RESEARCH ARTICLE

NEW RECORD OF *Fusarium* LEAF SPOT DISEASE OF *Philodendron hastatum*, AN ORNAMENTAL PLANT

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ABSTRACT

Philodendron hastatum is also known as Silver Sword *Philodendron* due to its shiny, silvery leaf appearance and belongs to the family Araceae. It is known to be a perfect addition to the home decors as ornamental plant with high economic value all over the world. Necrotic spots were observed on the leaves of *P. hastatum*, at the plant sales centers and in the adjoining home gardens in Chenkalady, Batticaloa, Sri Lanka. Symptoms initiated as brownish, tan or black spots and enlarged with 3 to 5 mm diameter lesions on the adaxial of the matured leaves. No defoliation was observed due to this unknown disease and also no symptoms were observed on either on flowers or stem. Present study was conducted with the main objective of identifying fungal pathogen causing the leaf spots in *P. hastatum*, using morphological and molecular data. *Fusarium* was isolated from five leaves of three different *P. hastatum* plants, showing the lesions from the study area. The morphological data showed that an isolate was sparse to abundant cottony mycelium with colony color and pigmentations from pale white to light violet. Conidiophores are short, single, lateral mono phialides in the aerial mycelium that are later arranged in densely branched clusters. Macroconidia are fusiform, slightly curved, and pointed at the tip, with three septate basal cells and a size of 23-54 x 3-4.5 µm. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved. The molesular data showed that the sequence of the fungal isolate was 99 % similar to the sequence with Accession Number AB369259, based on the BLAST search results with authenticated sequences. Thereby, the fungus associated with this leaf disease was identified as *Fusarium oxysporum*, based on the morphological and molecular data. Healthy inoculated *P. hastatum* leaves produced spot symptoms 7 days after the inoculation and they were similar to those observed originally on diseased leaves. The pathogenicity of the fungus was confirmed by performing the Koch's postulates. This is recorded as the first report of *Philodendron* leaf spot, caused by *Fusarium oxysporum*, in Sri Lanka.

Keywords: *Fusarium oxysporum, Leaf spot disease, Pathogenicity, Philodendron hastatum*

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1. INTRODUCTION

Philodendron hastatum is also known as Silver Sword *Philodendron* due to its shiny, silvery leaf appearance. It comes under the Tribe: Philodendreae, Plant Family: Araceae and the synonyms are *Philodendron elongatum*, *Philodendron hastifolium* and *Philodendron disparile*. The foliage is almost metallic, slender-pointed, heart to arrow-shaped, grey-green to grey-blue in color and has elongated leaf blades. Emerging leaves are protected by modified leaf structures known as cataphylls, and leaf shape changes with age, typically beginning heart-shaped and progressing to an arrow-shaped appearance. The leaves are 45-60 cm long. This species enjoys climbing and can reach a height of 3 m if given the right growing conditions [1].

This tropical plant, which originated in south-eastern Brazil, is a popular houseplant in many parts of the world, including the United Kingdom. But now the *P. hastatum* is listed on the Red List of endangered species despite the fact it is fairly common in plant collections [2]. This plant is perfect addition to the home decors as ornamental plant, because of its silverfish leaves and its metallic shine. Therefore, it has a high economic value all over the world [3]. Moreover, *P. hastatum* is quite resistant to diseases and pests [4].

However, in some unsuitable environments, the Silver Sword can be attacked by spider mites and mealybugs. Spraying the plant with water and applying insecticidal soap on a regular basis will help keep pests at bay. Another species that frequently attacks *Hastatums* are red spider mites, which are tiny pests that are difficult to notice but are found all over the world [5]. Leaf spot diseases are more common after relatively cool and wet spring, as the infection usually requires free water on the leaf surface. The most common ornamental trees and shrubs are hosts to one or more pathogens that affect the leaves [6]. In most cases, leaf spots are seen as more aesthetic than life threatening problems, although they do cause significant and sometimes premature leaf drop [7].

In *P. hastatum*, spots were brownish, tan or black. According to the data of morphological identification, 3 to 5 mm diameter lesions were observed in 12 leaves from the 18 diseased leaves sample (Figure 1).

