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Graphiola phoenicis (Mong.) Poit. : An enigmatic parasitic fungus from mangrove palm of Indian Sundarbans

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During a survey of foliicolous fungi of mangrove ecosystem of Sundarbans *Graphiola phoenicis* (Mong) Poit leaf spot disease (often reported as false smut) was detected on leaves of *Phoenix paludosa*, collected for the first time from mangroves of the Sundarbans. This communication deals with light microscopic and scanning electron microscopic characters of this mangrove fungi. The objective was to verify and report the occurrence of this foliar disease of mangrove plant from Indian Sundarbans.

Key words: Foliicolous fungi, *Graphiola phoenicis*, mangrove, *Phoenix paludosa*, Sundarbans

INTRODUCTION

Mangroves constitute a dynamic ecosystem with an excellent co-existence of varied forms of plants and animals of terrestrial and aquatic habitats. The term ‘mangrove’ is derived from the Spanish word ‘mangle’ and the Portuguese word ‘mangue’. Mangrove forests are dominated by salt tolerant halophytic seed plants including herbs, shrubs and trees and are restricted to the intertidal belts exposed to the high and low tides. There has been a growing interest in the exploration of fungi of tropical mangroves.

Although a number of fungi have been reported by different workers from Indian Mangroves (Alias *et al*. 2010; Borse *et al*.2013; Bhimba *et al.* 2011; Li *et al*. 2011; Pal, 2012; Pal, 2014; Pal, 2017; Pal, 2018). Sarma, (2012). Suciatmih and Rahmansyah, (2013) very little is known about fungal diversity and their parasitic relationship with mangrove plants of Sundarbans which is the largest mangrove forest in the world covering 1.48-1.73 million acres of land. Sundarbans has a humid, tropical maritime climate with an annual rain-fall about 1650 to 1800 mm in Central and Northern areas and about 2790 mm in outer coasts. The average maximum and minimum temperatures are 29° C (June-July) and 20° C (Dec.-Jan.) respectively and the humidity varies between 70-88%. Leaf-inhabiting fungi of Sundarbans play a significant role in litter decomposition apart from their involvement in various diseases of mangrove plants. Despite the fact, a large majority of them remains yet to be identified.

Graphiola leaf spot is a fungal leaf disease caused by *Graphiola phoenicis* (Moug.) Poit.. This disease is often referred to as “false smut”. The disease is easily diagnosed by direct examination of affected leaf tissue.

MATERIALS AND METHODS

Macroscopic examination

Infected leaves showing characteristics symptoms of diseases were examined with the help of an illuminated magnifying glass. The leaves with typical fructifications were carefully searched and macroscopic features were noted.

Microscopic exanimation

For microscopic examination of infected leaf materials, both dissecting and compound

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microscope were used to study the vegetative and reproductive structures of fungi. A small portion of the infected leaf tissue was first placed in the dissecting microscope, mounted in lactophenol, teased carefully, covered with a cover glass and observed under the microscope. Vertical sections (V.S.) of peridium were also examined under the compound microscope. Cotton blue – lactophenol (0.05 g Cotton blue in 100 ml lactophenol) was used for staining purpose. The slides were finally sealed with nail varnish, labelled and stored for further examinations, if necessary. Microscopic measurements of reproductive structures were made with an ocular and stage micrometer. Camera lucida drawings were also made under necessary magnifications depicting almost all the details. The photo micrographs were taken using a Leitz Binocular research microscope fitted with WILD MPS 12 camera attachment.

Scanning Electron Microscopy (SEM)

A portion (3 mm X 5 mm) of dried leaf bearing fructification was mounted on aluminium SEM stubs using carbon tacks. The specimen was sputter coated (Emscope SC500 cool sputter coater) with gold / palladium for 2 mins at 35 mm target distance, resulting in a deposition of ca.12 nm.Specimens were examined in a Hitachi, S-2300 scanning electron microscope and finally Scanning Electron Micrographs were taken.

RESULTS AND DISCUSSION

Description of the fungus

Graphiola phoenicis (Moug. ex Fr.) Poit. (Fig.1.C-I; Fig.2.A-F; Fig.3.A-E) Annls Sci. Nat., Bot., sér. 1: 473 (1824).

