P. mangiferae*. In the second subclade, *P. apetalae* and *P. disseminata* are joined together to form a clade. *P. bruguierae*, *P. guepini* and *P. moluccens* joined together to form another clade which is joined with *P. palmarum*. *P. caseolaris* is joined with this combined clade and with this combined clade, *P. versicolor* is joined. The goodness-of-

Fig. 1. A.: *Pestalotiopsis agallochae*– infected leaf of *Excoecaria agallocha* **B.** *Pestalotiopsis apetalae* – infected leaf of *Sonneratia apetala* **C.** *Pestalotiopsis disseminata*– infected leaf of *Aegiceras corniculatum.* **D.***Pestalotiopsis bruguierae*– infected leaf of *Bruguiera gymnorrhiza* **E.***Pestalotiopsis caseolaris*– infected leaf of *Sonneratia caseolaris* **F-G.** *Pestalotiopsis versicolor*– infected leaf of *Ceriops decandra* **H.** *Pestalotiopsis heritierae*– infected leaf of *Heritiera fomes* (Bar = 2 cm).

fit test of the similarity matrix with the corresponding dendrogram revealed high correlation (r = 0.87, normalized Mantel statistic Z) showing that the tree correctly reflects the similarities among different species. However, more morphological characters are needed for better understanding of their relationship.

The results of the Principal Coordinate Analysis *i.e.* PCOORDA (Fig. 6B) were mostly comparable with the UPGMA based cluster analysis. The first three most informative PC components explained 51.91% of the total variation. Here, *P. agallochae*, *P. heritierae* are placed closely and *P. mangiferae* are placed somewhat distantly but closer than the other species. From the picture, it is evident that the other species except *P. versicolor* are closer to each other than the previously mentioned three species. *P. versicolor*, indeed maintained its distance from the other species. So, from both UPGMA and PCOORDA analysis, the placement of different *Pestalotiopsis* species is well resolved.

