

**FUNGI ASSOCIATED WITH THE LEAF BLIGHT OF *OCHROMA PYRAMIDALE* (Cav.
ex Lam) Urb. IN THE UNIVERSITY OF BENIN, BENIN CITY**

BY

**Emmanuel Emeka IKPASEH
AGR1700281**

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WILDLIFE MANAGEMENT, UNIVERSITY
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**IN PARTIAL FUFILMENT OF THE REQUIREMNT FOR BACHELOR'S DEGREE
IN AGRICULTURE (FOREST RESOURCES AND
WILDLIFE MANAGEMENT) OF THE UNIVERSITY OF BENIN,
BENIN CITY, NIGERIA**

OCTOBER, 2023

CERTIFICATION

This is to certify that this project work was carried out by **Emmanuel Emeka IKPASEH** in the Department of Forest Resources and Wildlife Management, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria

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(Head of Department)

Date

Date

DEDICATION

I dedicate this work first and foremost to Almighty God who has been there right from the beginning to this very point. Special dedication also to my ever-supportive parents for their relentless support and motivation during the course of this project. Also, to my supervisor PROF. (MRS.) M.I IKHATUA who made this project work a success.

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TABLE OF CONTENT

	Page
Title.....	I
Certification.....	ii
Dedication.....	iii
Acknowledgement.....	iv
Table of Content.....	v
List of Tables.....	vii
List of Plates	viii
Abstract.....	ix

CHAPTER ONE

1.0 INTRODUCTION.....	1
1.1 Background to the Study.....	1
1.2 Uses of <i>Ochroma pyramidale</i>	2
1.3 Propagation.....	3
1.3 Pests and Diseases.....	3
1.5 Statement of Problem.....	4
1.6 Justification of the Study.....	4
1.7 Objective of the Study.....	4

CHAPTER TWO

2.0 Literature Review.....	5
2.1. Leaf blight	6
2.2 Common Tree Diseases	7
2.3 Control of Tree Diseases.....	8

CHAPTER THREE

3.0 Materials and methods.....	10
3.1 Collection of Samples	10

3.2	Sterilization of Plates.....	10
3.3	Preparation of the Medium.....	10
+		
3.4	Pouring of Medium.....	10
3.5	Isolation.....	11
3.5.1	Preparation of Samples.....	11
3.5.2	Inoculation of Plates.....	11
3.6	Sub-culturing.....	12
3.7	Preparation of Stock Culture.....	12
3.8	Identification of Isolates.....	12
CHAPTER FOUR		
4.1	Results.....	13
4.2	Discussion.....	16
CHAPTER FIVE		
5.1	Conclusion	18
5.2	Recommendation.....	18
REFERENCES.....		19

LIST OF TABLE

Table 1: Cultural and morphological characteristics of the fungal isolates associated with the diseased leaves.....	15
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LIST OF PLATES

Plate 1: Photograph of healthy (left) and diseased (right) leaves of *Ochroma pyramidale*
.....13

Plate 2: Photograph of 7-day old culture of *Sclerotium rolfsii* on PDA medium: From left to right are the upper and lower surfaces.....14

Plate 3: Photograph of 7-day old culture of *Penicillium* sp. on PDA medium: From left to right are the upper and lower surfaces14

Plate 4: Photograph of stock cultures of *Sclerotium rolfsii* (left) and *Penicillium* sp. (right).....14

ABSTRACT

This study was carried out to examine the fungi associated with the leaf blight of *Ochroma pyramidale* trees. The diseased leaves of *Ochroma pyramidale* were obtained from the Department of Forest Resources and Wildlife Management Arboretum, Faculty of Agriculture, University of Benin, Ugbowo Campus, Benin City. Isolation, sub-culturing, identification of isolates were carried out. The medium used was Potato Dextrose Agar (PDA). Identification of isolates under the light microscope was carried out. The symptoms observed on the leaves were darkish brown to black lesions that spread around the edges of the leaves and progressed into the lamina. Two fungi were found to be associated with the leaf blight of *Ochroma pyramidale*. These are *Sclerotium rolfsii* and *Penicillium* sp. More research work should be embarked upon to ascertain the pathogen of the disease and how to effectively control and prevent its development.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Ochroma pyramidale (Cav. ex Lam.) Urb. commonly known as the balsa tree belongs to the family Malvaceae. It is a large, fast-growing tree, and is the sole member of the genus *Ochroma*. *Ochroma pyramidale* is native from southern Mexico to southern Brazil, but can now be found in many other countries like Papua New Guinea, Indonesia, Thailand, Solomon Islands (Dahms 1991). It is the lightest known commercial timber, being even lighter than cork.

