UTILIZATION OF SELECTED WASTE EXTRACTS ON THE VEGETATIVE GROWTH OF *Marasmiellus inoderma* (Berk.) SINGER

BY

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BIOTECHNOLOGY TECHNIQUES

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.sc.) IN SCIENCE LABORATORY TECHNOLOGY.

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CERTIFICATION

This is to certify that this project work, titled "**UTILIZATION OF SELECTED WASTE EXTRACTS ON THE VEGETATIVE GROWTH OF** *Marasmiellus inoderma* **(Berk) Singer"** was carried out by Chinwendu Joyce MBATAHU with Matriculation Number LSC1907337, of the Department of Science Laboratory Technology, 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 work is dedicated to Almighty God

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ABSTRACT

Mushrooms are good degraders of wastes and plant litters. *Marasmiellus inoderma* is an edible and medicinal mushroom that usually grows on leaf litters and wood wastes. To test for the suitability of plant wastes on the vegetative growth of the mushroom, extracts from selected wastes were used to prepare media for its growth in the laboratory. Six plant wastes were selected for this study. They include Oil palm fruit fiber (OPFF), Banana leaves, *Terminalia catappa* leaves (TC**)**, paper wastes , cotton wastes and melon husk wastes. The extracts of each these wastes were prepared by weighing 100g of the waste into 1L of water and boiling for 15 minutes. The extracts were amended with Dextrose sugar and PDA and then inoculated a 10mm agar plug of a 7day old culture. The results obtained indicated that the extract from cotton waste (75.75 ± 12.71 mm) and cotton amended with PDA gave the highest mycelia extension (74.33 ± 1.61 mm) followed by Oil palm fruit fiber extract amended with PDA (73.5 ± 3.04 mm) and the least extension was observed in the extract of waste paper amended with PDA (39.33 ± 29.17 mm). The extract of cotton amended with PDA gave the highest biomass yield after 12 days of inoculation. The pure extract of Banana leaves compared well with PDA in the mycelia extension of the mushroom while Melon amended with PDA compared favorably with PDA in biomass yield of the mycelium. The density and morphology of the mushroom on the extracts differed considerably. While the mycelia growth and form was dense in pure banana and cotton extracts, it was slightly dense in the pure extracts of OPFF and *Terminalia catappa* leaf and very dense in melon husk and cotton waste extracts when both were amended with PDA. This study shows that the fungus can be cultivated on these wastes and can therefore be used to reduce the waste and convert these wastes into food. These substrates can also be useful in generating the mycelia of the fungus *Marasmiellus inoderma* to produce other value-added products.4

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Innovations in managing agricultural wastes or biomass is a continuous challenge and recent trends favor the utilization of this wastes for value added purposes (Rosentrater *et al.,* 2009). A mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and picked up by hand (Chang and Miles, 2004). Mushroom cultivation is the practice or process of growing edible or medicinal mushrooms for use by man as food, medicine or fodder for livestock. Mushroom cultivation is a direct utilization of their ecological role in the bioconversion of wastes generated from agriculture into edible biomass (Savoie *et al.,* 2011). Most fungi are usually unseen and go unnoticed as it colonizes and absorbs nutrient from woods, fallen leaves and other organic matter. As a group, fungi can grow on almost any carbon source. A fungus composes of tubular, branched filaments called hyphae and an aggregation of hyphae is called mycelium.

Many fungi including *Marasmiellus inoderma* are saprophytic in nature and can grow on a wide range of organic matter. In contrast to the cultivation of higher plants, the culturing of fungi and growing of mushrooms is a recent practice and just like any form of agriculture, seeds are needed for propagation. Just as seeds as used for propagation in plants, so are fungi or mushroom spores used for propagating mushrooms. These spores germinate and develop a mycelium which can grow in conditions that are favorable to the organism. The mycelium absorbs nutrient from the substrate or medium on which it grows. The mushroom *Marasmiellus inoderma* is an edible mushroom which belongs to the family *Marasmiaceae.* Most members of this genus are small, brown unimpressive mushrooms and several of the species are known to

grow in a characteristic fairy ring pattern. *Marasmiellus inoderma* is usually known to grow in clusters on decaying leaves and mixed wood. According to a study done by Okhuoya and Osenwegie (2009), this mushroom is found growing on dried fronds and trunks of oil palm (*Elaeis guineensis*). Interaction with locals revealed that this mushroom has medicinal properties which makes it useful. An oncological study carried out by Petrova *et al.,* 2007 showed that this mushroom is a potent and strong inhibitor of the effects of cancer-causing agents and reported it to have anti-microbial and phytotoxic properties.

