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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY

ISSN 2090-3812

www.eajbs.com

Vol. 16 No.1 (2025)

Citation: Egypt. *Acad. J. Biolog. Sci.* (H. Botany) *Vol.16 (1) pp 111-116 (2025)*
DOI: 10.21608/EAJBSH.2025.428260

Egypt. Acad. Journal Biology. Sci., 16 (1): 111-116 (2025)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbsh.journals.ekb.eg.

Fungal Diversity Associated with Leafspot Diseases of Some Horticultural Plants in The Botanical Garden, University of Lagos

Samuel, T.O. [1], Ebabhi, A. M. [2] and Odusipe, A. O. [1]

[1]Department of Botany, Faculty of Science, University of Lagos, Nigeria.
[2] Biology Unit, Department of Education, Distance Learning Institute, University of Lagos, Nigeria.

*E. Mail: tosamuel@unilag.edu.ng

ARTICLE INFO
Article History
Received:9/4/2025
Accepted:18/5/2025
Available:22/5/2025

***Keywords*:**
Fungi, identification, leafspot, horticultural plants.

ABSTRACT

Plants can be used for industrial, medicinal, nutritional, and aesthetic purposes. Leaf spot disease, which significantly lowers their horticultural yield, is mostly caused by fungal species. One way to mitigate this is to identify the causative agent or agents. This study aimed at isolating, identifying and characterizing pathogenic fungi associated with leaf spot diseases in some horticultural plants. Twenty horticultural plant species with leaf spot symptoms were obtained from the Botanical Garden, University of Lagos. Isolation was carried out using pour plate method and identification of fungi species was based on conventional and molecular methods. Results from this study revealed the presence of *Colletotrichum gloeosporioides*, species of *Periconia*, *Rhizopus*, *Mucor*, *Macrophomina*, *Mycosphaerella* and *Sclerotinia* as the causative agents. Further research on control and preventive measures is required in order to improve the health and yield of horticultural plant production and avoid a recurrence of similar incidents.

INTRODUCTION

Plants are always essential to meeting human requirements (Huang *et al.,* 2020). When it comes to ornamental plants, leaf spot diseases are most likely the prevalent and fungi are the primary cause of these diseases. Although other organisms like bacteria and nematodes can also cause leaf spot diseases. The plant and fungi species causing the infection might affect the symptoms of leaf spot. Usually, the earliest indications of disease are tiny, dark patches on the leaves, which may be uneven or round. The disease may cause the spots to grow and combine to form larger lesions, which may ultimately lower the yield and quality of the plants (Matić *et al*. 2020). Globally, phytofungal infections are a major issue for the food and agriculture industries (Khaskheli *et al.,* 2025). Furthermore, a lot of them also produce mycotoxins, which can seriously destroy crops and are dangerous to both human and animals. Whether a plant is suspected of being infected by the pathogen or shows obvious symptoms of illness, microbial pathogen detection is the process of identifying the ongoing existence of a certain target organism or organisms in a plant or its surroundings (Thilagam *et al.,* 2018). If diagnosed and detected promptly, it is possible to manage fungal leaf infections (Archana *et al.,* 2019).

MATERIALS AND METHODS

Study Area and Collection of Specimen:

The study was carried out in the Botanical Garden, University of Lagos, Yaba, Lagos, southwestern Nigeria among twenty plant species. It is geographically located on 6° 30' 40.10" N, 3° 23' 54.39" E and 07.01 m above sea level. The twenty plants species with leafspot signs studied were: *Dianthera secunda, Terminalia catappa, Jatropha carcus, Plumeria rubra, Dracaena hyancinthoides, Adenanthera pavonina, Banisteriopsis muricata, Cola cordifolia, Heliconia psittacorum, Abrus precatorius, Bischofia javanica, Philodendron giganteum, Pseuderanthemum maculatum, Acalypha wilkesiana, Capsicum frutescens, Tabernaemontana undulata, Capsicum chinense, Tabernaemontana pachysiphon, Milicia excelsa* and *Ficus nymphaeifolia.* Leaves with leafspot disease symptoms were collected by plucking with a sterile scissors and separately packed into sterile papers, folded on edges to form flat packet and these were labelled with plant's names. The collected samples were transported to the Plant Pathology Laboratory, Department of Botany, University of Lagos for further processes.