Ochroma pyramidale is a typical pioneer plant, which establishes itself in clearings in forests, either man-made or where trees have fallen, or in abandoned agricultural fields. (Fletcher 1951). It grows extremely rapidly, up to 27 meters (89ft) in 10–15 years. The speed of growth accounts for the lightness of the wood, which has a lower density than cork. (Angier and Ziegler 2011).

It produces flowers from the third year onwards, typically at the end of the rainy season when few other trees are in flower. The large flowers open in the late afternoon and remain open overnight. Each may contain a pool of nectar up to 2.5 centimetres (0.98 in) deep. Daytime pollinators include capuchin monkeys. However, most pollination occurs at night. The main pollinators were once thought to be bats, but recent evidence suggests that two nocturnal arboreal mammals, the kinkajou and the olingo, may be the primary pollinators. (Angier and Ziegler 2011).

In natural conditions it occurs up to 1000 m altitude, in areas with an annual precipitation of 1250–3000 mm and a mean annual temperature of 22–28°C. It tolerates a dry season of up to 5 months, but only if the relative humidity does not normally drop below 75%.

Balsa is one of the fastest growing wood species, reaching about 20 m in height and up to 75 cm in diameter in 5-8 years and because of its fast growth, the wood density is very low. (Fletcher 1951).

It is evergreen or dry-season deciduous, with large 30 to 40 centimetres (12 to 16 in), weakly palmately lobed leaves. It has a simple, alternate, triangular heart shaped or palm shaped leaves and pinnately veined with 7–9 pairs of lateral veins. The leaves and the stalks each grow up to 40 cm long. (Quimbo 1980).

Ecuador is known to be the largest supplier of commercial balsa of about 95% or more. In recent years, about 60% of the balsa has been plantation-grown in densely packed patches of around 1000 trees per hectare (compared to about two to three per hectare in nature). The trees are harvested after six to ten years of growth. The scattering of balsa trees over large forested areas makes their harvesting difficult and expensive. For this reason, most balsa wood used commercially is harvested from plantations, particularly from Ecuador.

1.2 Uses

- Balsa wood is the lightest commercial lumber and it is used for building model airplanes and floats. At one time, it was used in the aeronautics industry (Whitmore 1978).
- The bark of this tree is made into ropes and belts.
- The kapok in its fruit is used to stuff mattresses, pillows and plush animals.
- Balsa is used to make wooden crank baits for fishing
- Balsa is also used in the manufacture of "breakaway" wooden props such as tables and chairs that are designed to be broken as part of theatre, movie, and television productions (Whitmore 1978).

- For medicinal uses: The root bark is emetic, it is usually taken to cause vomiting (stomach ailment). Indigenous Peoples use its leaves in infusions to cure foot and mouth diseases of livestock.

1.3 Propagation

The seed needs high temperatures to germinate. Seeds can be sown directly in the field or in the nursery. Freshly collected seeds have only 10% germination.

Seeds contain an impervious testa which must be ruptured by heat (boiling water, fire) before they germinate. These pre-treated seeds show 65% -75% germination. The very small seeds should be collected from standing trees, and can be stored for several years in jute bags or in closed containers. (Huxley 1992).

1.4 Pests and diseases

According to Smith and Cognato (2013) a new species of *Xyleborine* ambrosia beetle has been found to attack balsa, *Ochroma pyramidale* (Cavanilles ex Lamarck) Urban, in Ecuador.

Coptoborus ochromactonus attacked balsa between 1.5 and 3 years in age. Successful attacks were more prevalent in smaller diameter trees and unhealthy trees. (Smith and Cognato 2013).