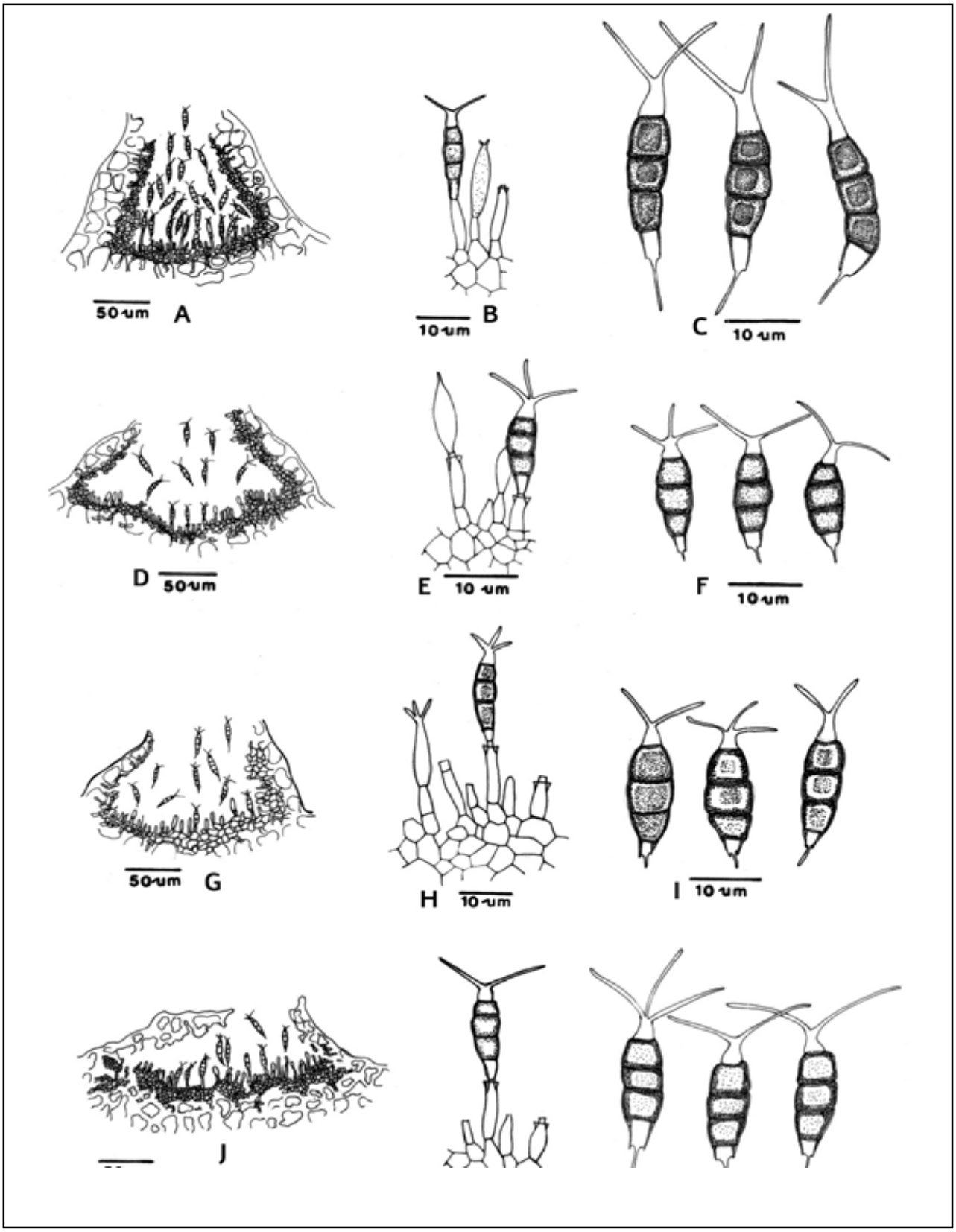

Fig. 2: A-C.*Pestalotiopsis agallochae*. **A.** V. S. of acervulus, **B.**Conidiogenous cells and developing conidia, **C.** Conidia. **D-F.** *P. apetalae*. **D.** V. S. of acervulus, **E.** Conidiogenous cells and developing conidia, **F.** Conidia. **G-I.** *P. bruguierae*. **G.** V. S. of acervulus, **H.** Conidiogenous cells and developing conidia, **I.** Conidia. **J-L.** *P. caseolaris*. **J.** V. S. of acervulus, **K.** Conidiogenous cells and developing conidia, **L.**Conidia.

