EFFECTS OF SELECTED SUBSTRATES ON THE MYCELIAL GROWTH OF TWO EDIBLE MUSHROOMS *Psathyrella atroumbonata* and *Pleurotus tuberregium*

BY

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(BIOTECHNOLOGY TECHNIQUES)

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BENIN CITY.

OCTOBER, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE(B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY

OCTOBER, 2025

CERTIFICATION

This is to certify that this Project work was carried out by Vera Ese EDEGAN with matriculation number LSC2007282, of the Department of Science Laboratory Technology Biotechnology Techniques, Faculty of Life Sciences, University of Benin, Benin City, Edo state under the supervision of. Dr. A.T. Dania.

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DEDICATION

This project is dedicated this to God Almighty, whose infinite grace and mercy have guided me throughout my undergraduate journey. I would also like to dedicate it to my parents and sibling for their unwavering love, encouragement, and support.

ACKNOWLEDGEMENTS

My utmost gratitude goes to God almighty for his love, grace, protection and guidance upon my life and for the wisdom, knowledge and perseverance needed for the completion of this work.

I wish to express my sincere appreciation to my project supervisor, Dr. Theophilus Dania for his invaluable guidance, supportive nature, and insightful feedback throughout this research project. His Knowledge, encouragement and commitment have been crucial in influencing the direction and quality of this work. I am truly thankful to my Head of Department, Prof. J. O OSARUMWENSE, for creating a supportive academic atmosphere and promoting a culture of excellence within the department. His leadership and vision have served as an inspiration, and I appreciate his support and encouragement. A huge thank you to my project coordinator, Dr. P. O. Alonge, for his patience and guidance during the research phase. I wish to acknowledge my project colleagues for their collaboration, support, and valuable contributions throughout this project.

Their involvement and positive attitudes truly enhanced this project and made it a team effort. To my parents, Mr and Mrs Sunday Edegan, you guys are the real “MVPs”. My sincerest gratitude for the financial, emotional, mental and physical support given to me . I also wish to express my appreciation to my dear friends, Damian, Mabel Divinegrace, Glory, and Ebosetale for their unwavering love and encouragement during my academic journey. Last but certainly not least, my heartfelt thanks go to My pastors Mr Romanus sabo , Rev Fred Owobu for their prayers throughout this journey, Sis treasure, Bro holiness, SUF Thelma and glory, with all my leaders and friends in kingdom builders lovelife church for their teachings, training, and for nurturing my love for God each day. I love and celebrate you all

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ABSTRACT

This study evaluated the effect of different lignocellulosic substrates on the mycelial growth performance of *Pleurotus tuberregium* and *Psathyrella atroumbonata*, two indigenous edible mushrooms of nutritional and economic significance in Nigeria. Five agro-residues including oil palm fiber corn straw, corn husk, plantain leaves, and sawdust were prepared as substrates following pasteurization procedures. Pure cultures of *Pleurotus tuberregium* and *Psathyrella atroumbonata* were inoculated into the substrates and incubated at 35°C under controlled humidity (75-80%). Mycelial growth parameters, including radial extension, growth rate, and morphological density, were measured at three-day intervals. The results revealed that substrate composition significantly influenced mycelial colonization and growth rate. For *Pleurotus tuberregium,* plantain leaves supported the highest mycelial extension after three days, followed by oil palm fiber, while corn husk exhibited the least growth. In contrast, *Psathyrella atroumbonata* recorded its best performance on oil palm fiber and sawdust, with the least growth again on corn husk. Mycelial density and morphology varied across substrates, with dense and woolly growth observed on oil palm fiber and plantain leaves, indicating vigorous metabolic activity, while sparse or thin mycelium was associated with corn husk, reflecting nutrient limitation and structural rigidity. The comparative growth rate analysis showed that *Psathyrella atroumbonata* (27.06 mm day') grew faster than *Pleurotus tuberregium* (22.39 mm day'), suggesting species-specific enzymatic adaptation and differential substrate utilization. The study establishes that substrate selection is a critical determinant of mycelial performance in mushroom cultivation. Plantain leaves and oil palm fiber emerged as the most efficient substrates for *Pleurotus tuberregium* and *Psathyrella atroumbonata*, respectively, due to their favorable carbon-nitrogen balance, moisture retention, and porosity. The findings highlights the potential of agricultural residues as contributing to both waste reduction and enhanced food.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background of the study

In recent decades, mushroom cultivation has emerged as an area of growing interest among entrepreneurs, farmers, and researchers across the globe. Numerous investigations have sought to improve profitability and promote wider adoption of mushroom farming while also examining the nutritional sources that sustain mycelial growth (Fasehah and Shah, 2017). Various substrates have been employed with different levels of success, including wheat straw, cottonseed hulls, cereal straws, corncobs, sugarcane residue, and sawdust. Nevertheless, compared with these well-studied substrates, information on the suitability of wood chips for mushroom production remains limited and inconclusive (Masevhe *et al.,* 2016). Nithyatharani *et al.* (2018) defined a substrate as any medium containing lignin, hemicellulose, and cellulose that allows mycelium to colonize until fruiting bodies are formed. The selection of an appropriate substrate is crucial, as it influences mushroom yield, nutritional composition, bioefficiency, quality, production cost, and cultivation duration (Masevhe *et al.,* 2016). Despite the potential benefits, mushroom farming in Africa remains underdeveloped. Fewer than 15% of Africans engage in mushroom cultivation due to limited financial support, inadequate technical knowledge, and lack of awareness. On a commercial scale, only South Africa, Zimbabwe, and Kenya have established significant production (FAO, 2002; Kivaisi, 2007). The type of substrate used in mushroom cultivation not only affects productivity but also determines the nutrient content available to the mushrooms. Carrasco *et al.* (2018) observed that although substantial progress has been made, there remain knowledge gaps concerning the specific nutritional needs of mushrooms. During growth, mushrooms release extracellular enzymes such as lignin peroxidases, quinone reductases, dehydrogenases, xylanases, cellulases, laccases, and cellobiose dehydrogenases.

These enzymes are central to the breakdown of lignocellulosic material in substrates, facilitating nutrient uptake. Successful mushroom metabolism further requires oxygen, an optimal pH, and macronutrients such as carbon and nitrogen for energy and structural development (Ashraf *et al.,* 2013). One promising option is the use of encroacher bushes, which are typically cleared from agricultural lands. Converting such biomass into mushroom substrates can enhance value addition while simultaneously generating income. Even if 3% to 5% of encroacher bushes were harvested, wood-based farming could emerge as a major subsector of primary agriculture, while the remaining biomass could be utilized in mushroom cultivation (Haukongo *et al.,* 2022). However, the success of this approach depends on the availability of quality spawn, reliable substrates, and controlled environmental conditions such as temperature and humidity (Uddin *et al.,* 2011).

As highlighted by Masevhe *et al.* (2016), suitable substrates are often locally sourced and are characterized by high carbon content, lignocellulosic compounds, and essential nutrients including nitrogen, potassium, phosphorus, and iron. These substrates must also be sterile to minimize contamination risks. A wide range of agricultural residues can serve this purpose, including wood, leaves, horticultural waste, wheat straw, cottonseed hulls, and sugarcane by-products (Siwulski *et al.,* 2019). Empirical studies have demonstrated the effectiveness of sawdust, corncobs, and sugarcane bagasse as substrates for mushroom production (Haukongo *et al.,* 2022). Similarly, oyster mushrooms have been cultivated successfully on diverse agro-waste substrates such as rice and wheat straw, paddy husks, paper, coffee pulp, cotton residues, bean straw, bagasse, and molasses waste (Owaid *et al.,* 2015). Cereal straws including wheat, rye, and oats are particularly suitable, while hardwoods such as elm, beech, alder, ash, and cottonwood, especially when decomposing are also valuable (Fasehah and Shah, 2017).

Different mushroom species vary in their capacity to degrade substrates. Many species efficiently utilize plant fibers from straw; however, straw substrates are highly vulnerable to microbial contamination, which restricts yields and often results in competition between mushroom mycelium and other microorganisms (Mashudu *et al.,* 2015). In addition, moulds and pests can severely compromise productivity or even prevent fruiting altogether (Ralph and Kurtzman, 2010). To address these challenges, growers commonly pasteurize substrates to suppress competing organisms (Diana *et al.,* 2006). Recent studies further suggest that combining or supplementing substrates enhances nutritional availability for mycelial growth. For instance, sawdust derived from specific tree species produces higher yields when supplemented with wheat bran. Other additives such as cornmeal and lime are sometimes introduced to improve nutrient balance and regulate pH (Carrasco *et al.,* 2018).