Fungal Isolation and Identification:

With the aid of a sterilized blade, diseased leaf part of the collected plants species was sectioned into sizeable pieces of about 5 mm x 5 mm. Each portion of the respective plant species was cut with a sterile tweezer, put in a Petri dish with 40% sodium hypochlorite, and shaken for 30 seconds (to get rid of opportunistic pathogens).

The cut pieces were then lifted out and rinsed in three Petri dishes containing sterile distilled water for five seconds respectively. The cut pieces were blot-dried and aseptically placed in a petri dish containing prepared Potato Dextrose Agar with chloramphenicol. These were incubated at room temperature (28-30 °C) and was observed daily for 5 days for fungal growth. Mycelial growth from each plate was subculture on freshly prepared PDA plates and the process was repeated until pure plates of isolates were obtained. The fungal isolate was identified using conventional taxonomic techniques as described by Talbot (1971). The morphology of each fungus was studied and compared with the descriptions of Alexopoulos *et al.* (2007) and Bryce, (1992).

Molecular Identification:

For the fungal isolates that were not identified conventionally, **t**he isolates were aseptically transferred from stock-culture into tubes containing potato dextrose broth (PDB) and incubated at room temperature for five days. The spore and mycelium of pure cultures were harvested for DNA extraction. The DNA extraction procedure of Zymo Research Bacterial/Fungi kit by Inqaba Biotec (South Africa) was adopted. A Cleaver Scientific Mini micro-centrifuge was used to spin down and slightly vortex the DNA samples. The concentration and purity of the samples were measured using 1 µl of the elution buffer as a blank. The concentration was measured in relation to the absorbance at 260 and 280 nm. An agarose gel stained with 1% (w/v) ethidium bromide was used to evaluate the purity of the isolated genomic DNA. Lambda DNA Hindlll, a 1 kb DNA ladder, was used as a molecular weight indicator. The electrodes were attached to the power pack so that the well was in the cathode's location. The parameters for the run were 90 volts for one hour. The gel was recorded using the OMNIDOC System from Cleaver Scientific.

Polymerase Chain Reaction (PCR):

Amplification of the ribosomal subunits was performed according to the New England Biolabs ITS1. Using One Taq quick load 2x master mix with standard buffer and the 16S-27F (5'AGAGTTTGATCMTGGCTCAG 3') and 16S-1492R (5'CGGTTACC TTGTTACGACTT 3'). The PCR reaction mixture (30 µl) containing 15 µl PCR master mix (New England Biolabs), 1 µl (10µM) of each primer, 7.5 µl nuclease free water and 4.5 µl template DNA 1.5 µl (10nm) of (ITS1 and ITS4). PCR amplification was performed in

cleaver scientific G-TC 965 following conditions 94 ℃ for 30 seconds; 94 ℃ for 30 seconds; 52 ºC for 1 minute; 68 ºC for 1 minute; 68 ºC for 5 minutes; 4 ºC hold in Cleaver Scientific GTC 96 S. PCR products were separated on 2% agarose gel, stained with ethidium bromide.

Sequencing and Phylogenetic Analysis:

The PCR amplicons of all fungal isolates were sequenced in Inqaba Biotec (South Africa). The sequences were trimmed and edited using Sequencher 5.4.6. Build 46289. Sequence Homology was done using the NCBI-BLAST tool and the phylogenetic tree using ClustalW in MEGA 11.