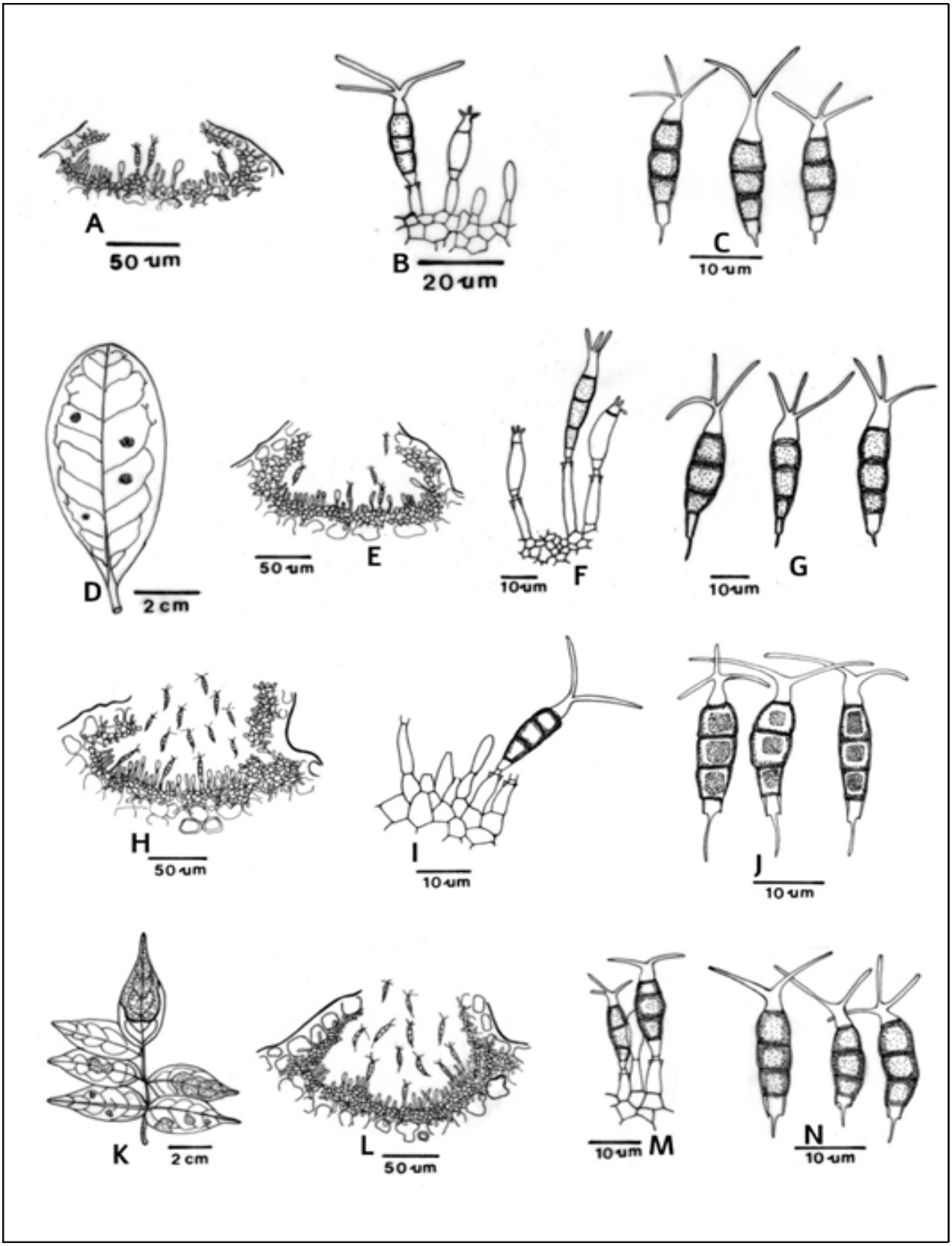

Fig. 3: A-C. *Pestalotiopsis disseminata*. **A.**V. S. of acervulus, **B.** Conidiogenous cells and developing conidia, **C.**Conidia. **D-G.** *P. guepini.* **D.** *P. guepini* – infected leaf of *Xylocarpus granatum*, **E.**V. S. of acervulus, **F.** Conidiogenous cells and developing conidia, **G.** Conidia. **H-J.** *P. heritierae*. **H.**V. S. of acervulus, **I.** Conidiogenous cells and developing conidia, **J.**Conidia. **K-N.** *P. mangiferae*. **K.** *P. mangiferae* – infected leaf of *Derris scandens*, **L.**V. S. of acervulus, **M.** Conidiogenous cells and developing conidia **N.** Conidia.