Psathyrella atroumbonata is a widely recognized edible mushroom that typically occurs in the wild and has not yet been brought into large-scale commercial cultivation. In many parts of Africa, particularly within the West African sub-region, mushroom farming remains in its early stages, with indigenous production techniques still underdeveloped. As a result, most communities continue to rely heavily on the collection and consumption of wild edible species (Okhuoya and Ajerio, 1988; Isikhuemhen and Okhuoya, 1995; 1996). This species is distributed across tropical Africa, with notable prevalence in West Africa, where it forms an important part of the local diet. Ecologically, *P. atroumbonata* is a lignicolous fungus that typically develops in small clusters on decayed tree trunks, stumps, or roots in advanced stages of decomposition. Morphologically, it possesses a distinct whitish stipe measuring approximately 5-9 cm in length, and a light brown, brittle pileus that ranges from 1.5 to 5 cm in diameter and often assumes a bell-shaped or conical form. Its spores are pale brown. Although superficially resembling *Coprinus* species, *P. atroumbonata* does not exhibit the autodigestive process characteristic of that genus. Attempts to cultivate *P. atroumbonata* under artificial conditions have been reported; however, yields are generally low when grown on synthetic substrates (Ayodele and Okhuoya, 2007).

Pleurotus tuberregium (Fr.) Sing. is a tuber-forming white-rot basidiomycete notable for its ability to produce fruiting bodies from a distinctive globose sclerotium, often compared to a giant truffle (Nwokolo, 1987). The species occurs predominantly in tropical and subtropical regions, colonizing both hardwood and softwood hosts such as *Mangifera indica*, *Daniellia oliveri*, and *Treculia africana*. It is the only member of the *Pleurotus* genus known to form a true sclerotium and is further distinguished by its non-pleurotoid growth habit (Isikhuemhen and Nerud, 1999). The sclerotia vary considerably in size, ranging from a few centimeters to several in diameter, and are typically spherical to oval, dark brown externally, and white internally (Okhuoya and Okogbo, 1991). In Nigeria, *P. tuberregium* holds dual significance as both a food source and a medicinal resource. Its sclerotia are highly nutritious, particularly rich in protein, and regarded as a delicacy with considerable market value (Okhuoya and Okogbo, 1990). The hard tuber is often peeled and ground for use in local dishes, while the fruiting bodies are commonly incorporated into vegetable soups (Oso, 1977). Traditionally, the sclerotium may serve as a substitute for melon (*Citrullus lanatus*) seed or groundnut (*Arachis hypogaea*) cake in the preparation of sauces and soups. Across tropical Africa, the sclerotia are frequently milled with melon or groundnut, seasoned, and molded into patties for baking or cooking. Groundnut-based patties are typically eaten as snacks, whereas the mushroom fruiting bodies are valued as an accessible and affordable protein source (Nwokolo, 1987).

Beyond its nutritional contributions, *P. tuberregium* is widely recognized for its medicinal applications. In Nigeria, traditional healers employ the sclerotia and fruiting bodies, either alone or in combination with herbs, to treat a range of ailments including headaches, stomach disorders, colds, fever, asthma, smallpox, hypertension, and coughs (Fasidi and Olorunmaye, 1994; Oso, 1997). Similar practices exist in Ghana, where the fungus is used by herbalists for conditions such as childhood undernutrition, asthma, and high blood pressure (Dzomeku, 2009). Research has also revealed additional biological properties: pure cultures of the fungus can kill and derive nutrition from nematodes (Hibett and Thorn, 1994), while its mycelial activity contributes to the remediation of crude oil-contaminated soils, enabling subsequent plant growth such as *Vigna unguiculata* (Isikhuemhen *et al.,* 2003; Adenipekun, 2008). Yongabi (2004) further demonstrated that the sclerotium acts as an effective coagulant and disinfectant suitable for natural and wastewater purification. The species also features in traditional medicine beyond Africa. In China, it is incorporated into folk remedies, where it is consumed as a tonic and prescribed for respiratory conditions such as coughs and asthma (Chengua *et al.,* 2000).

1.2. STATEMENT OF THE PROBLEM

Although edible mushrooms such as *Psathyrella atroumbonata* and *Pleurotus tuberregium* are highly valued in Nigeria for their nutritional, medicinal and economic benefits, their cultivation has not reached its full potential. The choice of substrate is a major factor determining the success of mushroom farming, as it directly affects the rate of mycelial colonization and eventual mushroom yield. Farmers often rely on trial and error in selecting substrates, leading to inconsistent growth outcomes and low productivity. While materials such as oil palm fiber corn residues, plantain leaves and sawdust are abundant in many regions, their relative effectiveness in supporting the growth of these mushroom species has not been sufficiently studied. This lack of systematic evaluation limits the expansion of commercial mushroom production and restricts the role of these species in addressing nutritional and livelihood challenges.

1.3. JUSTIFICATION OF THE STUDY

This study is necessary because the need to enhance mushroom cultivation through the use of readily available agricultural residues such as oil palm fiber corn straw, corn husk, plantain leaves and sawdust. Determining their effectiveness as substrates will provide practical solutions for smallholder farmers while reducing reliance on imported or costly cultivation materials. Furthermore, converting agricultural by-products into substrates supports waste recycling and environmental sustainability. Improved cultivation of *Psathyrella atroumbonata* and *Pleurotus tuberregium* will not only increase their availability as affordable sources of protein and bioactive compounds but also create income-generating opportunities.

1.4. AIM AND OBJECTIVES

The aim of this study is to evaluate the effect of selected substrates; oil palm fiber corn straw, corn husk, plantain leaves and sawdust, on the mycelial growth of *Psathyrella atroumbonata* and *Pleurotus tuberregium*.

The specific objectives of this study are to determine:

1. The effect of substrates in the mycelia extension of *Psathyrella atroumbonata* and *Pleurotus tuberregium*.
2. The effect of substrates on the growth rate of *Psathyrella atroumbonata* and *Pleurotus tuberregium*.
3. The effect of substrates in the mycelia density and mycelia morphology of *Psathyrella atroumbonata* and *Pleurotus tuberregium*.