The art of culturing started about 1894 in France. However, the methods for preparing a pure culture through tissue culture or spore culture was given by two scientists, Fergusen (1902) and Duggan (1905). A pure culture is one that contains only a single kind of microbe; not a mixture (Okhuoya *et al.,* 2012). Jenison (1948) and Kaul (1981) reported from their study that when meals of grains, legumes, oranges, banana, celery, alfafa, parsnip, corn steep and gluten extracts are incorporated into agar, the mycelia of mushrooms grow at a faster rate. The active mycelia which is used to produce mushroom spawn depends on the agar medium or food substances necessary for its growth (Chang and Miles, 2004). Mushroom growth is greatly influenced by the media or substrate on which it is growing and the influence of the media also depends on the available nutrient, pH, microbial activity, aeration and water content. The more nutrients are easily assessible, the denser the mycelia ramification (Edward, 2000). One of the most crucial and critical stages of mushroom cultivation is the production of viable or active mushroom cultures or spawn. It has also been described as the bedrock and the most limiting factor to mushroom cultivation all over the world (Stanley, 2010). Some steps taken to culture edible mushrooms are usually complex and require some specificity but the methods used in the

course of this study can help to assist farmers and people interested in the practice of cultivating mushrooms as a skill or hobby.

In Nigeria, wastes (including agricultural wastes) are usually disposed of in land dumpsites or burnt openly which is not proper as the decomposition of these wastes by other microbes results in the release of methane into the atmosphere and the burning releases Carbon dioxide into the atmosphere. These forms of waste disposal contribute immensely to Environmental pollution. Agricultural wastes are rich in various nutrients and lignocellulosic which are difficult to break down. However, studies have shown that these components can be effectively broken down by mushrooms with little or no pollution in the process. Microbial technology helps in large scale recycling of agro wastes as an alternative way to use agricultural residues or wastes (Chang and Miles, 2004; Khan *et al.,* 2010). Mushrooms are known to be degraders of wastes thereby converting these wastes into compost that can be used as manure for growing crops. The conversion of these wastes into usable products and the ability of mushrooms to do this conversion is a valuable source of income and also a good means of reducing pollution. In Nigeria, mushroom consumption habits and mushroom cultivation practices has not been well documented but it is believed that many parts of the country are suitable for mushroom growing. Publications containing biochemical studies of substrates and fungi confirm that fungi which are grown in rich biologically active substances, unconventional substrates provide a rich biochemical composition when compared with fungi grown on the normal conventional substrates. Mushroom cultivation has been viewed by different scientists as a very effective way to extract bioresources left behind in agricultural residues and as a strategy for protecting the environment. Various scientists have reported that mushrooms can be easily grown on different ligno-cellulose wastes such as banana leaves, cereal straw, paper wastes,

sawdust and poultry droppings. Mushroom cultivation can be used as technology for managing wastes from agricultural and agro-based industries.

According to Das and Mukherjee., (2007), cultivation of mushrooms such as the Oyster mushroom, *Pleurotus cornipuceae,* can play a vital role in managing wastes whose disposal has become a problem. These wastes can be recycled into food and the environment may be less endangered by pollution (Eswaran and Ramabadran., 2000). Furthermore, the use of these waste residues can help to convert unwanted or inedible biomass into nutritious food in form of mushrooms. Economic and nutritional importance of mushrooms increases as edible food, this is due to the high protein and fiber content in them. Mushrooms are rich in antioxidants which play a major role in human health and nutrition. Many mushrooms possess a range of metabolites that are of interest to pharmaceutical and phytochemical scientists and even to food nutritionists. Suresh *et al.,* 2017 reported that the methanol extracts of the mushroom *Pleurotus djamor* is able to inhibit the growth of some gram-negative bacteria such as *Escherichia coli*, *Pseudomonas putida* and *Vibrio cholera*. According to Petrova *et al.,* 2007, this inhibitory ability may be due to the elution of polar compounds in the methanol extract of this mushroom. Utilizing these wastes for mushroom cultivation can be a viable means of solving pollution problems in the country and at the same time produce edible products of high market value which in turn serves as source of income for the mushroom grower. Mondal *et al.,* 2010 reported that after cultivating the mushroom *Pleurotus florida* on different substrates, the highest mycelia growth was observed on banana leaves and rice bran. The process of cultivation mushrooms such as *P. pulmonaris* on different mixtures of cotton waste and cassava peels (Adebayo *et al.,* 2009), cotton waste alone and combined with rice husk (Khan *et al.,* 2010), and also using coir fiber, oil palm waste, sawdust of *Gmelina arborea* and rice straw (Jonathan *et al.,* 2013) have been carried out and