With the advent of large scale plantations of *Ochroma pyramidale*, serious pest and disease problems have emerged. The most common problems are caused by wood-boring beetles (Buprestidae and Scolytinae), leaf-feeding weevils (Curculionidae, *Heilipus* sp.) and fungal diseases such as *pataroja* (red foot), which is likely caused by *Phytophthora palmivora* (Butler) Butler (Tainter *et al.* 2001), *Phythium vexans* de Bary (Delgado and Holmes 2006). *Polyporus*

leprieurii var. *yasuniensis* is a prolific wood-decay fungus that attack mostly the branches or root of *Ochroma pyramidale*. (Toapanta-Alban, 2022)

Fusarium oxysporum is a soil-borne fungus that infect the root of *Ochroma pyramidale* causing wilting, yellowing, and eventually death of the plant. (Hussian and Muhammad, 2011).

1.5 Statement of the problem

Blight was observed on the leaves of *Ochroma pyramidale* tree and the incidence was very high (i.e. 80%) with the number of affected trees up to 4 out of the 5 sighted. The affected areas of the leaves showed necrotic lesions on the lamina and appeared dark-brown.

1.6 Justification of the study

Due to the high degree of incidence of blight experienced on the leaves of *Ochroma pyramidale* trees in the Arboretum of the Department of Forest Resources and Wildlife Management, Faculty of Agriculture, University of Benin, this study was undertaken to provide knowledge on the fungi associated with the diseased leaves.

1.7 Objective of the study

The main objective of the study is to investigate the fungi associated with the leaf blight on *Ochroma pyramidale*.

The specific objective is:

1. To isolate and identify the fungi associated with the blight on the leaves of *Ochroma pyramidale*.

CHAPTER TWO

2.0

LITERATURE REVIEW

Disease of a plant is an abnormality in the structure, function of the host plant cells and tissue as a result of continuous irritation caused by pathogenic agents or an environmental factor (Agrios, 2005).

A disease is not constant, it is a series of changes in plant. All plants, to an extent, are subjected to disease. Plant disease is the result of an infectious, or biotic agent (a living component of an ecosystem) or a non-infectious, or abiotic agent (non- living, physical and/or chemical component) factor. Biotic diseases are caused by living organisms (e.g. fungi, bacteria, and viruses) while abiotic diseases are caused by non-living environmental conditions, (e.g. Soil compaction, wind, frost, soil salt damage, and girdling roots (Ogunsiji *et al.*, 2020).

Pathogenic fungi cause plant diseases such as leaf wilting, discolouration of foliage (damping off, leaf blister, brown spot, leaf spot sooty moulds foliage rust, powdery mildews, anthracnose and blight) and early leaf drop (Iqbal *et al.*, 2018).

It has been shown that, the major causal pathogens of leaf diseases are a wide range of fungi (Maizatul-Suriza *et al.*, 2019) which are of great threat to nurseries and young plantations as it can lead to yield reduction (Coutinho *et al.*, 1998). High humidity favours development of the great majority of leaf diseases caused by fungi, water molds, and bacteria. Moisture is generally needed for spore germination, the multiplication and the initiation of infection.

Excessive moisture in the soil, overwatering, shade, high density seedlings and warm humid hot temperature are favourable for leaf disease development (Thiribhuvanamala *et al.*, 2017). Leaf disease is a serious threat to nurseries and young plantations (Coutinho *et al.*, 1998). This can

lead to yield reduction. It causes the reduction of available resources for plants, which fail to produce enough biomass, seeds, and thus yield (Sinclair *et al.*, 2004).

2.1 Leaf blight

Leaf blight is a fungal disease affecting plants in which portion of the leaves become discoloured, dried out but not rotten. Leaf blight is the rapid and complete chlorosis, browning of leaves which may result to death of the plant eventually (Agrios, 2005). Symptoms of early blight first appear at the base of affected plants where roughly circular brown spots appear on leaves (Sanoubar and Barbanti 2017).

Notable blight occurrences on forest trees are:

- Leaf blight disease of *Gmelina arborea* which is caused by *Glomerella cingulata* in association with *Fusarium solani*: The blighted leaves often show holes in the infected portion as a result of shedding of infected tissues during heavy rains. Infected plants exhibit blighting of shoots and leaves (Sharma and Matthew 1990). Rapid spread of this disease can cause large scale mortality of nursery stocks.
- Leaf blight disease of *Mansonia altissima* which is caused by *Cercospora mansoniicola*. *Cercospora mansoniicola* affect the leaves tissues by invading and colonizing the cells of the leaf, producing toxin that damage the cells and cause tissue death. The infected leaves may become distorted or twisted and eventually fall off the tree, causing defoliation. (Mizielinska and Szczepkowski, 2019).