CHAPTER TWO

2.1 LITERATURE REVIEW

Mushrooms have long been regarded as an essential component of gourmet cuisine worldwide, appreciated particularly for their distinctive taste, and revered as a culinary treasure. Although more than 2,000 mushroom species are found in nature, only about 25 are commonly consumed as food, with a limited number being commercially cultivated. They are considered a delicacy not only for their nutritional and functional properties but also as nutraceutical foods, gaining attention for their sensory appeal, medicinal benefits, and economic value (Chang and Miles, 2008; Ergönül *et al.,* 2013). Nonetheless, distinguishing between edible and medicinal mushrooms is not straightforward, since many edible varieties possess therapeutic qualities, while several medicinal ones are also edible (Guillamón *et al.,* 2010). Mushrooms are spore-producing structures, or sporocarps, that play a vital role in the sexual reproductive cycle of numerous fungal species (Maurice *et al.*, 2021). They are generally considered safe for human consumption due to their minimal toxicity and low antinutrient content, while being rich sources of proteins, dietary fiber, vitamins, minerals, and other essential nutrients (El-Ramady *et al.*, 2022). The nutritional composition of mushrooms varies considerably among species, with some containing up to 30% crude protein by weight, while crude fiber, fat, and carbohydrates can reach approximately 28%, 8%, and 95% by weight, respectively (Gargano *et al.*, 2017). As a sustainable and protein-dense alternative to meat, mushrooms offer a reduced environmental footprint compared to traditional livestock production. Additionally, bioactive compounds such as β-glucans, peptides, proteins, and phenolic compounds contribute to mushrooms' immunomodulatory, antibacterial, cytostatic, and antioxidant effects, which has led to their classification as 'medicinal mushrooms' in certain contexts (Carrasco-González *et al.*, 2017; Gargano *et al.*, 2017). The beneficial effects of mushroom consumption on human health are well documented, driving a global rise in demand and positioning mushrooms as a rapidly expanding commodity in international markets (de Frutos, 2020). Since 1978, global mushroom production has increased more than thirtyfold, with Asia, particularly China, dominating approximately 90% of output. Production in the European Union, led by the Netherlands and Poland, as well as in the Americas, has also expanded substantially in recent decades (Rouse *et al.*, 2017). Commercial cultivation encompasses over fifty species, with the four primary genera; Lentinula (e.g., shiitake), Pleurotus (oyster mushrooms), Auricularia (wood ear mushrooms), and Agaricus (button mushrooms) representing 74% of the worldwide market (Kumla *et al.*, 2020). Mushroom cultivation relies on plant biomass-based substrates such as crop residues and underutilized wood, which are increasingly available due to rising agricultural production driven by population growth. Conventional methods of managing these residues often involve burning, a practice that contributes to air pollution and squanders valuable biomass that could otherwise be converted into food, fuels, or chemicals (Kaushal and Prasher, 2021; Martín, 2020). Using crop residues for mushroom cultivation exemplifies sustainable recycling, transforming lignocellulosic biomass into edible products while reducing land use and enabling year-round production independent of environmental conditions (Chen *et al.*, 2022). Cultivation produces fruiting bodies for harvest and generates spent mushroom substrate (SMS) as a major by-product. Depending on the species and conditions, 3–5 kilograms of SMS are produced per kilogram of fresh mushrooms (Zisopoulos *et al.*, 2016). In 2018, global SMS production reached around 64 million tons, projected to exceed 100 million tons by 2026 (Atallah *et al.*, 2021). SMS management presents logistical and environmental challenges due to its bulk, high moisture, and low density, making transport costly. Improper disposal can also lead to greenhouse gas emissions, malodors, and water contamination (Beyer, 2011). Landfilling, once the primary disposal route, is now prohibited in the European Union under regulations addressing biodegradable waste (Council Directive, 1999). The composition of SMS is largely determined by the initial substrate and any supplements, with lignocellulosic by-products from agriculture, forestry, and agro-industrial processes serving as the main substrates for Lentinula, Pleurotus, and Auricularia species, which together account for 60% of global production. Species such as Agaricus require composted substrates incorporating chicken manure, with additives like cereal bran, legume flour, and mineral salts enhancing growth. Enzymatic degradation during cultivation releases nutrients that support fungal growth, resulting in mass losses of 26–46%, 57–77%, and 61–75% for cellulose, hemicellulose, and lignin, respectively, in species such as Pleurotus ostreatus, Pleurotus pulmonarius, and Lentinula edodes. Consequently, SMS primarily consists of plant cell-wall materials, residual mycelium, and minor quantities of non-cell-wall carbohydrates, proteins, and minerals. In Nigeria and worldwide, mushrooms are valued as a delicacy (Gucia *et al.*, 2011; Nnorom *et al.*, 2013). Pleurotus tuber-regium, in particular, is consumed for its nutritional content, medicinal properties, taste, and aroma (Falendysz and Gucia, 2008; Nnorom *et al.*, 2013). Its sclerotium, a dense storage structure of fungal mycelium, allows survival under adverse conditions, often attaining spherical or ovoid shapes up to 30 cm in diameter and weighing as much as 5 kg (Thorn *et al.*, 2000; Iwuagwu and Onyekweli, 2002). Nutritionally, it contains high levels of protein (approximately 64.31% wet weight, 71.21% dry weight), carbohydrates (20% wet weight, 22.15% dry weight), ash (2.2% wet weight, 2.44% dry weight), crude fiber (2.89% wet weight, 3.2% dry weight), and sugars, including significant galactose content (Ikewuchi and Ikewuchi, 2009). Traditional medicine utilizes extracts from P. tuber-regium sclerotia to treat headaches, digestive disorders, asthma, hypertension, and as potential antitumor and antihyperglycemic agents. The species also contributes to agricultural waste recycling, growing on substrates such as rice straw, wheat straw, corn cobs, cassava peels, palm residues, cottonseed hulls, peanut shells, and sunflower straw, offering a nutritionally rich food source, aiding bioremediation, and providing income generation opportunities (Onyeike and Ehirim, 2001; Basu *et al.*, 2007; Anoliefo *et al.*, 2002; Iskheuemhen *et al.*, 2003; Zhang *et al.*, 2007). Pleurotus tuber-regium (Fr.) Singer is a saprotrophic basidiomycete that predominantly colonizes decaying wood, including species such as *Daniellia oliveri* (Rolfe), *Treculia africana* Decne. ex Trécul, *Terminalia superba* Engl. andDiels, and *Terminalia ivorensis* A.Chev. (Zoberi, 1973; Okhuoya and Okogbo, 1990). This fungus is widely distributed across tropical Africa and parts of Australasia (Oso, 1977; Isikhuemhen and LeBauer, 2004) and is distinguished by a non-pleurotoid growth habit (Isikhuemhen and Nerud, 1999). During decomposition, the fungus forms a tuber-like sclerotium either within the wood or in the underlying soil, representing the only Pleurotus species that produces true sclerotia (Isikhuemhen *et al.*, 2000). Sclerotia are typically ovoid or spherical, reaching diameters up to 30 cm, and can sustain fruiting body development as long as nutrient reserves are available. Both the sclerotia and fruiting bodies are edible (Oso, 1977). The sclerotium generally develops under nutrient-limiting or harsh environmental conditions, allowing the fungus to survive unfavorable periods. In Nigeria, wild collection remains the primary source, though availability is declining due to herbicide use, agrochemical exposure, and frequent bush burning. Pleurotus tuber-regium exhibits a tetrapolar mating system (Chen and Huang, 2004) and has been documented across tropical and subtropical Africa; including Nigeria, Sierra Leone, Zimbabwe, Ghana, Kenya, Cameroon, Zambia, Uganda, Chad, the Democratic Republic of Congo, Burundi, and Ivory Coast as well as Australasia and the Pacific, including Australia, Hunan Province in China, Sri Lanka, India, Malaysia, Indonesia, Burma, and Papua New Guinea. Its potential medicinal applications are under global investigation (Akpaja *et al.*, 2003). Morphologically, it resembles *Pleurotus ostreatus* but can be differentiated by its upward-curved pileus, in contrast to the downward-curved pileus of *P. ostreatus*, while its microdroplets exhibit nematophagous activity (Hibbett and Thorn, 1994). Although industrial-scale cultivation is limited, studies have demonstrated that organic waste substrates such as cardboard, sawdust, and maize can support its growth (Okhuoya and Okogbo, 1991; Isikhuemhen and Okhuoya, 1995; Isikhuemhen and LeBauer, 2004; Afolabi *et al.*, 2021). Optimal mycelial growth occurs between 30°C and 35°C and at pH levels of 4-9, with the fungus tolerating temperatures from 15°C to 40°C (Oso, 1977; Fasidi and Ekuere, 1993). The species holds considerable economic value due to its edible sclerotia and mushrooms (Lau and Abdullah, 2016), with the sclerotia also serving non-food purposes, such as recreational use in toy-making (Kamalebo *et al.*, 2018). Oranusi *et al.* (2014) found no significant difference in colony-forming units per milliliter between heterotrophic organisms cultured on sclerotium agar, plate count agar, and nutrient agar, indicating its potential as a standardized, low-cost growth medium. Furthermore, sclerotia have been effectively used as coagulants and disinfectants for natural and wastewater treatment (Yongabi, 2004). Pleurotus tuber-regium, also known as *Lentinus tuber-regium*, is referred to as the "sclerotia-producing Pleurotus" or "King Tuber Oyster mushroom," and called “hunai” in China (Chen andHuang, 2004; Lau andAbdullah, 2016). In Nigeria, it is known as “olu ohu” by the Yoruba due to the sclerotium’s ability to produce fruiting bodies over extended periods in moist conditions (Akinyele, 2020). In southeastern Nigeria, it is called “osu” or “ero nsu,” and in Hausa, it is known as “katala” or “rumbagada” (Oso, 1977). Various African communities employ the tuber-like sclerotia for treating ailments such as skin diseases, malnutrition in children, inflammatory headaches, stomach disorders, colds, asthma, fever, diabetes, hypertension, and smallpox (Oso, 1977; Chen and Huang, 2004). Indigenous knowledge of its edible and medicinal properties has been transmitted across generations (Ayodele *et al.*, 2011; Oyetayo, 2020). Some traditional healers combine Pleurotus tuber-regium with herbal blends to manage hypertension, asthma, smallpox, headaches, stomach disorders, colds, and fevers