Figure 1: *Leaf spot symptoms of P. hastatum,* ***(A)*** *Healthy leaf of P. hastatum,* ***(B)*** *Leaf of P. hastatum showing symptoms of leaf spot disease*

Thereby, the objective of this study was to isolate and characterize the pathogen causing the leaf spot disease of ornamental plant, *P. hastatum.*

2. MATERIAL AND METHODS

2.1 Collection of diseased samples

Diseased leaves were collected from home gardens at Chenkalady, Batticaloa. Leaves which infested with fungi were selected for the experiment. The Ethno botanical study of these plants was carried out and taxonomical identification was done by the botanical survey.

2.2 Isolation of the pathogen

Five leaves from three different *P. hastatum* plants were used for the isolation of the pathogen. Small segments (5x5 mm^2), cut from the diseased areas of the leaf, were surface sterilized in sodium hypochlorite for 3 min. After rinsing the segments in sterilized distilled water, the excess liquid was removed by placing them on sterilized filter papers before transferring aseptically on to Potato Dextrose Agar (PDA) plates (4 segments per plate), supplemented with amoxicillin to suppress bacterial growth. The plates were incubated at 28 - 30 °C and 5-7 days to allow fungal growth. After colonies

grew out, the hyphal margins were chosen for subculturing onto fresh PDA and incubated at 28 °C for 14 to 21 days.

2.3 Single spore isolation and preparation of mono-conidial cultures

A suspension of conidia was prepared by scraping mycelia from two weeks old cultures and suspending in sterile distilled water in 50 ml flasks. The suspension was filtered through sterile glass wool and the concentration was adjusted to 1 x 10^6 conidia ml^{-1}. A loopful of the suspension from each isolate was streaked and spread on tap water agar plates and the plates were incubated at 26-28 ℃ for 18 h. A single germinated conidium was located by moving the objective lens (x 25) of a light microscope across the streak line of the inverted agar plate. A small piece of agar with the germinated conidium was cut and transferred on to fresh PDA plates. The plates were incubated for seven days at 28-30 ℃ [8].

2.4 Morphological characterization of the pathogen

Two weeks old mono-conidial cultures of the pathogen were used to study the colony morphology such as colony color, texture, and pigmentation underneath, the presence or absence of concentric rings, sectoring and reproductive morphology, acervuli or the conidial masses. The conidial characteristics such as shape of conidia, presence of septa etc. were recorded by using top mount eye piece biotec camera and the cellSens software. Camera was connected to the eyepiece of light microscope. The average length of the conidia was calculated by using the software.

2.5 Molecular characterization of the pathogen

The fungus isolated from leaf spots of *P. hastatum* was taken for molecular studies. Single spore culture of the pathogen was used for DNA extraction. Total DNA was extracted using DNeasy plant mini kit (QIAGEN, Germany) as described in the manufacturer's guidelines [9]. PCR amplification was done after the DNA extraction process was successfully completed. For this research project, universal primers ITS 1 and ITS 4 were used. DNA Ladder 100 bp was used as the marker. Marker is important

to compare the base pair size of the DNA sample and the marker is used according to desired analysis.

PCR reactions were performed with a reaction mixture volume of 20 µL containing 10 µL of ready-to-use PCR mixture (Promega, USA), 1 µL of forward and reverse primers each (10 µM) and 1 µL DNA sample. Agarose gel electrophoresis was used to analyze the quantity and quality of extracted genomic DNA, PCR product and purified PCR product. PCR products were electrophoresed on a 2 % agarose gel, stained with ethidium bromide (10 mg/ml) and examined in a gel documentation system (Enduro GDS, Labnet, USA) [10]. The PCR products were sequenced by sending the samples to the Macrogen, South Korea. Sequences were compared with the other related sequences in GenBank (NCBI) by using BLAST.

2.6 Pathogenicity test

For artificial inoculation, uniform-sized, healthy leaves of *P. hastatum* was used. A 1 x 10^6 conidia ml^{-1} suspension was prepared from pure cultures of the fungal isolates as described in section 2.3. Then 20 µl drops (6 drops per leaf) were applied on to wounded and unwounded sites of leaves. Six replicate leaves were used for inoculation and another three leaves treated with drops of sterile distilled water served as controls. Inoculated leaves were incubated inside moisture chambers at 28 – 30 ℃ for 24 h to provide humid conditions to favor pathogen infection and then the moisture chambers were opened. The symptoms developed on inoculated leaves were compared with those of the original diseased leaves. The pathogens were re-isolated from the symptomatic leaves on PDA. The colony morphology and micromorphology of re-isolated pathogen were compared with those of the original isolate [11].