positive have been reported. Pani (2012) studied the utilization of cotton wastes and sunflower stalks either alone or when combined with paddy straw for the production of the sporophore of milky mushroom *Calocybe indica.* The results obtained showed positive growth but the substrates which were combined with paddy straw grew better. A study carried out by Suganthi and Krishnakumari., 2018, shows that some mushrooms like *Pleurotus cronipucea*, show high mycelia growth when grown on waste from sugarcane, paddy straw, banana leaves and sawdust. The effects of different substrate on the mycelia growth and yield of *Volvariella* spp was evaluated by Tripathy *et al.,* 2011. The mushroom was cultivated on substrates of paddy straw, oil palm fiber and sawdust and it was observed that *V. volvacaea* was able to degrade these wastes completely. Other edible mushrooms such as *Agaricus* spp, *Lentinus* spp, *Pholiota nameko*, *Hypsizygus mamoreus, Garnoderma lucidum* are also regarded as active bio destructors. Stanley *et al.,* (2013) recommended that the use of farm waste supernatant extracts as culture media should be encouraged in culturing and boosting the vigor of *Pleurotus sajor caju* mycelia for spawn production. They also carried out a study to show that farm wastes such as cassava and yam peels are very suitable media for the production of *Pleurotus sajor caju* as they stimulate luxuriant mycelia growth better than synthetic culture media. These stimulatory substances that aids in the rapid mycelia growth are amino acids, vitamins and essential nutrients as investigated in a study by Kadiri and Kehinde (1990). Media can be locally formulated to determine specific features of a microorganism. A certain pathogen can be isolated from the part of the plant or host it infects and that part can be incorporated or used as a component of a conventional medium in order to establish a physiological relationship between the host and the pathogen (Okhouya *et al.,* 2012). It can be used to check if the host produces metabolites that encourages the pathogen to infect it. Dede and Okungbowa (2007) grew the fungus *Ceratocystis paradoxa* which was

isolated from oil palm on media supplemented with oil palm fruit extract. In their report, the vegetative growth of the fungi on the supplemented media compared well with growth on potato dextrose broth. This suggests that certain substances present in oil palm supports the growth of this fungus. It was recommended that this medium can be used to grow *C. paradoxa* when commercial or conventional mediums like potato dextrose medium are not available as it is much cheaper and easier to obtain. In another study, Okungbowa and Ogbimi (2007) cultivated a soil fungus *Trichoderma viride* on bean pod extract agar. The results obtained from their study was excellent and this led to the conclusion that bean pod extract agar could replace PDA in the culturing of *T. viride*. Different scientists have reported that certain moulds which infect fruits have shown good growth when cultivated in media which are partly or fully supplemented with the mesocarps of such fruits and as such, wastes from vegetables, fruits and farms show great promise for cultivation of mushrooms. Studies have also shown that the extracts and secondary metabolites of some basidiomycetes and their spent can be used as cattle feed, fertilizers or landfills. Tsegaya and Tefera (2017) report that many agricultural and agro-industrial by-products are important in mushroom production and mushroom cultivation is a useful method of environmental waste management and waste disposal. They also recommend that since mushrooms require carbon, nitrogen and other cellulosic nutrients, most organic matter contains hemicellulose, cellulose and lignin can be used as mushroom substrate for example; rice and wheat straw, cotton seed hull, sugar cane, bagasse, sawdust, waste paper, leaves and so on.

1.2 Statement of Problem

The study on the utilization of selected waste extracts for enhancing the vegetative growth of *Marasmiellus inoderma* addresses a significant issue in agricultural sustainability and waste management. *Marasmiellus inoderma*, an edible mushroom has the ability to degrade

waste matter while building its biomass while degrading waste can also be used to convert waste to the value added product.

Furthermore, the research seeks to contribute to the broader understanding of fungal ecology and its applications in biocontrol and bioremediation. Utilizing waste extracts not only promotes the growth of beneficial fungi but also aligns with environmental goals by reducing waste disposal challenges. This approach may lead to innovative strategies for harnessing fungal growth to combat plant pathogens, thereby enhancing crop resilience and productivity. The findings from this study could provide valuable insights into sustainable agricultural practices, offering a dual benefit of waste reduction and improved management of plant diseases caused by *M. inoderma*.