Pathogenicity Test:

Spore suspensions of each isolates were prepared by adding 10 ml of sterilized distilled water to a 21-day old PDA culture, dislodging the inoculum with a sterile glass rod and filtering through two layers of sterile muslin cloth. The spore suspension was made up to 200 ml by adding sterilized distilled water. Each prepared inoculum suspension was sprayed on a visually healthy species of same plant from which each isolate was initially isolated from with an atomizer. Test plant leaves were covered with sterile polythene bags for 24 hours, to allow the spores/mycelia to germinate on the leaves.

RESULTS

A total of 22 isolates were morphologically observed and found to belong to seven different fungal genera. These include*; Colletotrichum*, *Periconia*, *Rhizopus*, *Mucor*, *Macrophomina*, *Mycosphaerella* and *Sclerotinia* as shown on Table 1. Extracted genomic DNA from some fungal isolates that could not be identified morphologically showed intact bands which indicated that the extracted DNA is suitable for further downstream analysis. The purity of the extracted DNA was within the range of 1.8 - 2.0 ng/ul as expected for pure DNA and the agarose gels of PCR amplicons showed products ranging from 500 base pairs to 600 base pairs as expected for a successful amplification of the internally transcribed spacer regions of fungal species. After a preliminary identification, the ITS sequences of the extracted fungi obtained were analyzed to find their identical. The accession numbers issued by the GenBank and the percentage identification are summarized in Table 2. All of the fungi utilized in the pathogenicity test were re-isolated from artificially infected leaves with symptoms that were comparable to the initial leaf spot symptoms 20 days after the inoculation. This tends to validate the Koch's hypothesis.

Table 1: Sampled Plant Species and Isolated Fungal Species.

S/N	Plant species	Causative agent
1	*Dianthera secunda*	*Rhizopus* species
2	*Terminalia catappa*	*Macrophomina* species
3	*Jatropha carcus*	*Mucor* species
4	*Plumeria rubra*	*Rhizopus* species
5	*Dracaena hyancinthoides*	*Macrophomina* species
6	*Adenanthera pavonina*	*Periconia* species; *Sclerotinia* species
7	*Banisteriopsis muricata*	*Macrophomina* species
8	*Cola cordifolia*	*Macrophomina* species
9	*Heliconia psittacorum*	*Colletotrichum gloeosporioides*
10	*Abrus precatorius*	*Rhizopus* species
11	*Bischofia javanica*	*Mucor* species
12	*Philodendron giganteum*	*Rhizopus* species
13	*Pseuderanthemum maculatum*	*Colletotrichum gloeosporioides; Colleototrichum* species
14	*Acalypha wilkesiana*	*Macrophomina* species
15	*Capsicum frutescens*	*Macrophomina* species
16	*Tabernaemontana undulata*	*Mycosphaerella* species; *Colletotrichum gloeosporioides*
17	*Capsicum chinense*	*Macrophomina* species
18	*Tabernaemontana pachysiphon*	*Mucor* species
19	*Milicia excelsa*	*Macrophomina* species
20	*Ficus nymphaeifolia*	*Macrophomina* species

Table 2: Percent Sequence Similarity and GenBank Accession

Name of Sample	Organism	Percentage Identification	GenBank Accession
Tabernaemontana undulata	*Colletotrichum gloeosporioides*	99.83%	MG925324.1
Heliconia psittacorum	*Colletotrichum gloeosporioides*	95.01%	MH156760.1
Adenanthera pavonina	*Periconia species.*	98.23%	ON207652.1

DISCUSSION

The leafspots observed in the plant species in this study showed diverse symptoms. This corresponds to Sun *et al.* (2020) study who reported different several symptoms of leafspot on *Zea mays* caused by different species of *Bipolaris*. Typical leaf spots, on the other hand, typically have brown, black, tan, or reddish centres with quite distinct edges. Spots can clump together to cover entire leaves and range in diameter from pinhead to several centimetres. Some spots can be elevated or fallen out, giving the leaf a shot-hole look, while others are irregular or round in shape.