3. RESULTS AND DISCUSSION

3.1 Morphological characters of *Fusarium* species isolated from *P. hastatum*

The cultural appearances of *P. hastatum* isolates were sparse to abundant cottony mycelium with colony color and pigmentations from pale white to light violet. *Fusarium* species in *P. hastatum* have slightly curved and relatively thick macroconidia with a

slightly hook apical cell and foot shaped basal cell (Figure 3). The macroconidial septation of *Fusarium* species isolated from *P. hastatum* ranged from 3 to 5 and 21.8 to 63.9 µm length of macroconidia were observed.

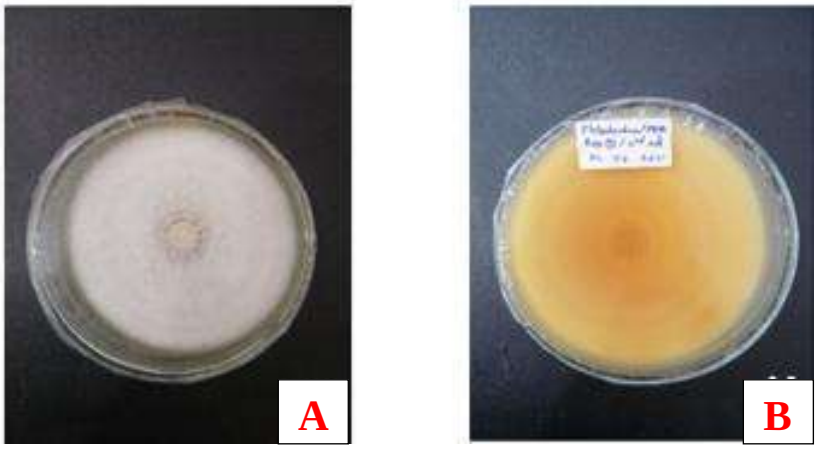

Figure 2: *Upper surface* ***(A)*** *and lower surface* ***(B)*** *views of the 7-days-old colony of mono-conidial fungal culture isolated from P. hastatum on PDA*

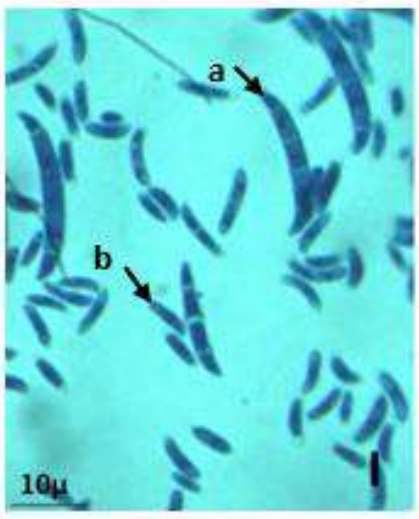

Figure 2: *Macro* ***(a)*** *and Micro* ***(b)*** *conidia of 7-day old monoconidial culture of Fusarium species isolated from P. hastatum mounted in lactophenol-cotton blue*

Colonies of *Fusarium* sp. grow quickly, 4.5 cm in four days, aerial mycelium white, becoming purple, with discrete orange sporodochia present in some strains, reverse hyaline to dark blue or dark purple. Conidiophores are short, single, lateral mono phialides in the aerial mycelium that are later arranged in densely branched clusters. Macroconidia are fusiform, slightly curved, and pointed at the tip, with three septate

basal cells and a size of 23-54 x 3-4.5 µm as described in 3.1. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved as showed in Figure 3. Chlamydospores are terminal or intercalary, hyaline, and 5-13 µm long [12].

3.2 Molecular characterization of the pathogen

Fungal culture, isolated from *P. hastatum* gave a clear band. The size of the PCR products is approximately 600 bp (Figure 6). The sequence of the fungal culture was 99 % similar to the sequence with Accession Number AB369259, based on the BLAST search results with authenticated sequences (GenBank, https://www.ncbi.nlm.nih.gov/). Pathogen was identified as *Fusarium oxysporum* based on the molecular and morphological data.