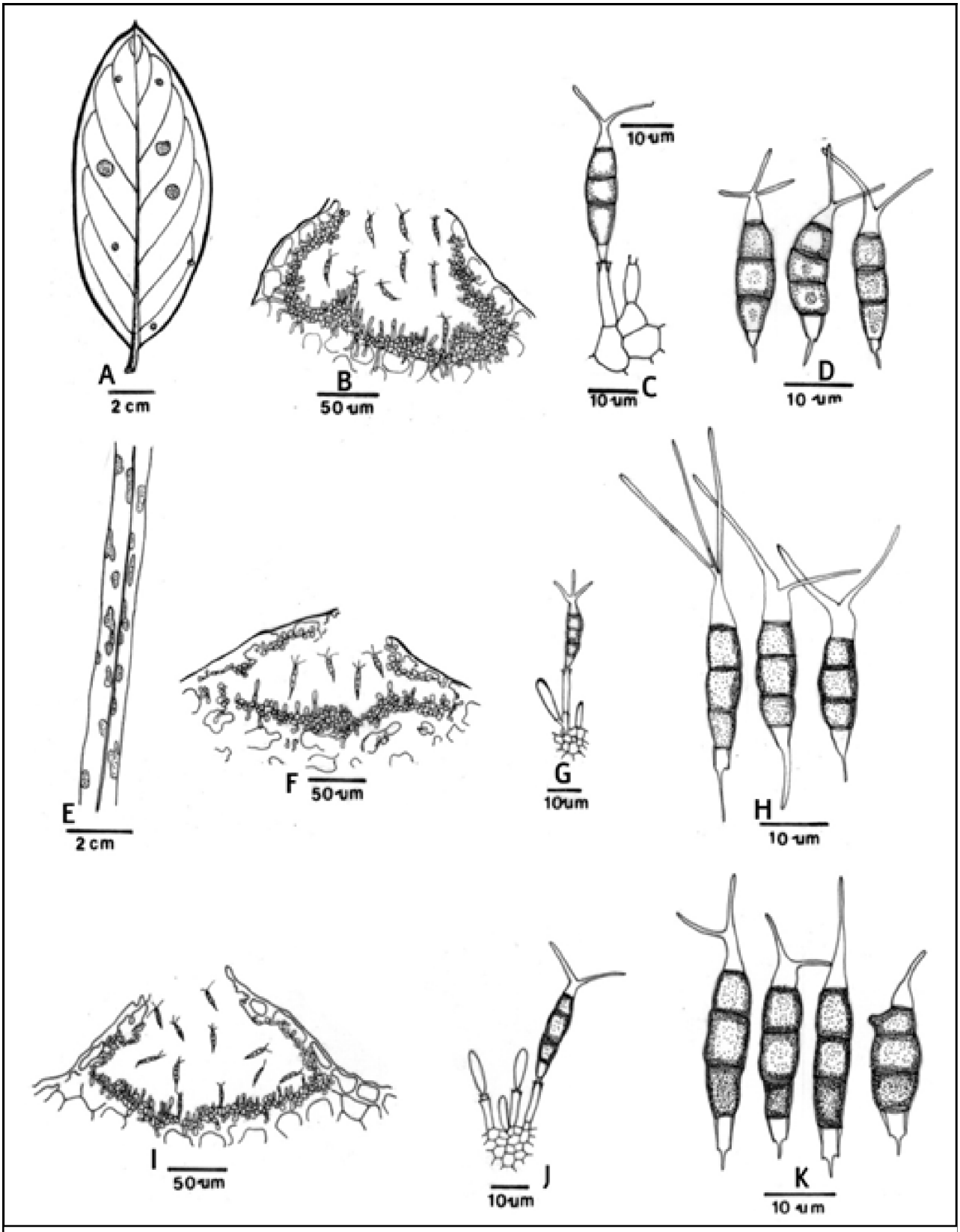

Fig. 4: A-D. *Pestalotiopsis moluccens*. **A.** *P.moluccens* – infected leaf of *Xylocarpus moluccensis*, **B.**V. S. of acervulus **C.** Conidiogenous cells and developing conidia, **D.** Conidia. **E-H.** *P. palmarum*. **E.** *P. palmarum* – infected leaf of *Phoenix paludosa*, **F.** V. S. of acervulus **G.** Conidiogenous cells and developing conidia **H.** Conidia. **I-K.** *P. versicolor*. **I.** V. S. of acervulus **J.** Conidiogenous cells and developing conidia **K.**Conidia.