(Akpaja *et al.*, 2003). In Edo State, it is applied for obesity, cough, and asthma, while in southeastern Nigeria, it is used for cardiovascular concerns (Isikhuemhen and Okhuoya, 1995; Isikhuemhen and LeBauer, 2004). In Ghana, it is used in funeral practices and as a remedy for childhood anemia and malnutrition (Okhuoya *et al.*, 1998), while local practitioners also employ it for hypertension, asthma, and underweight in children (Dzomeku, 2009). In the Democratic Republic of Congo, sclerotia are used to treat bedwetting, bronchitis, and to stimulate lactation, and in some areas, the powdered sclerotium is applied to deter birds from rice fields (Kamalebo *et al.*, 2018). Both sclerotia and fruiting bodies of Pleurotus tuber-regium are edible. The outer brown layer of the sclerotium is removed, and the inner white portion can be ground into fine powder and added to soups to increase volume (Iwuagwu andOnyekweli, 2002). It can also be mashed with melon or chopped into pieces for vegetable or okra soups, and may substitute for melon in certain recipes. In Nigerian cuisine, sclerotium is commonly used to thicken soups such as “nkwobi.” The sclerotia are widely consumed across sub-Saharan Africa, retaining nutritional and medicinal properties even after drying (Gbasouzor and Ileleji, 2023). The stipe and pileus can be chopped, boiled, and incorporated into soups, offering essential trace elements and minerals to support growth and tissue repair (Aloma *et al.*, 2018). Analyses of sporophores and sclerotia show low fat content but high levels of proteins, vitamins, carbohydrates, and minerals (Isikhuemhen and LaBauer, 2004). Proximate composition includes 16.5% dry matter, 7.4% crude fiber, 14.6% crude protein, and 4.0% lipids (Ogundana and Fagade, 1982). Total sugar content is 18.6%, predominantly galactose, with lower levels of maltose and glucose. Oxalic acid and hydrogen cyanide levels are minimal, while vitamin C is negligible. Lipid and ethanol-soluble sugar contents are low, rendering it suitable for individuals with diabetes, cardiovascular concerns, or weight management issues (Jonathan *et al.*, 2006). Among edible wild mushrooms, it has the highest crude fiber content. Young fruiting bodies contain more protein than mature ones, and sclerotia composition includes ash (2.20% wet weight, 2.44% dry weight), crude fiber (2.89% wet weight, 3.20% dry weight), carbohydrates (20.00% wet weight, 22.15% dry weight), and protein (64.31% wet weight, 71.21% dry weight) (Ikewuchi and Ikewuchi, 2011). Sclerotia provide 6.15 g aromatic amino acids, 1.50 g sulfur-containing amino acids, and 25.93 g essential amino acids per 100 g. Phenylalanine, leucine, and histidine are abundant, while cystine and methionine are limiting amino acids, and their chemical scores relative to egg protein, human milk, and adult protein requirements are 26%, 88%, and 36%, respectively (Ikewuchi andIkewuchi, 2011). The fruiting bodies and sclerotia thus offer a viable protein source in regions with limited access to meat or fish, and they are suitable for vegan diets. Sclerotia have also been used to support weight gain in malnourished infants (Ikewuchi andIkewuchi, 2011). Daily protein needs ranging from 23 to 56 g can be met in part by mushroom consumption (Chaney, 2006). The high crude fiber content may reduce the risk of obesity, hypertension, colon cancer, diabetes, and certain digestive disorders (Ikewuchi and Ikewuchi, 2008; Adeyi *et al.*, 2021), enhancing colon health by increasing stool bulk and lowering bile acid concentrations associated with high-fat diets (Dillard and German, 2000). Major minerals include potassium (60.66 ± 4.13 mg/kg) and magnesium (41.79 ± 3.14 mg/kg), while manganese (1.20 ± 0.10 mg/kg) and zinc (0.95 ± 0.07 mg/kg) are the predominant micronutrients. Amino acids such as glutamic acid (11.51 ± 1.01 mg/kg) and aspartic acid (5.52 ± 0.86 mg/kg) are abundant (Ohiri, 2018). Mushroom proteins are highly digestible and soluble compared to plant sources (Jonathan *et al.*, 2006; Zhou *et al.*, 2020). Given their richness in micronutrients and phytochemicals, they can be consumed directly, as food additives, or as herbal remedies. Dietary inclusion of Pleurotus tuber-regium has been shown to improve feed intake, weight gain, nutrient digestibility, and nitrogen utilization in West African dwarf goats (Jiwuba *et al.*, 2023) and enhance growth performance when incorporated into biodegraded groundnut shells (Wuanor *et al.*, 2018). In rabbits, diets containing 50–70% sclerotium powder improved weight gain, feed conversion, cholesterol, creatinine, alanine transaminase levels, and gastrointestinal health without adverse effects (Salami *et al.*, 2021). Polysaccharides and carbohydrates from sclerotia have demonstrated potential as prebiotics, promoting the growth of *Bifidobacterium* species and increasing short-chain fatty acid production (Lin *et al.*, 2020). Globally, *Agaricus bisporus* is the most widely cultivated mushroom, followed by *Lentinus edodes*, *Pleurotus* species, and *Flammulina velutipes*. Mushroom cultivation has shown a steady increase, with China ranking as the largest producer worldwide (Chang and Miles, 2008; Aida *et al.,* 2009; Patel and Goyal, 2012). At the same time, wild mushrooms are gaining significance for their nutritional, sensory, and particularly pharmacological properties (Ergönül *et al.,* 2013). Mushrooms also represent a potential source of novel antimicrobial agents, mainly derived from secondary metabolites such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones, as well as primary metabolites like oxalic acid, peptides, and proteins. Among them, *Lentinus edodes* has been the most extensively researched and exhibits antimicrobial activity against both gram-positive and gram-negative bacteria (Alves *et al.,* 2012). Nutritionally, mushrooms are highly valuable as they contain significant amounts of protein, including essential amino acids and fiber, with low fat content but enriched with beneficial fatty acids. Furthermore, edible mushrooms are a good source of vital vitamins such as B1, B2, B12, C, D, and E (Heleno *et al.,* 2010; Mattila *et al.,* 2001). Therefore, they can serve as an excellent reservoir of diverse nutraceuticals, suitable for direct inclusion in the human diet to enhance health through the synergistic effects of their bioactive compounds (Barros *et al.,* 2007; 2008).

2.2. Some growth media

For over two millennia, the practice of cultivating mushrooms on wood has been widespread, particularly in Asia. Hardwood species such as birch, hickory, beech, and oak are typically employed, either as logs or sawdust substrates. Log cultivation involves inoculating spawn into holes drilled into freshly cut logs, which are then incubated under shaded conditions for approximately one year before the first flush appears. Thereafter, the logs may continue to yield mushrooms for three to four years (Stamets, 2005; Levine, 2020). Hardwood substrates are especially suitable for *Lentinus edodes* (shiitake), *Ganoderma lucidum* (reishi), and *Tremella fuciformis*, all of which are widely recognized for their medicinal properties (Stamets, 2000). *G. lucidum* is commonly cultivated on hardwood logs cut to lengths of 1-2 m and at least 1 m in diameter, a technique popularized because of its rich bioactive compounds (Samantha, 2010). Fruiting bodies are harder than most mushrooms, typically presenting a flat, rounded cap with a red to reddish-brown coloration (Siwulski *et al*., 2015). *T. fuciformis*, commonly known as snow fungus, silver ear, or white jelly mushroom, is a valued edible medicinal species cultivated mainly in China. Belonging to the family Tremellaceae, it is often found on decayed wood in tropical regions, producing white to pale-yellow fruiting bodies (Shahrajabian *et al.,* 2020; Haukongo *et al*., 2022).

Straw remains one of the most widely used substrates for mushroom cultivation due to its abundance, affordability, ease of handling, and suitability for pasteurization. It is frequently used for growing oyster mushrooms (*Pleurotus* spp.) and *Volvariella volvacea* (Cai *et al.,* 1999; Haukongo *et al.,* 2022). Cultivation typically involves soaking straw in water for 10-12 hours, draining the excess water, and occasionally supplementing it with chalk ($CaCO_3$) and wheat bran. Sterilization is achieved by autoclaving at 121 °C for 30 minutes or pasteurization for 2-4 hours in drums to suppress competing microbes (Owaid *et al.,* 2015). The inoculated substrate is usually packed in transparent plastic bags under sterile conditions and incubated for 1-2 months until the first flush is produced. Supplementation of straw not only improves yields but also influences the nutritional profile of the harvested mushrooms (Haukongo *et al.,* 2022).