The majority of leaf spot diseases are brought on by fungi, while nematodes and bacteria can also cause foliar diseases. Some genera of fungi associated with leaf spots

isolated from this study are *Alternaria, Ascochvta, Blumeriella, Cercospora, Colletotrichum, Entomosporium, Gnomonia, Guignardia, Mvcosphaerella, Phyllosticta, Septoria, Tubakia, and Venturia*. These findings are consistent with the findings of Douglas (2020) who stated that these fungi species are commonly associated with leaf spots. He also found that heavily infected leaves turn yellow and brown, shrivel and drop prematurely. *Macrophomina* species was identified in *Terminalia catappa, Dracaena hyacinthoides, Banisteriopsis muricata* and *Cola cordifolia* This fungus is known to cause charcoal rot in *Glycine max,* leaf spot and rotting of developing pods and seed in *Arachis hypogaea* while in *Zea mays,* it causes a stalk rot during hot, dry conditions (Samuel and Adekunle, 2015; Rustam Ugli *et al.* 2024).

Mucor species was isolated from diseased leaf spots of *Jatropha carcus, Heliconia psittacorum* and *Bischofia javanica*. These findings are consistent with the findings reported by Rodriguez and Hernández (2004) who concluded that *Mucor hiemalis* was consistently found in the leaf lesions of young peach trees. They reported that the pathogenicity tests confirmed *Mucor hiemalis* as the cause of the foliar spots in peaches. The disease developed more quickly and extensively when injured tissues were infected on the lower side of the leaves. Lesions started as water-soaked patches that progressively grew as the pathogen colonized them. Severe necrosis and death of leaf tissues happened seven days after inoculation.

It was recorded as India's first instance of *Periconia macrospinosa* producing pointed gourd leaf necrosis. *Adenanthera pavonina* sick leaf patches were used to isolate and identify *Periconia* species (Sarkar *et al.*,2019). Twenty-two isolates of *Sclerotium rolfsii* causing spotted leaf rot from Varanasi, India were grown on 6% *Cyperus rotundus* rhizome meal agar (CRMA) medium for the induction of athelial stage (Sarkar *et al.*,2019). *Sclerotinia,* which was isolated from *Adenanthera pavonina,* is one of the fungi that have been isolated from the leaf spot diseases of twenty plant species in the University of Lagos botanical garden. Understanding the various morphological and anatomical effects of a disease as well as its infection process is essential when determining how to control it (Adekunle, 2011). Leafspots on leaves decrease plant's photosynthetic area, which in turn affects how much assimilate enters the sink. Crop failure can occur when severe leaf spot infections cause complete defoliation. Similar to this, a large number of organisms that cause leaf spots can partially or completely kill the host by destroying the tissues directly as well as by spreading poisonous chemicals throughout the body well beyond the initial infection site. As a result, the leaves are of lower quality and quantity for use as vegetables and cattle feed, respectively (Ilondu, 2013).

CONCLUSION

Using molecular and morphological identification methods, this current study provides the precise identities of the pathogenic fungi associated with leaf spot diseases in some horticultural plants. To combat the diseases caused in these horticultural plants, the information provided here serves as an update on the current understanding of these fungal pathogen groups. To enhance the health and productivity of these horticultural plant productions and prevent a repeat of similar situations, more studies on control and preventive measures is needed.

Declarations:

Ethical Approval: There is no need for ethical issues because no human, animal, or plant subjects were used in this study.

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content of this paper.

Authors Contributions: I hereby verify that all authors mentioned on the title page have contributed significantly to the idea and planning of the research, has carefully read the work, attested to the veracity and correctness of the data and its interpretation, and has given their approval for submission.

Funding: For this study, the authors did not receive any kind of funding.
Availability of Data and Materials: Upon reasonable request, the corresponding author will make all datasets analyzed and described in this paper available.
Acknowledgements: Special thanks to the staff of the Department of Botany, University of Lagos who were of great assistance throughout this study.

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