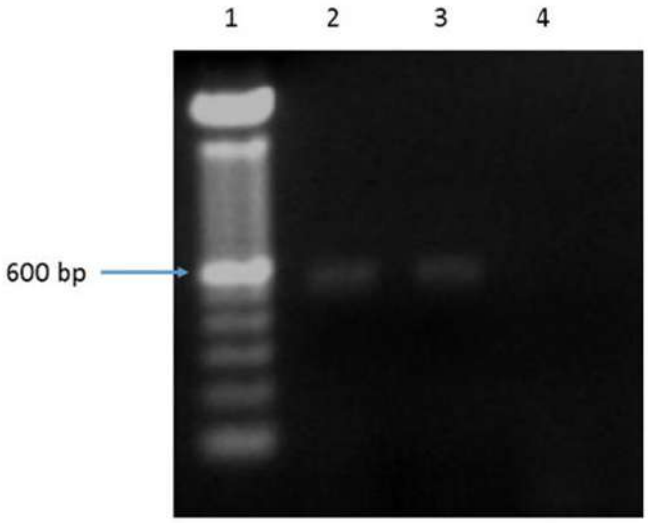

Figure 6: *Gel Electrophoresis of PCR product,* ***Lane 1:*** *DNA Ladder (Tracklt 100bp),* ***Lane 2 & 3:*** *PCR product of fungus culture isolated from leaf spots of P. hastatum (DNA Samples),* ***Lane 4:*** *Control.*

3.3 Pathogenicity test for *Fusarium* species

The pathogen with tapered and curved (falcate) conidia, subsequently identified as *Fusarium* species, was isolated from diseased leaves of *P. hastatum* and the pathogen indicated its consistent presence associated with the leaf spots. Seven days after inoculation, *Phylodendron* leaves artificially inoculated with *Fusarium* species

developed brown color, circular, necrotic lesions typical to *Phylodendron* leaf spot observed in originally used leaf samples for pathogen isolation (Figure 5A). The leaves that were inoculated without wounding, initiated lesions 3 - 4 days later and took few more days for expansion than those in wound-inoculated leaves. There were no symptoms observed in the control leaves. The culture plates prepared by re-isolation were morphologically similar to those used for inoculation (Figure 5B).

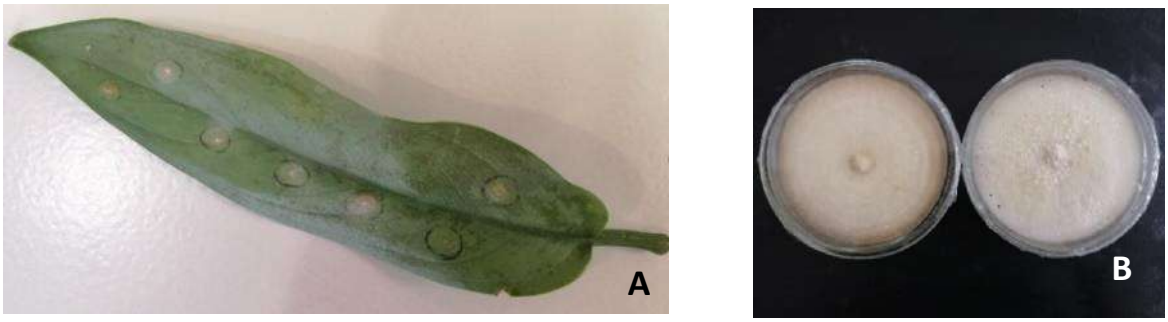

Figure 5: (A) *– P. hastatum leaf at 7 days after inoculation with Fusarium sp.* ***(B)*** *– Upper surface view of the culture plate on PDA prepared by re-isolation of Fusarium species from artificially inoculated leaf of P. hastatum (Right side), Upper view of the initial monoconidial culture of Fusarium sp. isolated from P. hastatum on PDA (Left side).*

Lilies are also susceptible to a variety of fungal and viral diseases as an ornamental plant. The most common disease in many Araceae plants is *Fusarium* leaf spot. Several *Fusarium* species have been linked to Lilies and *F. oxysporum* was the major species out of them. It causes symptoms such as oval brown spots on the leaves, leaf or sheath blights as described in *P. hastatum* and sometimes spreading until most of the leaf is destroyed [13].

Severe leaf spot disease has been observed on *Aloe vera* plants in the winters, during a survey of various nurseries of Gwalior, India. Irregular, sunken, dark cream-brown spots having reddish brown margin were noticed on both surfaces of the leaves as described in *P. hastatum*. According to the data of molecular identification of the research, the total 59 isolates of fungi were recovered from diseased *A. vera* leaves, and 37 isolates were identified as belonging to the genus *Fusarium* [14].

CONCLUSION

The results conclude that *Fusarium oxysporum* causes leaf spot disease in *P. hastatum* in Sri Lanka and there were no previous reports on the association of *Fusarium* species with the *P. hastatum* in Sri Lanka. The present work may lay the foundation for future studies related to management of the leaf spot disease under nursery or marketing conditions.

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