Certain mushrooms are better adapted to compost-based substrates, particularly those that thrive on decomposed organic matter or manure. *Agaricus bisporus* (button mushroom) is a prime example, requiring microbial activity to degrade cellulose for mycelial growth (Funda *et al.,* 2017). Compost is generally prepared by mixing manure with straw and allowing it to decompose for about three weeks, after which it undergoes pasteurization at 57-59 °C for 6-8 hours before spawning (Haukongo *et al.,* 2022). Optimal compost moisture, typically 65-72%, is crucial, as excess moisture restricts aeration while dryness hinders mycelial colonization (Haukongo *et al.,* 2022). Nutrient supplementation, often with bran, poultry manure, nitrate, or ammonia, enhances productivity (Stamets, 2000). Button mushroom cultivation, if optimized, holds significant potential for addressing food insecurity, malnutrition, and poverty, particularly in developing regions (Haukongo *et al.,* 2022).

Mushrooms that establish symbiotic associations with plant roots are classified as mycorrhizal fungi. These include truffles (*Tuber melanosporum*), which are cultivated in orchards by inoculating seedlings of host trees with truffle spores prior to planting (Stamets, 2000). Harvesting typically occurs 4-7 years after inoculation (Fischer *et al.,* 2017). Truffles differ morphologically from conventional mushrooms, being round, firm, warted, and variable in size (Rajarathnam, 2013; Haukongo *et al.,* 2022). Their cultivation requires calcium-rich soils while avoiding environmental stressors such as acid rain and soil pollution, which compromise the nutritional balance essential for truffle mycelium development (Stamets, 2000).

2.3. Nutritional and medicinal benefits of mushrooms

Mushrooms offer diverse nutritional, medicinal, and environmental benefits, many of which are shaped by the choice of substrate. They are versatile food products that can also be processed into tea, paper pulp, cosmetics, and soups, thereby extending shelf life and economic value (Stamets, 2005). With a long history in medical mycology dating back to the first century (Wani *et al.,* 2010), mushrooms are now widely recognized as functional foods with roles in treating various ailments. Their documented properties include antimicrobial, anticancer, antiviral, immunomodulatory, and cholesterol-lowering effects (Alam *et al.,* 2007). Among the most studied are *Pleurotus* species, which demonstrate antioxidant, antitumor, hypoglycemic, and antihypertensive effects (Alam *et al.,* 2007). Oyster mushrooms, in particular, have been linked to the prevention and management of chronic diseases such as diabetes, cardiovascular disorders, hypertension, hepatitis B, kidney disease, and certain cancers (Mowsurni and Chowdhury, 2013). Their bioactive compounds also exhibit antibacterial, antifungal, and antiparasitic activities (Owaid *et al.,* 2015). In Ghana, *Ganoderma lucidum* and other tropical mushrooms have been utilized in developing nutraceuticals such as "Immune Assist 24/7," a supplement designed to enhance immunity in HIV/AIDS patients (Anchang, 2014). Straw mushrooms (*V. volvacea*), another widely cultivated species, produce enzymes that efficiently convert cellulose into glucose, thereby offering a rich source of proteins, essential amino acids, minerals, and vitamin C (Cai *et al.,* 1999). Their antioxidant compounds further protect cellular structures against oxidative damage, thereby contributing to cancer prevention and DNA protection (Haukongo *et al.,* 2022).

Large volumes of lignocellulosic residues from agricultural and industrial activities remain underutilized, often discarded through burning or left to decay. However, these by-products can serve as valuable substrates for mushroom cultivation, transforming waste into economically significant resources (Haukongo *et al.,* 2022). Spent mushroom substrate (SMS) retains substantial nutritional value and can be repurposed as organic compost for vegetable production, animal feed, biofuel generation, or even firewood (Carrasco *et al.,* 2018). Mushroom farming is relatively low-cost, requires minimal skill, and offers an avenue for income generation, particularly for marginalized groups such as women and people with disabilities (Tesfaw *et al.,* 2015). Globally, *A. bisporus*, *L. edodes*, and *Pleurotus* species remain the most commercially cultivated mushrooms, forming a cornerstone of the industry due to their high market demand and adaptability (Owaid *et al.,* 2015).

Ayodele and Okhuoya (2007) investigated the cultivation of *Psathyrella atroumbonata* using different agricultural residues to evaluate their effectiveness in supporting both mycelial extension and sporophore production. Their findings revealed that the most extensive mycelial growth occurred on oil palm fruit fibers (OPFF) and corn cobs, followed in order by rice straw, corn stems, guinea grass, banana leaves, and sawdust. In contrast, the highest sporophore yield, measured by fresh weight, was obtained from sawdust, with rice straw and banana leaves producing the next best results, while OPFF produced the lowest yield. Interestingly, although mycelial growth was observed on corn cobs, corn husks, and guinea corn shaft, these substrates failed to support sporophore formation. The study also noted that sporophore initiation was preceded by a gradual rise in temperature, which decreased slightly just before fruiting occurred. These results underscore the potential of specific agricultural wastes as viable substrates for the cultivation of *Psathyrella atroumbonata*.

Chiejina and Olufokunbi (2010) examined the influence of seven different substrates on the cultivation, yield, and protein composition of *Pleurotus tuberregium* (Fries) Singer. Using a completely randomized design with seven treatments and ten replicates, they reported that the highest fresh weight yield was achieved when mushrooms were cultivated on a mixture of river sand and sawdust, whereas the lowest yield occurred on topsoil combined with sawdust. Interestingly, oil palm fruit fiber (OPFF) did not support the formation of fruit bodies. In terms of nutritional composition, protein analysis indicated that mushrooms grown exclusively on river sand contained the highest protein content, while those cultivated on sawdust alone had the lowest. Based on these findings, the study recommended the use of river sand mixed with sawdust as the most suitable substrate for *P. tuberregium*, since this combination produced the greatest fruit body weight and maintained a protein content comparable to that obtained from river sand alone.

Apetorgbor *et al.* (2013) investigated the optimal environmental conditions for the growth of *Pleurotus tuberregium* as well as the suitability of selected organic materials as cultivation substrates. Their study assessed temperature, pH, and light regimes alongside the use of water hyacinth (*Eichhornia crassipes*), plantain (*Musa sapiens*) leaves, millet (*Eleusine coracana)* stalk, and composted *Triplochiton scleroxylon* (‘*wawa’*) sawdust. Results revealed that the fungus exhibited its fastest growth at 35°C, pH 6, and under continuous darkness. Among the substrates tested, only plantain leaves supported the production of both fruit bodies and sclerotia, while ‘*wawa’* sawdust facilitated the development of sclerotia alone when cultivated in bags. Fruit bodies from plantain leaves recorded a biological efficiency (B.E) of 54.47%, and their sclerotia yielded 62.05%. By contrast, sclerotia from ‘*wawa’* sawdust achieved a much higher B.E of 99.65%. Nutritional analysis further indicated that crude protein was most abundant in the sclerotia, followed by the pileus and then the stipe. These findings highlight the critical role of environmental factors and substrate selection in enhancing the commercial-scale cultivation and productivity of *P. tuberregium.*

Onuoha and Obi-Adumanya (2010) evaluated the growth performance and nutritional composition of *Pleurotus tuberregium* sclerotia cultivated on four different substrates: humus soil, a mixture of sawdust and humus soil, sawdust alone, and shreds of *Treculia africana* wood. Growth assessment was based on several parameters, including the number and height of fruit bodies, fresh and dry weights, pileus diameter, and stipe length. Among the tested substrates, sawdust, which served as the control, supported superior performance in terms of stipe length, fruit body number, and overall height. The mixture of sawdust and humus soil yielded mushrooms with higher fresh and dry weights, while humus soil alone produced fruit bodies with a larger pileus diameter. In contrast, *T. africana* wood shreds failed to support fruit body development. Nutritional analysis revealed significant variations ($P \leq 0.05$) across substrates. Mushrooms grown on sawdust mixed with humus soil showed higher ash and lipid content, whereas those from sawdust alone recorded significantly higher protein and crude fibre values. The sclerotia also exhibited notable differences in carbohydrate and energy values, while fruit bodies cultivated on humus soil had significantly higher moisture content. These findings suggest that substrate composition influences not only the yield and morphology of *P. tuberregium* but also its nutritional profile.