1.3 Significance of the Study

The study on the is significant due to its many contributions to sustainable development, waste management, and agricultural innovation. In light of escalating worldwide environmental concerns, the study corresponds with initiatives aimed at mitigating the issues associated with organic waste accumulation. This research investigates the viability of specific waste extracts as a growing medium for *M. inoderma*, offering a method to transform waste materials into useful bioresources, thus mitigating environmental degradation and fostering a circular economy.

M. inoderma, a fungus known for its role in nutrient cycling and organic matter decomposition, offers significant promise for biotechnological and ecological applications. Comprehending its vegetative development reaction to waste-derived extracts may result in advancements in the cultivation of this organism under regulated conditions. This affects both sustainable waste recycling and the improvement of soil fertility via fungal biomass production, hence fostering environmentally friendly agriculture practices.

This study has the potential to reveal new insights into the adaptability and growth mechanisms of fungus in unconventional substrates. This can facilitate the development of cost-effective, scalable techniques for fungal cultivation, benefiting sectors such as agriculture, medicines, and biotechnology. Optimizing the growth of *M. inoderma* may result in advancements in organic fertilizers, biopesticides, or the synthesis of bioactive chemicals having therapeutic potential.

The research will significantly advances the global sustainable development goal by illustrating practical uses of waste valorization. It will tackles urgent concerns of waste management while enhancing biodiversity and advocating the ecological advantages of fungi. The findings may bolster community-based initiatives in local economies, especially in areas rich in agricultural or organic waste, to utilize garbage for profitable and sustainable enterprises.

This work will enhance the understanding of fungal biology and mycelial growth dynamics, specifically concerning the *Marasmiellus inoderma*, in academic and scientific contexts. It encourages additional investigation into the biochemical interactions between fungi and organic waste extracts, perhaps fostering future research collaborations and advancements. This work, emphasizing sustainability, waste management, and fungal biotechnology, has the potential to significantly contribute to both science and society.

1.4 Aim

This study aims to test the suitability of selected wastes extracts for the cultivation of *Marasmiellus inoderma*, an edible mushroom from Nigeria

1.5 Objectives

1. To determine the effect of waste extracts of banana leaves, cotton wastes, paper waste, melon seed peels and *Terminalia catappa* leaves on the mycelia extension of *Marasmiellus inoderma*.
2. To evaluate the effect of these waste extracts on the biomass of *Marasmiellus inoderma*.
3. To evaluate the effect of these waste extracts on the growth morphology of *Marasmiellus inoderma*.
4. To evaluate the effect of these waste extracts on the growth rate of *Marasmiellus inoderma*

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungal Growth and Development

Fungi are eukaryotic organisms estimated to comprise over 4 million species. Fungal cell structures, while akin to those of other eukaryotic cells, are distinguished by the presence of ergosterol in their cell membranes and chitin in their cell walls. The cytoplasm of cells has elevated quantities of salt and sugar compared to other eukaryotes. This governs cellular homeostasis and modulates the exchange of substances (Peraza-Reyes & Malagnac, 2016). With the exception of yeasts, the majority of fungi possess microscopic structures known as hyphae, which aggregate to create discernible formations referred to as mycelium. The hyphae exhibit apical growth. In the apical regions of hyphal growth, there exist secretory vesicles known as "Spitzenkörper" and Wooronin bodies that function as peroxisomes (Dyer *et al.,* 2016). Due to the characteristics of hyphae, fungus may inhabit environments unsuitable for other eukaryotic cells and can utilize diverse substrates. Fungi participate in several degradations, transformation, and cyclical processes in nature. Despite being heterotrophic organisms, they can exist as saprophytic, mutualistic, and parasitic entities. Their global distribution and adaptability to many settings can be attributed to the exceptional reproductive capabilities of fungus (Li *et al.,* 2017).

Fungi has the capability to reproduce both sexually and asexually. Asexual reproduction in fungi occurs by vegetative reproduction through hyphae or through the spores they generate. Sexual reproduction involves the formation of a diploid nucleus through the fusion of haploid spores, followed by the germination of this nucleus, thus perpetuating the cycle. Asexual reproduction occurs more frequently than sexual reproduction in fungus (Peraza-Reyes &

Malagnac, 2016). This occurrence enhances the adaptive capacity of fungi and inhibits the accumulation of deleterious mutations and their transmission between generations. Moreover, their survival prospects and competitive edge are secured (Dyer *et al.,* 2016).