Synonymy

♦*Phacidium phoenicis* Moug. ex Fr.Systema Mycologicum 2: 572 (1823).

♦*Trichodesmium phoenicis* (Fr.) Chevall., Flore Générale des Environs de Paris 1: 382 (1826).

♦*Roestelia phoenicis* (Moug. ex Fr.) Bonord., Handbuch der allgemeinen Mykologie: 55 (1851).

Leaf spots (Fig.1.D) epiphyllous, distinct, elongated, sometimes lens shaped or irregular, coalescing forming patches, usually reddish brown with dark brown spot at the centre, distributed on both sides of the mid-vein, often concentrated towards the margin, frequency high, more on older leaves, 1-7 mm wide.

Fructifications (Fig.1.C-I) amphigenous, found in much higher frequency on lower surface of leaves, black, cupulate, 0.5-1.5 mm in diam. at maturity, peridiate; ***Peridium*** thick partially encloses the fertile tissue and forms a collar around the ostiole through which the narrow, white, flexuous filaments emerge (Fig.3.B), occasionally 2-3 fruiting bodies fused together forming a common ostiole. ***Filaments*** (Fig.3.D) tangled, hygroscopic, in fascicles, composed of rows of rectangular cells fused in a parallel fashion, extending up to 2-3 mm above the mouth of fruiting body, sometimes covered with several propagules produced by the fertile cells (basidia) within the cavity of the fructification. ***Propagules*** (primary basidiospores) ellipsoidal, verruculose, 2 celled, showing a smooth, slightly concaved cross-wall when forcibly separated into two secondary spores (Fig.3.E), dimension of propagules 4-5.8 X 2-5 µm, yellowish-brown in mass.

On living leaves of *Phoenix paludosa* Roxb. (Fig.1.A) (Arecaceae) collected from Bhagbatpur, South 24 Parganas, West Bengal, India on 9 March, 1996, CUPH 812 (holotype).

SEM micrographs revealed fructifications (Fig.2.A-C) showing thick, cracked outer wall (peridium), with enclosed fertile tissue and thick collar circumscribing ostiole (Fig.2D) through which elaters protrude; propagules (Fig.2.E) 2 celled, dimension 4-5.8 X 2-5 µm, with verruculose outer surface (Fig.2.F) and reveal a smooth slightly concave cross-wall.

G. phoenicis is the most common widely distributed and well known species of *Graphiol*a. It is a biotrophic parasite on palm (*P.paludosa*) often referred to as “false smut”. The disease is easily diagnosed by direct examination of affected leaf tissue. The disease is primarily cosmetic and does not adversely affect plant growth in landscape. Severe infection and damage was observed in areas of higher humidity when numerous sori are present on a leaf, the tissue can turn yellow and the entire leaf can die prematurely and reduces the tree growth and date production.

G. phoenicis has been reported from tropical and subtropical regions all over the world. It is known from all around the Mediterranean Sea (Algeria, Corsica, Crete, Cyprus, Egypt, France, Greece, Libya, Morocco, Palestine, Spain) and many countries of Africa (Ethiopia, Ghana, Kenya, Mauritania, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Zimbabwe) and adjacent islands (Canary Islands, Mauritius). In subtropical and tropical parts of numerous other countries (Australia, Barbados, Brazil, Burma, China, Cuba, Dominican Republic, Fiji, French Guiana, India, Jamaica, New Caledonia, New Zealand, Papua New Guinea, Peru, Philippines, Puerto Rico, Seychelles, Taiwan, Trinidad & Tobago, USA, Venezuela, Vietnam, Virgin Islands) *G. phoenicis*