Okhuoya and Etugo (1993) investigated the growth behavior of *Pleurotus tuberregium* on different natural substrates, including agricultural wastes. Their study showed that all substrates inoculated with the sclerotia of the fungus, with the exception of poultry manure and sawdust, successfully produced fruit bodies without the addition of supplementary nutrients. Among the substrates tested, oil palm fiber waste was particularly effective in promoting vigorous mycelial growth and was subsequently identified as a suitable medium for spawn preparation. The researchers further observed that both peeled sclerotia and their peelings were capable of independently initiating fruit body development, suggesting that the capacity for fruiting is not confined to a specific part of the sclerotium. In addition, they noted that larger sclerotial inocula yielded proportionally higher fruit body production, underscoring the role of inoculum size in influencing productivity.

CHAPTER THREE

3.1 MATERIALS AND METHODS

The study was conducted at The African Centre for Mushroom Research and Technology Innovation (ACMRTI) in the University of Benin, Benin-city, Edo state, Nigeria. The University is located within the humid tropical rainforest belt of southern Nigeria, with conditions that are generally favourable for mushroom cultivation and mycelial growth.

3.2. Sample Collection

Pure cultures of *Psathyrella atroumbonata* and *Pleurotus tuber-regium* were obtained from The African Centre for Mushroom Research and Technology Innovation (ACMRTI) in the University of Benin.

3.3. MATERIALS

The materials used in the experiment include;

- Guinea corn (*Sorghum bicolor*)
- Irish potato
- Potato dextrose agar
- Agar-agar medium
- Glucose
- Petri dishes
- Measuring cylinder
- Conical flasks

- Beakers
- Foil paper
- Cotton wool
- Masking tape
- Weighing balance
- Filter paper
- Knife
- Stove
- Pressure pot
- Methylated spirit
- Gloves
- Laminar flow
- Nose mask
- Ruler
- Jar bottles

3.4. Substrate Collection and Preparation

Five locally available agricultural and lignocellulosic residues were used as substrates. They include; Oil palm fiber Corn straw, Corn husk, Plantain leaves and Sawdust. All substrates were collected from farms and agro-processing sites around Benin City. The substrates were air-dried for 5-7 days, cut into pieces of 2-5cm to increase surface area. 100g of each substrates were soaked in hot water for 24 hours to obtain their extracts. Substrates were pasteurized by steaming at 80-90°C for 1 hour using a pressure pot. The substrates were then cooled to room temperature under aseptic conditions before inoculation.

3.5. Preparation of Potato Dextrose Agar

Potato Dextrose Agar (PDA) was prepared following standard protocols for fungal culture media. 200g of peeled and diced Irish potatoes were boiled in 500mL of distilled water for about 30-60 minutes, and the infusion was filtered through a fine clothe and adjusted to 1 L with distilled water. 20g of dextrose and 15g of agar were added and dissolved by heating. The medium was adjusted to a pH of 5.6 ± 0.2 and sterilized by autoclaving at 121°C for 15 minutes. After cooling to 45-50 °C, the medium was aseptically poured into sterile petri dishes and allowed to solidify (Ribeiro *et al.,* 2025).

3.6. Inoculation

The sterilized grains were aseptically inoculated with actively growing mycelial discs (5 mm) from PDA cultures. Sterile polypropylene bags were filled with 250 g (dry weight) of each substrate, adjusted to 65-70% moisture content. Each bag was then inoculated with 10 g of spawn of either *P. atroumbonata or P. tuberregium,* sealed, and perforated with sterile pins to facilitate aeration.

Plate 1: Pouring of media into plates

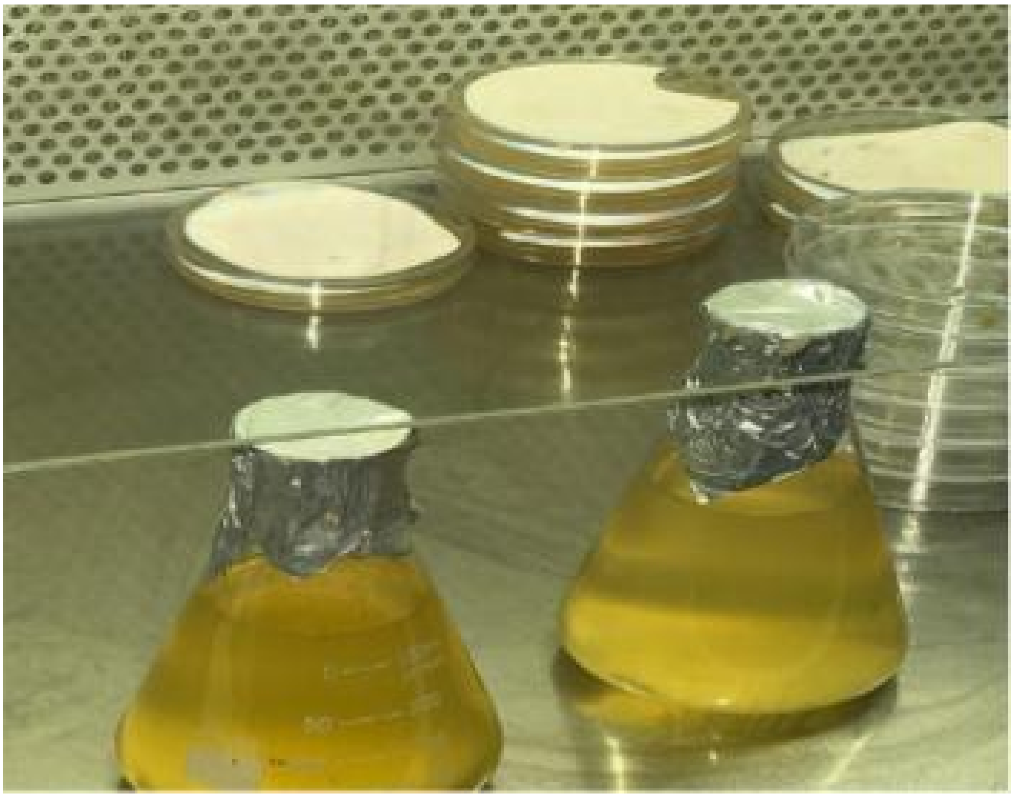

Plate 2: Inoculated plates

3.7. incubation

All inoculated bags were incubated in the Laboratory at 35°C and relative humidity of 75-80% under dark conditions to favour mycelial colonization. The bags were monitored daily for signs of mycelial growth and contamination.

3.8. Data Collection

Mycelial growth was evaluated by measuring radial extension on the substrates. Measurements were taken every three days from the day of inoculation until full substrate colonization. The following parameters were recorded:

- Rate of mycelial growth (cm/day)
- Time required for full substrate colonization (days)

- Mycelial density and vigor (assessed visually on a 1-5 scale, where 1 = very sparse and 5 = very dense growth).

CHAPTER FOUR

4.0 RESULTS

The mycelial growth performance of *Pleurotus tuberregium* and *Psathyrella atroumbonata* on different lignocellulosic substrates was evaluated over a three-day period. The results show the mycelia growth extension in *Pleurotus tuberregium* to be highest in plantain leaves (67.17±3.01), closely followed by Oil palm fiber with the least being Corn husk (35.33±5.03) as shown in Table 1. For *Psathyrella atroumbonata,* Oil palm fibre recorded the highest at (81.17± 4.91), closely followed by Sawdust, with the least being Corn husk as shown in Table 2. The Mycelial density and morphology and growth rate are presented in Tables 3-5.