2.2 Mushrooms

Fungi comprise a varied array of creatures, such as mushrooms, rusts, smuts, puffballs, truffles, morels, and yeasts, in addition to many lesser-known species (Blackwell *et al.,* 2011). As of now, more than 700,000 fungal species have been documented, while estimates indicate that the actual number may surpass 1.5 million (James *et al.,* 2006). In this expansive kingdom, edible mushrooms have been distinguished, acknowledged for generations as both a culinary delicacy and a significant nutritional asset to human diets. Mushrooms are abundant in proteins, lipids, amino acids, glycogen, vitamins, and vital minerals (Okhuoya *et al.,* 2010). Their mineral salt concentration significantly exceeds that of meat and fish and is almost double that of typical vegetables (Rambeli, 1983).

Mushrooms are the fruiting bodies of certain fungi, functioning primarily as reproductive structures. They belong predominantly to the phylum Basidiomycota, though some also arise from Ascomycota. These fruiting bodies emerge from a network of microscopic filaments called hyphae, which together form the mycelium. The mycelium resides within soil or organic matter, absorbing nutrients and enabling the fungus to thrive. Structurally, mushrooms consist of several parts: the cap (pileus), which protects the spore-bearing surface; gills (lamellae) or pores beneath the cap, which produce spores; the stem (stipe), which supports the cap; and the mycelium, which serves as the vegetative body. The production of spores is crucial to the fungal lifecycle. Spores germinate upon landing in favorable environments, producing hyphae. When hyphae

from compatible spores meet, they fuse to form a dikaryotic mycelium, which eventually produces mushrooms under appropriate environmental conditions (Alexopoulos *et al.,* 1996).

The diversity of mushrooms is staggering, with over 14,000 known species worldwide. They vary significantly in shape, size, color, and ecological function. Some mushrooms are highly valued for their culinary and nutritional attributes, while others are renowned for medicinal properties or present significant dangers due to toxicity. Edible mushrooms, such as the widely consumed button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* ostreatus), and shiitake mushroom (*Lentinula edodes*), are rich in proteins, vitamins, and minerals. These mushrooms not only enhance the flavor of meals but also contribute to a balanced diet. For example, they are excellent sources of B vitamins, vitamin D (when exposed to sunlight), selenium, and potassium. Their low-calorie content makes them suitable for weight management, while their antioxidants, such as ergothioneine, protect cells from oxidative damage (Kalac, 2009).

Certain mushrooms possess notable medicinal properties. Traditional medicine in Asia and other parts of the world has long utilized species like Reishi (*Ganoderma lucidum*), known for its immune-boosting and stress-reducing properties, and Turkey Tail (*Trametes versicolor*), which contains polysaccharides believed to enhance chemotherapy efficacy and inhibit cancer cell growth. Cordyceps species, another medicinal mushroom, are valued for their ability to improve energy and stamina (Wasser, 2002). The therapeutic potential of these fungi is supported by bioactive compounds, including beta-glucans, terpenoids, and polysaccharide-protein complexes. These compounds modulate the immune system, reduce inflammation, and exhibit antioxidant properties (Kidd, 2000).

However, not all mushrooms are safe for consumption. Toxic varieties such as the death cap (*Amanita phalloides*) and the destroying angel (*Amanita bisporigera*) have caused numerous fatalities due to their potent toxins, which damage the liver and kidneys. These species closely resemble edible mushrooms, making foraging dangerous without expert knowledge. Additionally, certain mushrooms, like Psilocybe species, contain psychoactive compounds such as psilocybin, which can alter perception and cognition. While these so-called "magic mushrooms" have been used recreationally, emerging research suggests potential therapeutic applications for mental health disorders, including treatment-resistant depression (Carhart-Harris *et al.,* 2018).

Ecologically, mushrooms are indispensable to the functioning of terrestrial ecosystems. They act as decomposers, breaking down organic matter such as dead plants and animals, recycling essential nutrients into the soil. This process not only enriches the soil but also supports plant growth and the broader food web. Moreover, many fungi form symbiotic relationships with plants through structures known as mycorrhizae. In these associations, the fungus provides plants with nutrients like phosphorus, while the plant supplies carbohydrates to the fungus. This mutualistic interaction enhances plant resilience to environmental stressors (Smith & Read, 2008). On the other hand, parasitic mushrooms such as Armillaria species can harm plants and trees, illustrating the complex and dualistic roles fungi play in ecosystems.