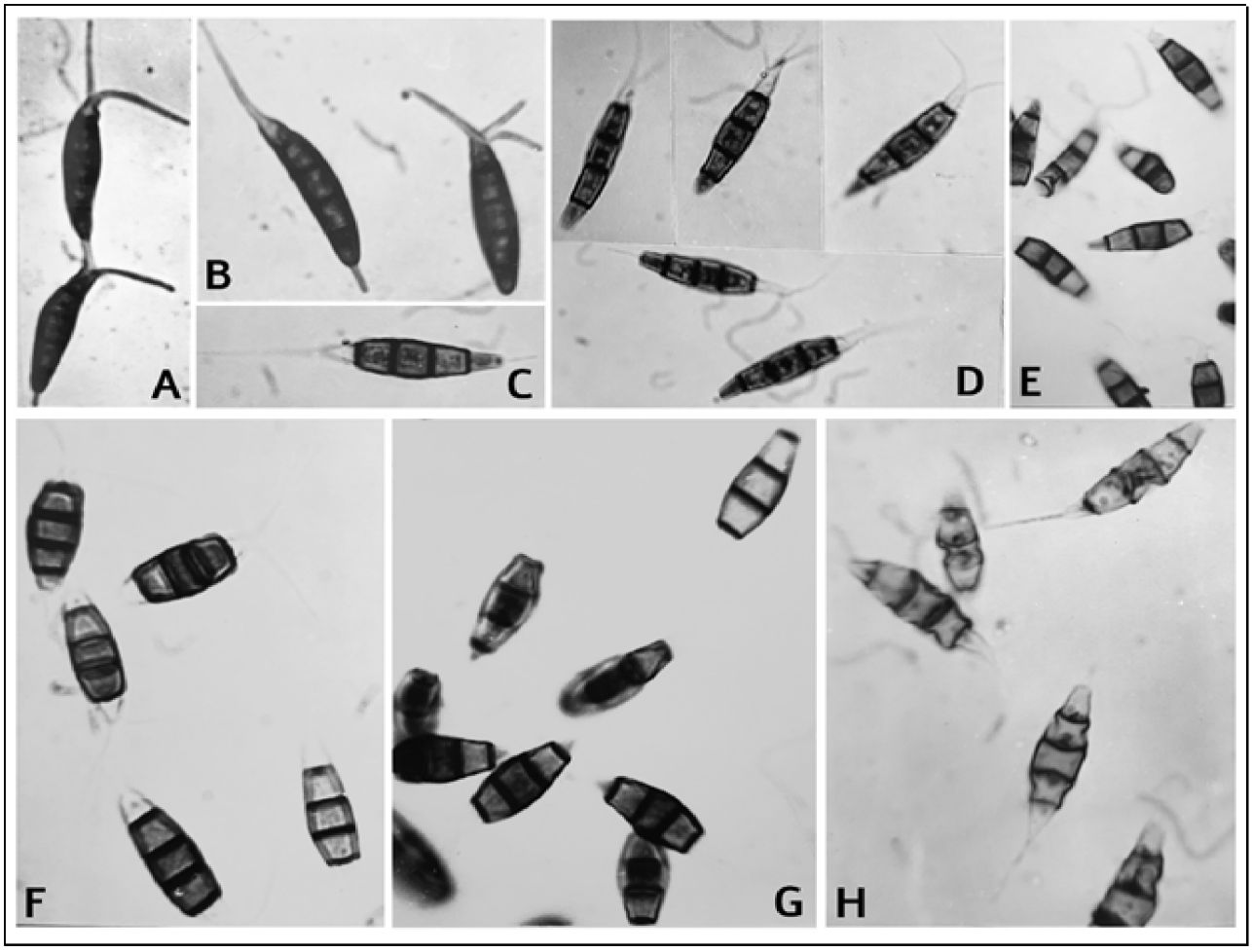

Fig. 5: Microphotographs of conidia of different species of *Pestalotiopsis*.**A-D.** *P. agallochae* **E.** *P. mangiferae* **F.** *P. caseolaris* **G.** *P. palmarum* **H.** *P. versicolor*.