Table 1: effect of substrates on daily Mycelial extension of *Pleurotus tuberregium*

	Mycelia extension (mm)		
Substrate	**1 DAI**	**2 DAI**	**3 DAI**
Oil Palm fibre	28.67 ± 3.62	38.83 ± 8.13	61.33 ± 6.25
Corn straw	30.0 ± 3.0	34.33 ± 3.62	48.67 ± 7.57
Corn husk	24.0 ± 1.32	26.0 ± 1.0	35.33 ± 5.03
Plantain leaves	36.83 ± 2.02	44.83 ± 1.26	67.17 ± 3.01
Sawdust	26.5 ± 7.81	31.5 ± 10.21	48.5 ± 12.76

Note: values – mean of three replicates ± standard deviation; DAI – Day after inoculation

Table 2: effect of substrates on daily Mycelial extension of *Psathyrella atroumbonata*

	Mycelia extension (mm)		
Substrate	**Day 1**	**Day 2**	**Day 3**
Oil Palm fibre	56.5 ± 1.5	71.5 ± 2.18	81.17 ± 4.91
Corn straw	34.17 ± 0.58	41.0 ± 0.5	55.0 ± 0.5
Corn husk	23.0 ± 6.06	25.83 ± 2.75	14.17 ± 2.02
Plantain leaves	34.0 ± 1.32	40.0 ± 1.32	42.17 ± 1.04
Sawdust	43.5 ± 1.32	54.83 ± 1.04	74.83 ± 1.76

Note: values – mean of three replicates ± standard deviation; DAI – Day inoculation

Table 3: Mycelial density and morphology of *Pleurotus tuberregium*

Substrates	**Density**	**morphology**
Oil Palm fibre	++++	wooly
Corn straw	+++	concentric
Corn husk	+	wooly
Plantain leaves	++++	concentric
Sawdust	++	serated

Note: + (sparse), ++ (thin), +++ (dense), ++++ (very dense)

Table 4: Mycelial density and morphology of *Psathyrella atroumbonata*

Substrates	**Density**	**morphology**
Oil Palm fibre	++++	wooly
Corn straw	+++	concentric
Corn husk	+	wooly
Plantain leaves	++	concentric
Sawdust	++++	serated

Key: + (sparse), ++ (thin), +++ (dense), ++++ (very dense)

Table 5: Mycelial growth rate of *Psathyrella atroumbonata and Pleurotus tuberregium*

Substrates	*Psathyrella atroumbonata*	*Pleurotus tuberregium*
Oil Palm fibre	27.06	20.44
Corn straw	18.33	16.22
Corn husk	4.72	11.78
Plantain leaves	14.06	22.39
Sawdust	24.94	16.16

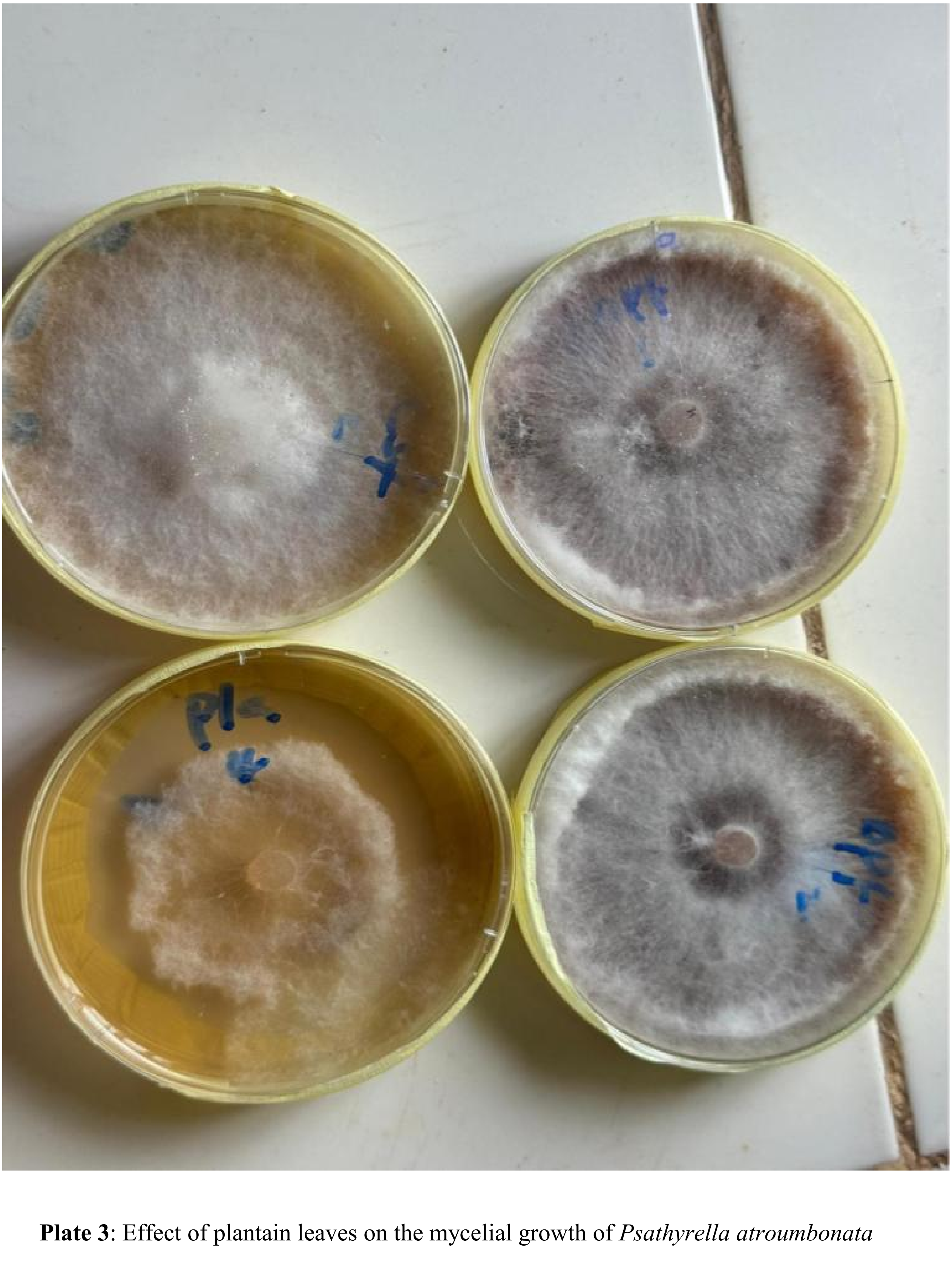

Plate 3: Effect of plantain leaves on the mycelial growth of *Psathyrella atroumbonata*

Plate 4: Effect of Oil palm fibre on mycelia growth of *Pleurotus tuberregium*

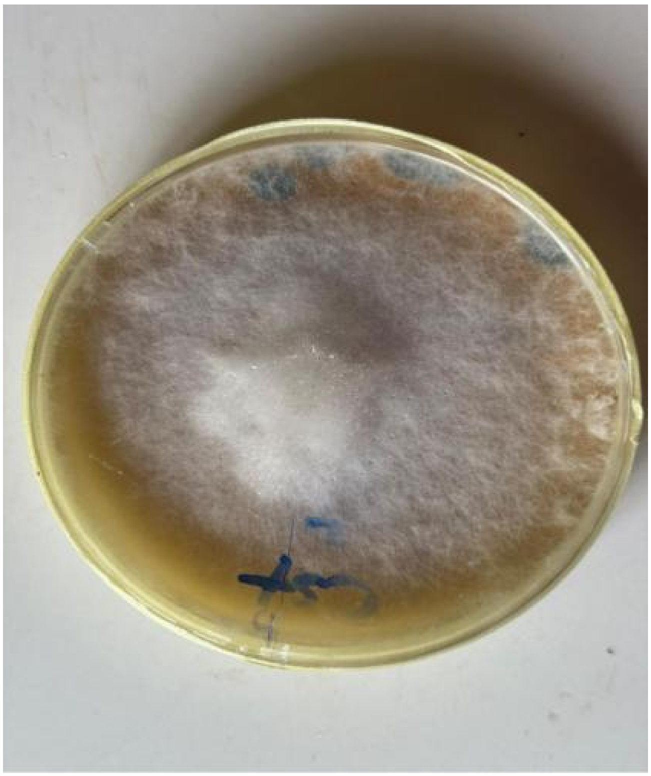

Plate 5: effect of Corn straw on the mycelial growth of *Psathyrella atroumbonata*