Mushrooms' uses extend beyond their ecological and nutritional roles to include industrial and environmental applications. Biodegradation is one such innovative use, where fungi are employed to break down pollutants, including plastics and petroleum products. Mycofabrication, which uses fungal mycelium to create sustainable materials, offers a potential replacement for plastics and synthetic leather. Additionally, fungal enzymes are being explored for biofuel production, presenting an eco-friendly solution to energy demands (Stamets, 2005).

Despite their myriad benefits, mushrooms also present challenges. The misidentification of wild mushrooms poses significant health risks, especially for inexperienced foragers. Even cultivated mushrooms may cause allergic reactions in some individuals. Furthermore, large-scale mushroom farming, while generally sustainable, can lead to habitat disruption and waste management issues if not carefully managed (Chang & Miles, 2004).

The future of mushrooms is promising, with ongoing research uncovering new applications in medicine, agriculture, and sustainability. Their potential to address pressing global challenges, from food security to environmental conservation, underscores their importance as a natural resource. As scientists continue to study their properties, mushrooms will likely play an increasingly vital role in human well-being and the health of the planet.

Figure 2.1: Some mushrooms found in Nigeria (Source: Osemwegie, 2006)

2.2.1 Categories of Mushrooms

Mushrooms can be categorized into four major groups. Changs and Miles (2004) categorize mushrooms into the following groups, namely: edible mushrooms, medicinal mushrooms, poisonous mushrooms and miscellaneous.

Table 1: Categories of mushrooms

Category	Defining Characteristics	Examples
Edible	Safe for human consumption and often cultivated as a food source High in nutrients like protein, vitamins (B-complex), minerals and antioxidant It has low calories, fat and cholesterol	*Agaricus bisporus*, *Marasmiellus inoderma*
Poisonous	It contains toxic and harmful substance that can cause illness, organ failure or death if ingested. Some are brightly coloured as a warning, while others are deceptively ordinary	*Amanita muscaria* (fly agric), *Amanita virosa* (destroying angel)
Medicinal	It contains bioactive compounds that promote health or are used as remedies in traditional and modern medicine Boost immune system, reduces inflammation and may have anti-cancer and anti-viral or bacterial properties	*Ganoderma lucidum* (reishi), *Hericium erinaceus* (lion's mane)
Miscellaneous	Serves purpose beyond food or medicine, including spiritual, recreational, industrial or ecological uses. May have psychoactive compounds, unique structural applications or dye producing capabilities Some species contributes to the ecosystem balance by decomposing waste or supporting plant growth.	*Lycoperdon perlatum.* *Psilocybe* spp.

2.3 Overview of *Marasmiellus inoderma*

Marasmiellus inoderma is a notable species within the family Marasmiaceae under the order Agaricales. It represents a critical ecological and ethnomycological resource in tropical and subtropical regions. This fungus was initially described as Marasmius inoderma by Berkeley in 1851 but was reassigned to the genus Marasmiellus by Singer in 1955. This reassignment

reflected a more refined understanding of its structural and ecological distinctions compared to other genera (De Kesel *et al.,* 2008). The species is recognized for its saprotrophic lifestyle, which enables it to decompose organic matter from monocotyledons such as oil palm (Elaeis guineensis) and banana (Musa spp.), making it a vital part of nutrient cycling in its ecosystems (Bastos *et al.,* 2023).

Ecologically, *M. inoderma* thrives in environments with high humidity and organic matter, such as banana plantations and oil palm residues, where it plays a dual role. On the one hand, it is a decomposer contributing to nutrient cycling and agricultural waste management; on the other, it can act as a pathogen under specific conditions, causing pseudostem rot in bananas (Thiruchchelvan *et al.,* 2012). This ecological duality has prompted scientific interest in its biology, management, and potential for sustainable agricultural practices.

In addition to its ecological significance, *M. inoderma* is an important dietary component for many communities, especially in West Africa. It is harvested and sometimes cultivated on agricultural waste materials, highlighting its cultural and nutritional value (De Kesel *et al.,* 2008). Its edibility and potential for small-scale, low-cost cultivation make it a candidate for poverty alleviation and food security in resource-limited settings (Bastos *et al.,* 2023).

2.3.1 Classification of *M. inoderma*

Taxonomically, *M. inoderma* is classified as follows:

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Marasmiaceae

Genus: *Marasmiellus*

Species: *Marasmiellus inoderma*

The genus *Marasmiellus* is distinguished by its small fruiting bodies, resilience to desiccation, and preference for nutrient-rich decomposing plant material. These traits set it apart from closely related genera such as *Marasmius*, which lack similar morphological and ecological traits (De Kesel *et al.,* 2008). The genus name itself emphasizes its diminutive and marasmic (withering) appearance, aligning with its ability to thrive under dry conditions and regenerate after rehydration.