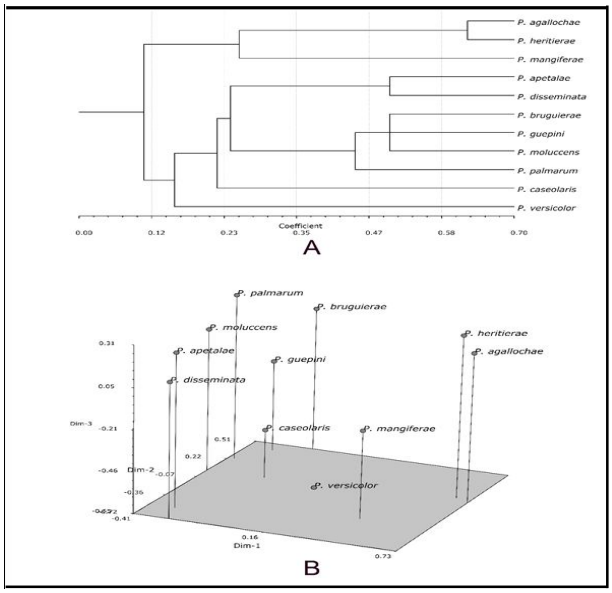

Fig. 6:A. UPGMA-based phenetic tree of species of *Pestalotiopsis*. **B.**Three-dimensional plot obtained from Principal coordinate analysis (PCOORDA) of species of *Pestalotiopsis*.

In conclusion, the present study reports eleven species of the fungal genus *Pestalotiopsis* from mangroves of Indian Sundarbans. Among them 4 species (*P. disseminata, P. guepini, P. mangiferae, P. palmarum*) were recorded for the first time on mangroves – *Aegiceras corniculatum, Xylocarpus granatum, Derris scandens, Phoenix paludosa* respectively. The pathogenicity test confirms their parasitic relationship with their respective host plants. They showed ample morphological variations among them and phenetic study revealed their taxonomic relationships. Knowledge gathered from the present study reveals a number of new and interesting facts which would provide useful biological information to researchers working in the field of fungal taxonomy and can create a new avenue for future conservation of mangroves of Sundarbans. Moreover, these endophytic parasitic fungi may be potential source of different metabolites that can be utilized for human use in future. Thus, this study has opened up a new

path for further research on potential foliar fungi of mangrove plants.

ACKNOWLEDGEMENT

We are grateful to Late Prof. R.P. Purkayastha (former Rashbehari Ghosh Professor, Dept. of Botany, University of Calcutta) for giving us the idea of this work. Financial assistance received from the Ministry of Environment & Forests, Government of India is gratefully acknowledged.

REFERENCES

Aly, A.H., Debbab, A., Kjer, J., Proksch, P. 2010. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. *Fung. Divers.***41**: 1–16.

Ariyawansa, H.A., Hyde, K.D. 2018. Additions to *Pestalotiopsis* in Taiwan. *Mycosphere***9**: 999–1013.

Cadamuro, R.D., Bastos, I.M.A.D.S., Pavi, C.P., Prá, I.D., Souza, D.S.M., Steindel, M., Sivla, I.T.D., Treichel, H., Fongaro, G. 2023. 1 - Pathogens control using mangrove endophytic fungi. Endophytic Association: What, Why and How - *A volume in Developments in Applied Microbiology and Biotechnology*. Academic Press. pp : 1-19.

Cannon, P.F. and Simmons, C.M. 2002. Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. *Mycologia* **94**: 210–220.

Chen, Y.X., Zeng, L., Shu, N., Jiang, M. et al. 2018. *Pestalotiopsis* like species causing gray blight disease on Camellia sinensis in China. *Plant Dis.***102**: 98–106.

Cruz, T.E.E.D., Bennett, R.M., Apurillo, C.C.S. 2023. Diversity and applications of fungi associated with mangrove leaves in the Philippines. Chapter 2.In *Mycology in the Tropics*(Eds.J. J. G. Guerrero, T.U. Dalisay, M.P. De Leon, M.A. O. Balendres, K.I. R. Notarte, T.E. E. D. Cruz) pp. 17-32.

Debbab, A., Aly, A.H., Proksch, P. 2013. Mangrove derived fungal endophytes a chemical and biological perception. *Fung. Divers.***61**: 1–27.

Hauser, L.A., Crovello, T.J. 1982. Numerical Analysis of Generic Relationships in Thelypodieae (Brassicaceae). *Syst. Bot.* **7**: 249-268.