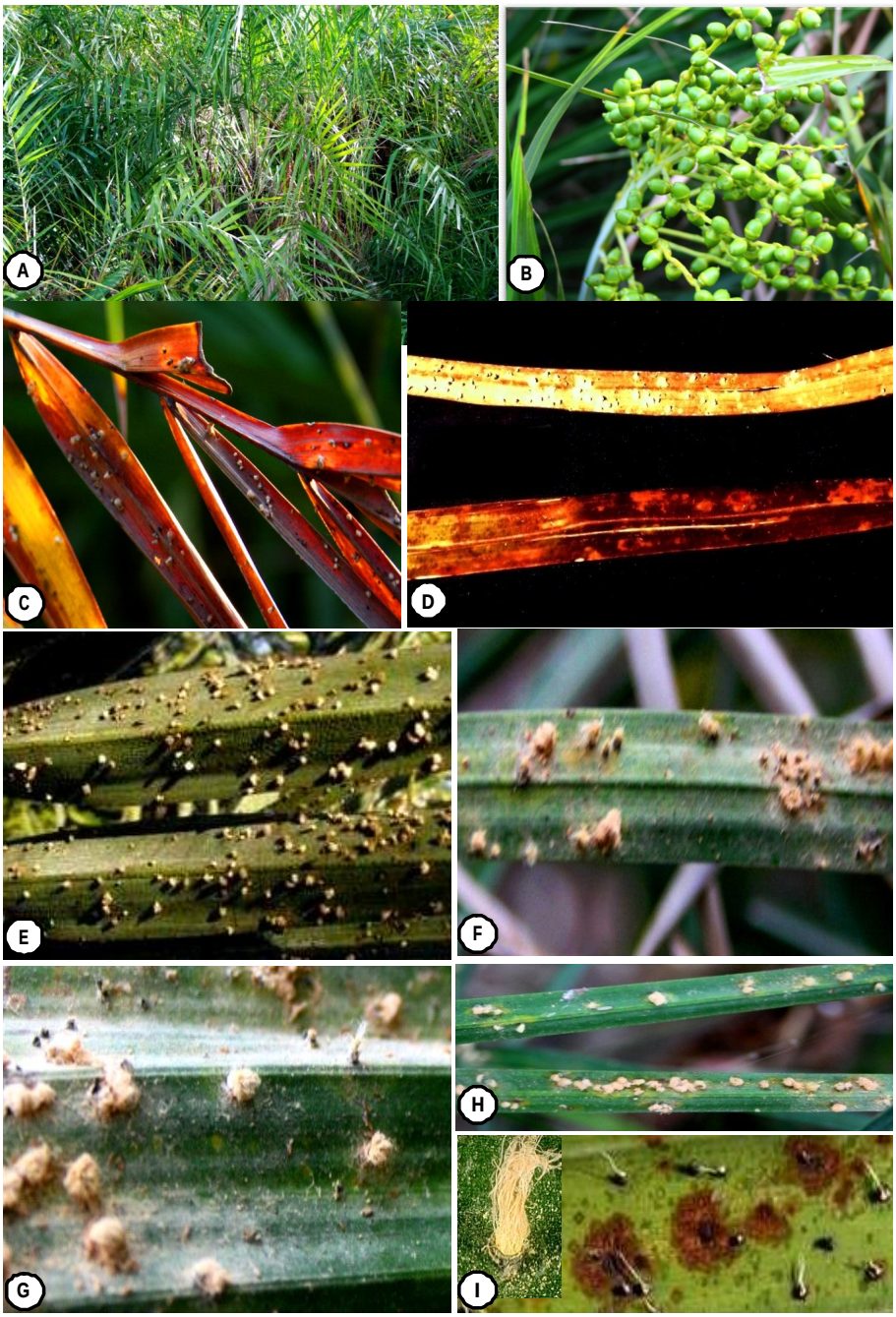

Fig 1: A. *P. paludosa* in tidal forest. B. Fruits. C. Fruiting bodies of the false smut fungus on the backside of the *P. paludosa* leaf. D & E. Heavy infestation of Graphiola leaf spot. F. *G. phoenicis* sori on the backside of the leaf. G & H. Sori and sterile flexuous hyphae of *G. phoenicis* on the leaves. I. Carbonaceous fruiting bodies (sori) have erupted through the leaflet epidermis. (Inset- Close up of sorus of *G. phoenicis* with yellowish white filaments protruding.)

probably is present due to the introduction of *P. canariensis* or other species of *Phoenix* as ornamental plant, in countries with cold climate (Belgium, Denmark, England, France, Germany, Hungary, Netherlands) in greenhouse.