The taxonomic history of *M. inoderma* is complex, reflecting its distribution across diverse ecological zones. Initially described from tropical South America, the species has been documented in various regions, including Africa and Asia, where its ecological roles and morphological traits have been re-evaluated to enhance its classification (De Kesel *et al.,* 2008; Pegler, 1968).

2.3.2 Morphological Characteristics

The morphology of *Marasmiellus inoderma* is both distinctive and highly adaptive, reflecting its saprotrophic lifestyle. The fruiting body, or basidiocarp, is subcartilaginous, measuring 10-30 mm across. The pileus starts as convex but transitions to a more planar or depressed shape as it matures. It is initially brownish-orange but turns white with faint pinkish hues upon drying. This hygrophanous nature, where the color changes with moisture content, is a hallmark of many species in the Marasmiaceae family (De Kesel *et al.,* 2008). The surface of the pileus is subtomentose to pruinose, contributing to its powdery appearance.

The lamellae of *M. inoderma* are thin, subdistant, and white in color, becoming pale yellowish when dried. They are adnate to subadunate with minimal forking, which enhances

their flexibility and longevity. The stipe, slender and cylindrical, tapers slightly towards the base, which is often reddish or pinkish and pubescent. This basal coloration is a unique feature that aids in distinguishing the species (De Kesel *et al.,* 2008).

Microscopically, *M. inoderma* exhibits smooth, ellipsoid spores measuring 6.9–10.6 × 4.2–5.7 µm. These spores are hyaline and inamyloid, indicative of its Basidiomycota lineage. The presence of clavate cheilocystidia with knotty outlines adds to its diagnostic features. The hymenophoral trama is subregular to regular, and clamp connections are observed in all tissues, signifying its evolutionary lineage within the Agaricales (De Kesel *et al.,* 2008).

2.3.3 Ecological Significance

The ecological role of *M. inoderma* is multifaceted, encompassing decomposition, nutrient cycling, and potential applications in sustainable agriculture. As a saprotroph, it colonizes decomposing organic matter, particularly monocotyledons like oil palm and banana. This function is critical in tropical agroecosystems, where organic waste accumulates and requires efficient decomposition to maintain soil fertility (De Kesel *et al.,* 2008; Thiruchchelvan *et al.,* 2012).

In banana plantations, *M. inoderma* plays a paradoxical role. While it contributes to breaking down plant debris, it can also act as a pathogen, causing pseudostem rot. This disease manifests as necrosis of the pseudostem's outer layers, stunting, and plant death, with significant impacts on crop yields (Thiruchchelvan *et al.,* 2012). Such dual functionality underscores the importance of understanding its biology and management.

Its seasonal appearance during the rainy months, from May to October, aligns with optimal moisture conditions. The species is commonly found in ruralized areas and managed plantations, suggesting its adaptability to both natural and anthropogenic environments. This

ecological plasticity highlights its potential as a model organism for studying fungal adaptation and resilience (De Kesel *et al.,* 2008).

2.3.4 Ethnomycological Importance

The ethnomycological value of *Marasmiellus inoderma* is well-documented, particularly in West Africa. In Benin and Togo, the species is harvested for consumption and sometimes cultivated on agricultural waste like oil palm residues. Local communities value it for its mild fungoid flavor and nutritional content, making it a critical component of traditional diets (De Kesel *et al.,* 2008). Its ability to grow on waste materials reflects its adaptability and underscores its potential for low-cost, sustainable farming practices.

Ethnobotanical surveys have revealed traditional cultivation practices, where *M. inoderma* is grown on readily available substrates. This practice not only provides a source of income but also promotes environmental sustainability by reducing waste accumulation. The simplicity of its cultivation makes it an accessible option for rural communities (De Kesel *et al.,* 2008).

Additionally, its potential for integrated pest management has been explored. Studies have shown that antagonistic fungi, such as *Trichoderma* spp., can inhibit its pathogenic growth, offering insights into sustainable disease management in crops (Thiruchchelvan *et al.,* 2012).

2.4 Culinary and Medicinal Uses of *Marasmiellus inoderma*

The culinary and medicinal uses of *Marasmiellus inoderma* are deeply rooted in its ecological versatility, cultural significance, and nutritional properties. This saprotrophic fungus, belonging to the Marasmiaceae family, has been recognized for its edibility and potential medicinal applications across various regions, particularly in tropical and subtropical environments. The dual roles it plays as both a food source and a subject of

ethnopharmacological interest make it a species of notable importance. Its presence in traditional diets and its potential therapeutic properties underscore the need for a comprehensive exploration of its uses.