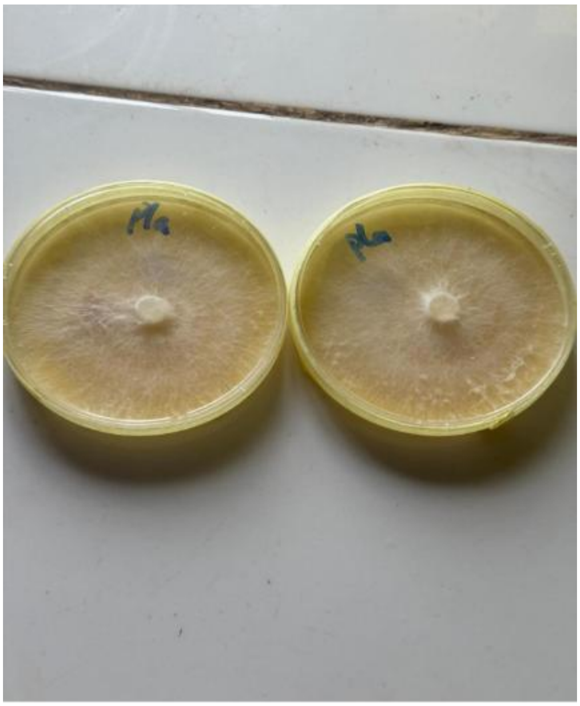

Plate 6 effect of plantain leaves on the mycelial growth of *Pleurotus tuberregium*

Plate 7: effect of corn husk on the mycelial growth of *Pleurotus tuberregium*

CHAPTER FIVE

5.0. DISCUSSION

This study is motivated by the need to explore sustainable, low-cost, and locally available substrates for the cultivation of edible mushrooms, particularly *Pleurotus tuberregium* and *Psathyrella atroumbonata*, which are widely consumed and valued for their nutritional, medicinal, and economic importance in Nigeria and across tropical Africa. Despite their high demand, the cultivation of these indigenous species remains limited due to inadequate information on suitable substrate materials that can support optimal mycelial growth and yield. Agricultural residues such as oil palm fiber corn husk, corn straw, sawdust, and plantain leaves are abundantly generated in most rural and semi-urban communities, yet they are often discarded as waste, contributing to environmental pollution. Converting these lignocellulosic by-products into growth substrates for mushrooms offers a dual benefit of waste management and food production. The results obtained from the study provide critical insight into the influence of different lignocellulosic substrates on the mycelial growth performance of *Pleurotus tuberregium* and *Psathyrella atroumbonata*. Generally, mycelial extension increased progressively from inoculation to complete plant colonization. The rate and extent of mycelial advancement differed significantly ($P \geq 0.5$) among the substrates. The mycelial growth of *Pleurotus tuberregium* exhibited significant variation across the tested substrates. The highest mean mycelial extension was observed on plantain leaves (67.17 ± 3.01 mm after three days), followed by oil palm fibre (61.33 ± 6.25 mm), while the least growth was recorded on corn husk (35.33 ± 5.03 mm). This differential response indicates that the nutritional composition and physical structure of plantain leaves provided a more favourable environment for enzymatic activity and nutrient assimilation by the fungus. Plantain leaves are rich in organic carbon, moderate nitrogen content, and essential minerals such as potassium and magnesium (Barshteyn and Krupodorova, 2016), which are important for fungal metabolism and protein synthesis. The rapid colonization on plantain leaves corroborates the findings of Okhuoya and Etugo (1993), who reported that *Pleurotus tuberregium* performs optimally on substrates with high cellulose-to-lignin ratios and good moisture retention capacity. The dense and concentric mycelial morphology observed in this study further supports that plantain leaves provided a conducive substrate structure that allowed for efficient oxygen diffusion and hyphal penetration. Conversely, corn husk produced the slowest mycelial extension and the sparsest growth density. This poor performance can be attributed to the high silica and lignin content of corn husk, which are resistant to enzymatic degradation, thereby limiting the availability of simple sugars for fungal metabolism (Chang and Miles, 2008; Barshteyn and Krupodorova, 2016). The sparse and woolly mycelial morphology observed on corn husk suggests that the substrate was nutritionally imbalanced and structurally rigid, which impeded effective colonization. Sawdust and corn straw supported moderate growth, although their mycelial densities were lower than those of plantain leaves and oil palm fibre. The growth response of *Psathyrella atroumbonata* across the substrates showed a similar trend of variability, though with different substrate preference. Oil palm fibre supported the highest mycelial extension (81.17 ± 4.91 mm after three days), followed by sawdust (74.83 ± 1.76 mm), whereas the least growth was again observed on corn husk (14.17 ± 2.02 mm). The superior growth on oil palm fibre may be due to its favorable structural composition, which includes moderate lignin, cellulose, and hemicellulose content, allowing for balanced nutrient availability and aeration (Obodai et al., 2003). Additionally, oil palm fibre retains moisture effectively, which prevents desiccation of the substrate surface and promotes continuous mycelial expansion. The dense and woolly mycelial morphology on oil palm fibre observed in this study indicates vigorous colonization, suggesting that *Psathyrella atroumbonata* efficiently utilized the available nutrients. This finding aligns with Amadioha and Nosike (2023), who reported enhanced mycelial growth of *Psathyrella atroumbonata* on oil palm waste due to its high carbon and nitrogen balance. The relatively high growth observed on sawdust corroborates similar studies by Pandey (2022), who noted that sawdust, being rich in lignocellulose and capable of retaining moisture, can serve as a good substrate for tropical mushrooms. In contrast, the poor growth performance of *Psathyrella atroumbonata* on corn husk mirrors that of *Pleurotus tuberregium* and can be attributed to the same limitations; high lignin content, poor aeration, and low nutrient accessibility (Isikhuemhen and Nerud, 1999). This substrate may not provide the ideal carbon-to-nitrogen ratio required for active vegetative growth. The thin, sparse, and irregular mycelial morphology observed further indicates physiological stress and suboptimal substrate utilization. The differences in mycelial density and morphological patterns observed among the substrates provide further evidence of substrate influence on fungal physiology. *Pleurotus tuberregium* exhibited very dense and concentric mycelial morphology on plantain leaves and oil palm fiber suggesting active metabolic processes and healthy vegetative growth. In contrast, the sparse mycelium on corn husk reflects limited nutrient absorption and retarded hyphal development. Indicating that substrate type influences mycelial density (Agba *et al.,* 2021). For *Psathyrella atroumbonata*, dense and woolly growth forms were observed on oil palm fibre and sawdust, while thin or sparse growth occurred on corn husk and plantain leaves. The morphological features observed are consistent with the findings of Guadarrama-Mendoza et al. (2014), who emphasized that dense, woolly, or concentric mycelia are indicators of vigorous vegetative expansion, while sparse or irregular growth patterns signal nutrient limitation or substrate incompatibility. The comparative growth rate analysis revealed that *Psathyrella atroumbonata* grew faster than *Pleurotus tuberregium* across most substrates, attaining its highest rate (27.06 mm day^{-1}) on oil palm fiber while *Pleurotus tuberregium* achieved its maximum rate (22.39 mm day^{-1}) on plantain leaves. This differential growth potential may be linked to species-specific enzymatic systems and ecological adaptation. Studies indicate that *P. atroumbonata* is capable of producing lignin-degrading enzymes (ligninase) and has been noted for its ability to digest cellulose, suggesting potential for efficient degradation of lignocellulosic materials (Ayodele and Okhuoya, 2009). The slower mycelial growth of *Pleurotus tuberregium* may be associated with its ecological preference for well-decomposed organic matter, as reported by previous studies which observed enhanced performance of this species on substrate mixtures rich in partially decomposed lignocellulosic material (Olutayo and Friday, 2020). However, both species demonstrated adaptability to agricultural wastes, confirming their potential for large-scale cultivation using locally available and low-cost materials

5.1. CONCLUSION

The results of this study demonstrate that substrate selection significantly affects the growth and colonization efficiency of edible mushrooms. Plantain leaves and oil palm fibre emerged as the most efficient substrates for *Pleurotus tuberregium* and *Psathyrella atroumbonata*, respectively, due to their favorable physicochemical properties, nutrient composition, and structural porosity. The ability of both species to utilize various agro-wastes highlights their ecological versatility and the potential of agricultural residues as sustainable raw materials for mushroom cultivation.

5.2. RECOMMENDATION

Based on the results, Mushroom farmers and biotechnologists can significantly enhance production efficiency by carefully selecting substrates tailored to the nutritional and ecological requirements of each mushroom species. Substrates with high lignocellulosic content and good moisture retention, such as plantain leaves and oil palm fiber have been shown to promote dense mycelial growth, rapid colonization, and improved yield.

Incorporating these substrates into cultivation practices not only optimizes growth performance but also provides a sustainable and locally available resource for mushroom production. Future studies could focus on refining substrate formulations and combinations to further maximize productivity and nutritional quality of cultivated mushrooms.

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