

**CHARACTERISATION AND MANAGEMENT OF FUNGAL
DISEASES OF *Carica papaya* (L.) IN A RESEARCH FARM**

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**A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE
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SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY,
COVENANT UNIVERSITY, OTA.**

JUNE, 2021

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Microbiology, in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria.

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DECLARATION

I, **ONIHA, MARGARET IKHIWILI (CUGP100271)** declare that this research was carried out by me under the supervision of Dr. Angela O. Eni and Dr. Olayemi O. Akinnola of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

ONIHA, MARGARET IKHIWILI

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Signature and Date

CERTIFICATION

We certify that this thesis titled “**Characterisation and Management of Fungal Diseases of *Carica papaya* (L.) in a Research Farm**” is an original research work carried out by **ONHA, MARGARET IKHIWILI (CUGP100271)**, in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Dr. Angela O. Eni and Dr. Olayemi O. Akinnola. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Microbiology.

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DEDICATION

I dedicate this project to God, the Almighty Father, for the manifestation of His divine and everlasting power. To Him alone be all the glory, honour and praises forever more.

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ABBREVIATIONS

ABI- Applied Biosystems	VHT- Vapour Heat Treatment
BLAST- Basic Local Alignment Search Tool	V- Volts
bp- base pair	
CMC- Carboxyl methyl cellulose	
DNA- Deoxyribonucleic acid	
DI- Disease Incidence	
DMRT- Duncan's Multiple Range Test	
DMSO- Dimethyl sulphoxide	
DPI- Days Post Inoculation	
DS- Disease Severity	
FAO- Food and Agriculture Organization	
GPS- Global Positioning System	
ITS- Internal Transcribed Spacer	
kg- Kilogram	
LSU- Large subunit	
LSD- Least Significant Difference	
MEGA- Molecular Evolution Genetic Analysis	
MIC- Minimum Inhibitory Concentration	
MFC- Minimum Fungicidal Concentration	
Mg- Milligrammes	
ml- Millilitres	
mm- Millimeters	
m/s- meters/second	
Mt- Metric Tonnes	
NCBI- National Center for Biotechnology Information	
NJ- Neighbour Joining	
PCR- Polymerase Chain Reaction	
PBS- Phosphate Buffered Saline	
RNA- Ribonucleic acid	
SSU- Small subunit	
TBE- Tris boric ethylene diamine tetracetic acid	
µl- Microliter	

ABSTRACT

Fungi are the most important group of microorganisms that attack *Carica papaya* leading to economic losses. Previous research on papaya disease management tend to focus on post-harvest diseases compared to field diseases which may be directly linked to the post-harvest diseases. Chemical fungicides are the major management mechanism employed to control preharvest fungal diseases with consequent toxicological risks to biota. This study was conducted to evaluate fungal diseases of *C. papaya* obtained from the pawpaw research demonstration farm in Covenant University. The study also evaluated the antifungal activity of some plant extracts as potential biocontrol agents. The fungal disease symptoms observed during the study, were evaluated and analyzed using disease incidence and severity as measurement tools. Fruit, leaf and stem samples were collected from infected papaya to isolate fungal pathogens during the early and late preharvest seasons. The isolates were first phenotypically characterised and then genotypically identified based on the Internal Transcribed Spacer genes. To assess the pathogenicity of the isolated fungi, the isolates were screened *in vitro* for cellulolytic and pectinolytic enzyme production using plate screening assay. Then n-Hexane, ethyl acetate, ethanol, methanol and aqueous leaf extracts of *Moringa oleifera* Lam, *Chrysophyllum albidum* G. Don, *Celosia argentea* L, *Telfaira occidentalis* Hook F. and *Bauhinia monandra* BmoLL were evaluated for antifungal activity *in vitro*. Three aqueous extracts with best activity were further evaluated *in vivo* on papaya fruits. Results of the study showed that disease severity was significantly higher in the late than the early preharvest season. Phenotypically characterised fungal isolates were confirmed genotypically as *Aspergillus niger* Van.Tiegham, *Aspergillus aculeatus* Lizuka, *Aspergillus flavus* Link, *Aspergillus fumigatus* Fresineus, *Trichoderma reesei* QM6a, *Trichoderma longibrachiatum* Rifai, *Rhizopus oryzae* Went and Geerlings and *Rhizopus delemar* Boidin. *Aspergillus niger* was the most frequently isolated fungus from fruit (29.41%) and stem (27.94%) samples while *Geotrichum* spp Link was the most frequently isolated from leaves (20.90%). Phylogenetic analysis of the amino acid sequence data showed the evolutionary relatedness of the characterised fungal species with their nearest neighbours. Secretion of cell wall degrading enzymes varied significantly among the fungal isolates indicating significant variation in pathogenicity ($p < 0.05$). *In vitro* antifungal, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assessments showed that the aqueous extracts had significant effects ($p = 0.000$) against all assessed fungal isolates than other extract types except for the ethanolic and methanolic extracts of *C. argentea* and *C. albidum* respectively. The highest zones of inhibition of the various aqueous extracts ranged from 23.33-39.00 mm and compared positively against Ketoconazole (11-15 mm; control antifungal agent). The aqueous extract of *B. monandra* had the highest *in vivo* activity with 150 mg/ml and 75 mg/ml inhibiting fungal growth for up to 5 and 3 days respectively. This study has generated fresh fungal sequence data which have been deposited into the genebank database. The study also identified that members of *Aspergillus* and *Rhizopus* genera are the major fungi responsible for papaya diseases. It also further validated the potentials of

some medicinal plants as antifungal agents for the sustainable management of papaya diseases.

Keywords: *Carica papaya*, Fungi, Antifungal plants, Papaya disease management, Pawpaw farm