2.2 Common tree diseases

Macrophoma mangifera caused fungal leaf spot disease of mango (*Mangifera indica*) in Nigeria which resulted in significant loss of crop (Okigbo and Osunde 2003).

Cercospora sisso attacked the leaves of *Dalbergia sisso* mostly on the lower surface producing yellowish to grayish-green discolouration (Shamsi *et al.* 2012).

Fusarium solani and *Lasiodiplodia theobromae* were discovered to be linked to stem dieback of *Ceiba pentandra* seedlings in both the nursery and field (Apetorgbor *et al.*, 2003). *Fusarium solani* has also been associated with dieback that led to decline of *Milicia excelsa* seedlings. (Apetorgbor *et al.*, 2003).

Rhizoctonia leaf web blight of *Albizia lebbek* is caused by *Rhizoctonia solani*. This disease was also reported on other broad leaved trees like *Tectona grandis*. It first appears on leaves close to the ground, as water soaked silvery grey blotches. During the rainy season the fungal invasion of foliage is rapid over the wet leaf surface (Mehrotra, 1990). This disease was reported for the first time in Assam (Mehrotra, 1990).

Anthraxnose was found to be connected with majority of guava trees grown in the humid forest region of southern Nigeria. This anthracnose was frequently found on the leaves. Guava production in the area is now all but unattractive to farmers and gardeners at home due to this disease (Amusa *et al.*, 2006).

According to Mordue and Rahayu (1974) three pathogens potentially causing disease in the nursery: *Pythium* sp., *Colletotrichum* sp. and *Sclerotium* sp. were present on the leaves of *Gmelina arborea* which caused leaf blight and leaf spot diseases only on seedlings. They are commonly present on vegetative than generative propagation material. This may be related to the

characteristics of *Sclerotium* sp. as a facultative parasite able to develop sclerotia that can survive for long time in the soil (Mordue and Rahayu, 1974).

According to Abraham *et al.*, (1988) anthracnose disease affects Breadfruit (*Artocarpus altilis*) and jackfruit (*Artocarpus heterophyllus*). The symptoms develop on twigs, leaves and fruit. Lesion on leaf starts as small dark spot that expands gradually to become grey at the center with dark brown margins.

2.3 Control of plant diseases

Trichoderma harzianum found in olive rhizosphere soils in northern Algeria had great biocontrol potential against *Verticillium dahliae*, the causal agent of wilting on olive trees. These isolates (*Trichoderma* spp.) showed an effective potential in reducing the in-vitro mycelial growth of this pathogenic fungus (Reghmit *et al.*, 2021).

Trichoderma harzianum has also been proven to be effective in suppressing *Sclerospora graminicola*, the causal agent of pearl millet downy mildew disease. *Trichoderma* spp. prominent role in directly suppressing the pathogen in the rhizosphere and establishing systemic resistance has been well demonstrated (Nandini *et al.*, 2021).

The inoculation of *Theobroma cacao* leaves with the foliar fungal endophyte *Colletotrichum tropicale* stimulated the expression of several host genes involved in the defense against pathogen (*Phytophthora palmivora*) and herbivore attack (*Thysanoptera*). (Porras and Bayman 2011).

Lumsden *et al.*, (1992) found out that suppressive activity of *Trichoderma virens* (GL-21) to damping-off of zinnias, incited by both *Rhizoctonia solani* and *Pythium ultimum*, was correlated with production of the antibiotic gliotoxin by the biocontrol agent.

Copping and Duke, (2007) reported that Neem oil has been used to kill fire blight of *Malus sylvestris* caused by *Erwinia amylovora*, a bacterial disease that causes the leaves of the tree to wilt and appear as though they have been burned.