Hu, H.L., Jeewon, R., Zhou, D.Q., Zhou, T.X., Hyde, K.D. 2007. Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and Ribes spp.: evidence from rDNA and â tubulin gene phylogenies. *Fung. Divers.***24**: 1–22.

Hyde, K.D., Norphanphoun, C., Maharachchikumbura, S.S.N., Bhat, D.J. et al. 2020. Refined Families of Sordariomycetes. *Mycosphere* **11:** 305–1059.

Jaccard, P. 1908. Nouvelles rescherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat***44**: 223-270.

Jeewon, R., Liew, E.C. and Hyde, K.D., 2002. Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters. *Mol. Phylogenet. Evol.***25**: 378-392.

Kamil, D., Prameela Devi, T., Mathur, N., Singh, O.P., Pandey, Pankaj, Prabhakaran, N., Patil, V. 2012. Addition of two new species of *Pestalotiopsis* to the fungal diversity in India. *J. Mycopathol. Res.***50**: 185–191.

Kathiresan, K., Bingham, B.L. 2001. Biology of mangroves and mangrove ecosystems. *Adv. Marine Biol.* **40**: 81-251.

Kumar, D.S.S., Hyde, K.D. 2004. Biodiversity and tissue–recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Divers.* **17**: 69–90.

Lateef, A., Sepiah, M. and Bolhassan, M.H., 2018. Molecular identification and diversity of *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis* species from four host plants in Sarawak, Borneo island (Malaysia). *J. Sci. Technol.***10**: 33-43.

Lee, S., Crous, P.W., Wingfield, M.J. 2006. Pestalotioid fungi from Restionaceae in the Cape Floral Kingdom. *Stud. Mycol.* **55**: 175–187.

Liu, A.R., Chen, S.C., Wu, S.Y., Xu, T. et al. 2010. Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in *Pestalotiopsis* taxonomy. *Mol.Phylogen.Evol.***57**: 528–535.

Liu, A.R., Xu, T. and Guo, L.D. 2007. Molecular and morphological description of *Pestalotiopsis hainanensis* sp. nov., a new endophytic from a tropical region of China. *Fung. Divers.***24**: 23–36.

Maharachchikumbura, S.S., Guo, L.D., Chukeatirote, E., Bahkali, A.H. and Hyde, K.D., 2011. Pestalotiopsis—morphology, phylogeny, biochemistry and diversity. *Fung. Divers.***50**: 167-187.

Maharachchikumbura, S.S.N., Guo, L.D., Cai, L., Chukeatirote, E. et al. 2012. A multi locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. *Fung. Divers.***56**: 95–129.

Maharachchikumbura, S.S.N., Hyde, K.D., Groenewald, J.Z., Xu, J. and Crous, P.W. 2014. *Pestalotiopsis* revisited. *Stud. Mycol.***79**: 121–186.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**: 209-220.

Monden, Y., Yamamoto, S., Yamakawa, R. 2013. First case of fungal keratitis caused by *Pestalotiopsis clavispora*. *Clinical Ophthalmol.* **7**: 2261–2264.

Norphanphoun, C., Jayawardena, R.S., Chen, Y., Wen, T.C. 2019. Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand. *Mycosphere***10**: 531–578.

Pal, A.K. 2012. Fungi in mangrove forests of Indian Sundarbans: biodiversity and parasitism. In: *Some aspects of coastal vegetation in Indiaincluding Andamans and Sundarbans*.(Eds. A.K. SamantaandS. Panda). pp. 58-77.

Pal, A.K. 2014. SEM studies on the conidial morphologoy of some *Pestalotiopsis* species occurring on mangrove plants. *J.Mycopathol. Res.***52:** 53 –57.

Pal, A.K. 2017. Folicolous fungi of mangrove plants of Indian Sundarbans – A treasure house of ethnobotanical medicine. *J. Mycopathol. Res*. **54**: 485-493.

Pal, A.K. 2018. Biodiversity, parasitism and screening of potential phytopathogenic strains of foliar fungi of Mangrove plants of Indian Sundarbans. In: *Advancement of Bological Sciences towards new generation*(Eds.A. Sarkar, ArghyaandC. Sa). pp.1-28.