A biotrophic parasitic fungus of a palm was collected from Sundarbans and identified as *G. phoenicis*. This fungus was originally identified as

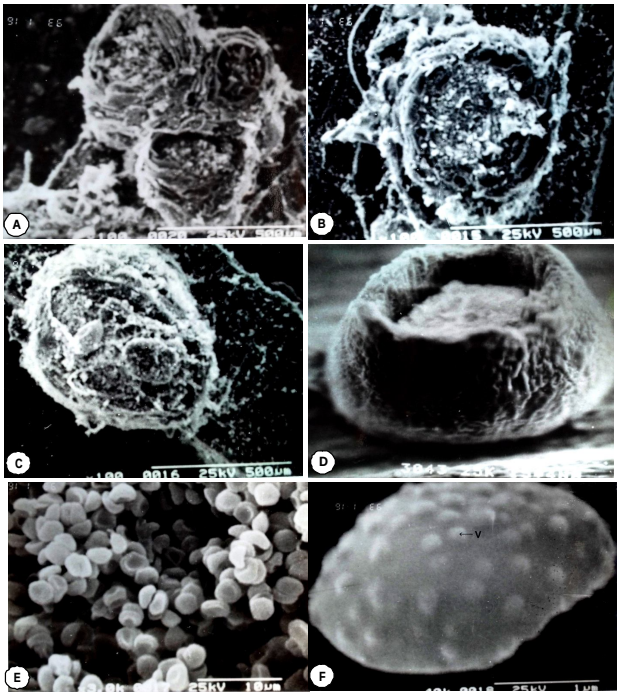

Fig 2 : Scanning electron micrographs of *G. phoenicis.* A-C. Fructifications on the leaflet. D. Magnified view of a fructification showing thick cracked outer wall (peridium), with enclosed fertile tissue and thick collar circumscribing ostiole. E. Propagules (spores) and elaters. F. A magnified propagule (secondary spore) with verruculose (V) surface.

a Myxomycete and subsequently identified by Mougeot and other workers as a Discomycete, a rust, a Pyrenomycete, a smut like fungus, a member of Hypomycetes and finally a member of Exobasidiales under Basidiomycota (Courtecuisse, 2009). The taxonomic confusion was persisted for about 160 years primarily due to lack of understanding of fructification development and the nuclear cycle of the fungus.This fungus is a member of Heterobasidiomycetes and should be accommodated in a separate order Graphiolales. On the basis of ontogenetic and caryological similarities to the Ustilaginales, *G. phoenicis* is phylogenetically related to the smuts but through steps of evolution changes occur in distict morphogenetic characters which separate this species from other smuts. Hence placement of this species in a separate order Graphiolales appears to be justified. However, Webster (1980) placed the genus *Graphiola* under Ustilaginales.

Although microfungi on palms were treated intensively, species of *Graphiola* were hardly studied in the past. The genus *Graphiola* is known for almost 200 years, the life cycle of *G. phoenicis* is figured out and some species of *Graphiola* are represented by molecular data in Gene Bank, but several basic morphological data and taxonomic conclusions are still missing. Important morphological details depict the position of primary spores on basidial cells and spore ornamentations (SEM), which are documented by the present investigation for *G.phoenicis*. Characteristics of basidia still lack for some known species of *Graphiol*a because it was not possible to observe these ephemerous cells in old, dry scanty specimens. Fresh specimens can also be used for the isolation of DNA, which is obtained easily from fresh specimens than from dried herbarium specimens (Piepenbring *et al.* 2012). Species of Graphiolaceae are unique among Ustilaginomycotina because of specific parasitism on palms, catenate basidia, and in the case of *Graphiola* spp. the fission of primary spores into