Enyiukwu and Awurum, (2011) reported that phytochemicals from some tropical plants (*Carica papaya* and *Piper guineense*) were found to strongly retard the germination of spores of *Collectotrichum destructivum*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Diseased leaves of *Ochroma pyramidale* were obtained from the Arboretum of the Department of Forest Resources and Wildlife Management, University of Benin. Photographs of the diseased leaves were taken

3.2 Sterilization of Petri Dishes

Clean Petri dishes 6cm and 9cm in diameter were washed, dried and stacked in a series of six, each wrapped with brown paper which was held with masking tape and then covered with aluminum foil paper. The dishes were placed in the oven at 160°C for 2 hours for sterilization. After sterilization the temperature of the oven was allowed to drop to normal room temperature before bringing out the dishes.

3.3 Preparation of the Medium

The solid agar medium used for culturing the micro-organism was Potato Dextrose Agar (PDA). 39 grams of PDA was weighed on an electric scale balance and dissolved in 1000ml of water i.e 39g/L in a calibrated conical flask. The medium was sterilized at a temperature of 121°C at 15psi (pounds per square inch) for 15 minutes using the autoclave. The medium was allowed to cool before pouring into the Petri dishes.

3.4 Pouring of Medium

The pouring of the medium was done within the lamina flow hood. The lamina flow hood was lit up with the fan switched on and was surface sterilized using cotton wool soaked in Methylated

spirit. Before pouring, the neck of the conical flask was sterilized using the flame from the spirit lamp to prevent contamination of the PDA medium. The medium (approximately 15ml) was poured into each sterile Petri dish and allowed to cool and solidify. Five Petri dishes each were arranged in transparent polythene bags, sealed and put in the refrigerator to preserve the medium.

3.5 ISOLATION

3.5.1 Preparation of samples

Samples of the diseased leaves were obtained from the field. The zone between the healthy and diseased portion of *Ochroma pyramidale* leaves were cut using a pair of scissors. The tissues were further cut into pieces of about 2mmx2mm. The samples were surface sterilized using bleach solution (1:4, V: V of bleach to water) in a universal bottle for three minutes after which the solution was decanted.

3.5.2 Inoculation of plates

The procedure was carried out in the lamina flow hood which was cleaned with 70% methylated spirit to ensure a sterile environment. The light and fan were switched on for at least 15 minutes. Materials to be used were placed in the lamina flow hood. The sterile tissue were removed from the universal bottle using a pair of sterile forceps and were placed on sterile absorbent papers. The forceps was dipped in methylated spirit and flamed using the spirit lamp to avoid contamination. With the sterile forceps, four tissue chips were inoculated into each plate of PDA medium. The inoculated plates were sealed with cling film, kept at room temperature ($30\pm 2^{\circ}\text{C}$) and daily observation was made on the microbial growth and records kept.

3.6 Sub-culturing

The procedure was done to obtain pure cultures of the isolates. Using a sterile inoculating needle, the inoculum from the isolates was introduced into sterile plates containing PDA medium within the lamina flow hood. The plates were labelled and sealed with the cling film and kept at room temperature ($30\pm 2^{\circ}\text{C}$). Daily observations were made and records kept.

3.7 Preparation of Stock Cultures

This was prepared by pouring 10ml of dissolved potato dextrose agar medium into several washed bottles (Bijou bottles). Sterilization of the medium in the bottles was done in an autoclave at 121°C at 15 psi for 15 minutes. After sterilization, the bottles were arranged in a slant position to solidify. After 24 hours, the bottles were inoculated with hyphae obtained from the pure cultures of isolates and incubated at room temperature ($30\pm 2^{\circ}\text{C}$) for growth.

3.8 Identification of Isolates

Slides of individual isolates were prepared. The sterile needle was used to transfer hyphae or spores from the pure culture of isolates to drops of water on or lactophenol cotton blue (to stain the white isolate) clean glass slides. The hyphae were teased with two inoculating needles and cover slips were placed on them. These were viewed under a light microscope in order to identify the organisms based on the morphological characteristics of vegetative parts and spores (Barnett and Hunter 1998).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The symptoms observed on the leaves were darkish brown to black lesions that spread around the edges of the leaves and progressed into the lamina. (Plate 1)



Plate 1: Photograph of healthy (left) and diseased (right) leaves of *Ochroma pyramidale*.