Pal, A.K. 2020. Analysis of strain variation in *Colletotrichum gloeosporioides*, a phytopathogenic fungus of mangrove plants of Sundarbans (Eastern India). *J. Mycopathol. Res.* **58**: 149-156.

Peng, J., Chen, P., Li, C., Liu, J., Sun, Y., Yang, M., Ding, B., Huang, H. and Tao, Y. 2022. Phthalide metabolites produced by mangrove endophytic fungus *Pestalotiopsis* sp. SAS4. *Magn. Reson. Chem.* **60**: 525-529.

Rayner, R.W. 1970. *A mycological colour chart*. Commonwealth Agricultural Bureaux, Kew, Surrey, England, pp. 34

Rohlf, F.J. 2009. NTSYSpc: numerical taxonomy system. ver. 2.21c. *Exeter Software*, Setauket, New York, USA.

Russell, J.R., Huang, J., Anand, P., Kucera, K. et al. 2011. Biodegradation of Polyester Polyurethane by Endophytic Fungi. *Appl. Environ.Microbiol.* 77: 6076–6084.

Senanayake, I.C., Lian, T.T., Mai, X.M., Jeewon, R., Maharachchikumbura, S.S.N., Hyde, K.D., Zeng, Y.J., Tian, S.L. and Xie, N., 2020. New geographical records of *Neopestalotiopsis* and *Pestalotiopsis*species in Guangdong Province, China. *Asian J. Mycol.***3**: 512-533.

Shara, M., Basyuni, M. ,Hasanuddin 2023. Potential of Phylloplane Fungi from Mangrove Plant (*Rhizophora apiculata* Blume) as Biological Control Agents against Fusarium oxysporum f. sp. cubense in Banana Plant (*Musa acuminata* L.). *Forests***14** : 1-16.

Shukla, S.T., Habbu, P.V., Kulkarni, V.H., Jagadish, K.S., Pandey, A.R., Sutariya, V.N., 2014. Endophytic microbes: a novel source for biologically/pharmacologically active secondary metabolites. *Asian J.Pharmacol.Toxicol.***2**: 1-6.

Smith, R.S. 1967. Control of tarsonemid mites in fungal cultures. *Mycologia*. **59**: 600-609.

Tejesvi, M.V., Nalini, M.S., Mahesh, B., Prakash, H.S. et al. 2007. New hopes from endophytic fungal secondary metabolites. *Bol. Soc. Qum. Mx*. **1**: 19–26.

Tejesvi, M.V., Tamhankar, S.A., Kini, K.R. et al. 2009. Phylogenetic analysis of endophytic Pestalotiopsis species from ethnopharmaceutically important medicinal trees. *Fung. Divers*. **38:** 167–183.

Tsai, I., Maharachchikumbura, S.S.N., Hyde, K.D., Ariyawansa, H.A. 2018. Molecular phylogeny, morphology and pathogenicity of *Pseudopestalotiopsis* species of Ixora in Taiwan. *Mycologic. Progress* **17**: 941–952.

Wijayawardene, N.N., Hyde, K.D., Lumbsch, H.T., Liu, J.K. et al. 2018. Outline of Ascomycota 2017. *Fung. Divers.* **88**: 167–263.

Xu, J., Ebada, S.S., Proksch, P. 2010. *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. *Fung. Divers.***44**: 15–31.

Xu, J., Yang, X., Lin, Q. 2014. Chemistry and biology of *Pestalotiopsis* derived natural products. *Fung. Divers.* **66**: 37–68.

Younis, T., Rahaman, L., Rahaman, S., Shinwari, A.K., Iqrar, I., and Shinwari, Z.K. 2022. Endophytes: Potential Source of Bioactive Compounds of Pharmaceutical Importance. *Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences* **59**: 1-13.

Zhang, Y.M., Maharachchikumbura, S.S.N., Tian, Q., et al. 2013. *Pestalotiopsis* species on ornamental plants in Yunnan Province, China. *Sydowia* **65**: 59–74.