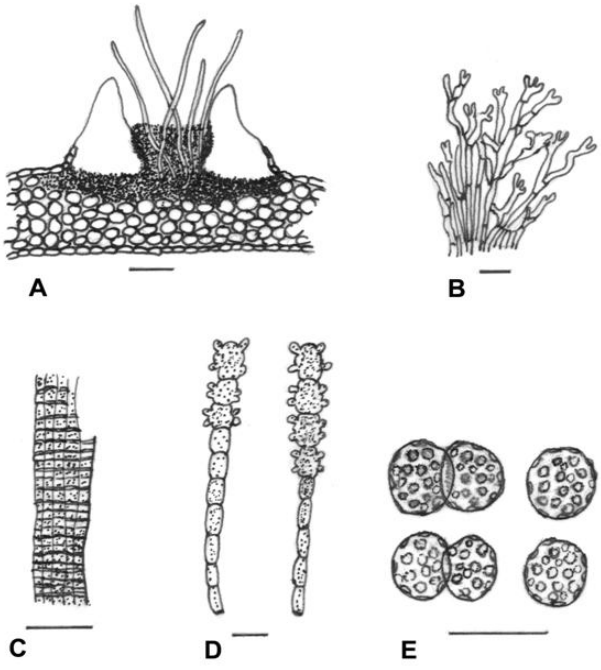

Fig. 3: A-E. *G. phoenicis.* A. Vertical section of a basidioma. Scale bar = 0.2 mm. B. Peridial hyphae. Scale bar = 10 µm. C. Part of an Elater. Scale Bar = 10 µm . D. Sporogenous filaments (chains of basidia with primary spores). Scale bar = 20 µm. E. Two septate primary spores and two secondary spores. Scale bar = 5 µm.

equal secondary spores. The jump of an ancestral fungus of Graphiolaceae onto palms which are the first extant family of monocots which is undoubtedly represented in the fossil record, might correspond to an early point of radiation of Exobasidiomycetidae among Ustilaginomycotina (Piepenbring *et al.* 2012).

Graphiola species seem to be patchily distributed. Some species (*G. compressa, G. phoenicis*) have followed the spread of their host palms by humans. Though many palms became pantropically distributed by humans, *Graphiola* species are rarely collected inspite of the broad distribution of potential hosts. By more extensive studies, further records and new species of *Graphiola* will certainly be discovered (Piepenbring *et al*. 2012).

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REFERENCES

Alias, S.A., Zaimuddin, N. and Jones, E.B.G. 2010. Biodiversity of marine fungi in Malaysian mangroves. *Botanica Marina.* **53**: 545-554.

Bhimba, B.V., Franco, D.A.A.D., Jose, G.M., Mathew, J.M. and Joel, E.L. 2011. Characterization of cytotoxic compound from mangrove derived fungi *Irpex hydnoides* VB4. *Asian Pacific Journ. of Trop. Biomed*. pp 223-226

Borse, B.D., Borse, K.N., Pawar, N.S. and Tuwar, A.R. 2013. Marine fungi from India – XII. A revised check list. *Indian Journal of Geo-Marine Sciences*. **42**: 110 – 119.

Courtecuisse, R. 2009. *Référentiel taxonomique des Basidiomycètes de France métropolitaine. National des Forêts. (Réseau naturaliste mycologie*). Mycological Society of France.

Li, H., Huang, H., Saho, C., Huang, H., Jiang, J., Zhu, X., Liu, Y., Liu, L., Lu,Y., Li, M., Lin, Y. and She, Z. 2011. Cytotoxic norsesquiterpene peroxides from the endophytic fungus *Talaromyces flavus* isolated from the mangrove plant *Sonneratia apetala. Journal of Natural Products*. **74**: 1230 – 1235.

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

Pal, A.K. 2014. SEM studies on the conidial morphologoy of some *Pestalotiopsis* spcies 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. *Isolation and characterization of leaf-inhabiting fungi from medicinal mangrove plants of Sundarbans*, Eastern India. In: Dynamics in Biology (Life Science for next generation). Edited by Dr. Ashim Chakraborty. Akinik Publications, New Delhi. pp. 57-80.

Piepenbring, M., Nold, F., Trampe, T. and Krischner, R. 2012. Revision of the genus *Graphiola* (Exobasidiales, Basidiomycota). *Nova Hedwigia*. **94**: 67-96.

Sarma, V.V. 2012. *Diversity and distribution of marine fungi on Rhizophora* spp. In Mangroves, C. Raghukumar (ed) Biology in marine fungi, Progress In molecular and subcellular biology. Springer-Verlag, Berlin, Heidelberg. pp: 243 – 275.

Suciatmih and Rahmansyah, M.2013. Endophytic fungi isolated from mangrove plant and have antagonism role against *Fusarium* wilt. *ARPN Journal of Agricultural and Biological Science***. 8**: 251 – 257.

Webster,J. 1980. *Introduction to fungi*. Cambridge University Press, Cambridge. pp 669.