Two fungal isolates were associated with the disease. A dark isolate identified as *Sclerotium rolfsii* (Plate 2) and a whitish isolate identified as *Penicillium* sp. (Plate 3). Their stock cultures are shown in Plate 4.

The cultural and morphological characteristics of the isolates are shown in Table 1.



Plate 2: Photograph of 7-day-old culture of *Sclerotium rolfsii* on PDA medium: From Left to Right are the upper and lower surfaces



Plate 3: Photograph of 7-day-old culture of *Penicillium* sp. on PDA medium: From Left to Right are the upper and lower surfaces



Plate 4: Photograph of stock cultures of *Sclerotium rolfsii* (left) and *Penicillium* sp. (right)

Cultural and morphological characteristics of the fungal isolates associated with the diseased leaves

Isolates	Cultural characteristics	Morphological characteristics	Identification
Isolate 1	Black growth which is slightly raised from the medium surface. It's under surface showed black colouration.	The hyphae are brownish and septate. Does not produce regular spores but produces globose or irregular sclerotia from the mycelium.	<i>Sclerotium rolfsii</i>
Isolate 2	White fast-spreading growth which is slightly raised from the medium surface. The undersurface has a brownish colour which later turned pinkish.	Hyphae are hyaline, septate and conidiophores arising from the mycelium singly or less often in synnemata. Spores borne on hyphae in broom like structures.	<i>Penicillium</i> sp.

4.2 DISCUSSION

The identified isolates *Sclerotium rolfsii* and *Penicillium* sp. were found to be the fungi associated with the leaf blight of *Ochroma pyramidale*. The symptoms observed were typical of blight and were similar to those reported by Rai and Mammatha, (2005) in which affected leaves showed dark brown lesions on the upper leaf surface which led to leaf distortion and defoliation.

Sclerotium rolfsii was found to be the pathogen responsible for stem rot and mortality of *Acacia mangium*, *Intsia palembanica* and *Shorea parvifolia* forest trees in Malaysia (Karim *et al.*, 2013).

Studies carried out in India reported that *Sclerotium rolfsii* was the causative agent of stem blight in *Tectona grandis* and *Grevillea robusta* plantations. Symptoms observed were softening stem, discolouration, and rotting of the affected tissues. (Kuppuswamy *et la.*, 2005)

The fungus *Sclerotium rolfsii* is also known to attack the root and stem of Eucalyptus trees, leading to the wilting, yellowing and eventually death of the tree. (Doley *et la.*, 2014).

Two new diseases of forest tree seedlings caused by *Sclerotium rolfsii* i.e collar rot of *Swietenia macrophylla* and leaf blight of *Pterocarpus santalinus* were reported for the first time in India. Symptoms observed on the *Swietenia macrophylla* were yellowing and wilting of leaves, water soaked lesions were seen at the collar region of the seedlings near the soil surface. On *Pterocarpus santalinus*, appearance of small dull brown spots on the leaves was the first symptom observed. Later, these necrosed spots coalesced to cover large range of the leaf giving a typically blight appearance. (Sankaran *et al.*, 1984).

Penicillium cellarum was first discovered to be the pathogen causing rot disease on *Dioscorea polystachya* (Chinese yam) in China. The diseased part of Chinese yam tuber rot expands from

the outside to the inside and sags, with a brown or dark brown discoloration, and the surface covered with a thick grayish green mold. (Wang *et al.*, 2023).

Penicillium purpurogenum was found to be the fungus causing root and collar rot in *Aloe vera* plants in various nurseries in India. Infected collar showed dark maroon–brown spots of variable sizes, while root rot infection appeared in the form of browning and decaying of root tips followed by rotting of total root system. (Avasthi *et la.*, 2015)

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the results from the isolation carried out on the leaves of diseased *Ochroma pyramidale* in the Faculty of Agriculture, University of Benin, Ugbowo Campus, it can be concluded that the fungi associated with the leaf blight are *Sclerotium rolfsii* and *Penicillium* sp.

5.2 Recommendations

Realizing the economic and medicinal values of the leaves of *Ochroma pyramidale*, it is recommended that more research work should be embarked upon to ascertain the pathogen of the disease and how to effectively control and prevent its development.

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