Edible mushrooms have long been valued as a critical component of traditional diets in many parts of the world, particularly in regions with limited access to animal-based proteins. *Marasmiellus inoderma*, like many other saprotrophic fungi, thrives on decaying plant matter and can be easily collected or cultivated in environments rich in agricultural residues, such as banana pseudostems or oil palm waste (De Kesel *et al.,* 2008). This ecological adaptability has made it a significant dietary resource in parts of West Africa, including Benin and Togo, where it is harvested and consumed as a delicacy. Its inclusion in traditional diets highlights its culinary value, particularly among the Adja people of Benin, who consider it a tasty and nutritious addition to meals (De Kesel *et al.,* 2008). The species is often prepared fresh, dried, or incorporated into soups and stews, serving as an affordable and accessible protein substitute. This dietary role is particularly critical in regions with protein deficiencies or where the cost of animal-based proteins is prohibitively high.

The mild fungoid flavor and appealing texture of *Marasmiellus inoderma* make it a favored choice among local communities. Its preparation methods, which include drying and smoking for preservation, reflect traditional practices designed to extend its usability throughout the year. Such techniques are common in tropical regions where the fruiting season is limited to a few months, typically during the rainy season. By leveraging simple preservation methods, communities have ensured a steady supply of this valuable food source. Moreover, its use extends beyond direct consumption; in some cases, dried forms of the mushroom are traded in local markets, contributing to the rural economy and providing an additional income source for

women and youth engaged in foraging and small-scale mushroom farming (De Kesel *et al.,* 2008; Bastos *et al.,* 2023).

The cultivation of *M. inoderma* is gaining attention due to its compatibility with low-tech farming methods. In West Africa, it has been successfully cultivated on agricultural byproducts such as oil palm residues, which are abundant in the region. These substrates not only provide a sustainable cultivation medium but also offer a solution to waste management challenges. This practice aligns with global efforts to promote food security through sustainable agriculture. The adaptability of *M. inoderma* to various substrates suggests that it could be an integral component of small-scale farming systems, particularly in regions where land and financial resources are limited (De Kesel *et al.,* 2008). The promotion of its cultivation could significantly enhance its availability as a food resource, while also addressing environmental concerns associated with agricultural waste.

Beyond its culinary applications, *Marasmiellus inoderma* holds potential medicinal value, although this aspect remains underexplored compared to its dietary role. Ethnomycological studies have identified related species within the *Marasmiaceae* family as sources of bioactive compounds with antimicrobial, antioxidant, and immune-modulating properties (Bastos *et al.,* 2023). These findings suggest that *M. inoderma* may also possess similar therapeutic benefits. In traditional medicine, mushrooms have often been regarded as health-promoting foods, used to strengthen immunity, improve vitality, and address various ailments. This perception has driven interest in their bioactive components, which include polysaccharides, phenolic compounds, and terpenoids. While specific studies on the pharmacological properties of *M. inoderma* are lacking, its close relatives provide a basis for further investigation into its potential medicinal uses.

The ability of *M. inoderma* to grow on nutrient-rich agricultural waste may contribute to its chemical profile, as substrates influence the production of bioactive metabolites in fungi. For example, its growth on oil palm waste and banana pseudostems could lead to the accumulation of specific secondary metabolites, which might exhibit therapeutic properties. In traditional West African practices, fungi like *M. inoderma* are often associated with health benefits, though the mechanisms underlying these effects are not well-documented. This gap in knowledge presents an opportunity for scientific exploration, particularly in identifying compounds with pharmaceutical potential (De Kesel *et al.,* 2008; Bastos *et al.,* 2023).

The dual role of *M. inoderma* as both a culinary and a medicinal resource is particularly significant in the context of global food security and health. Its cultivation on waste materials aligns with sustainable development goals, addressing food scarcity and waste management simultaneously. Furthermore, its potential therapeutic properties could contribute to the development of functional foods and nutraceuticals, which combine nutritional and medicinal benefits. This dual functionality highlights the importance of preserving and studying fungi like *M. inoderma* within their ecological and cultural contexts.

The ecological resilience and adaptability of *M. inoderma* further enhance its value as a resource. As a saprotrophic species, it decomposes lignocellulosic materials, playing a critical role in nutrient cycling within its ecosystem. This process not only supports agricultural productivity but also reduces the environmental impact of organic waste. By integrating its cultivation into agroecosystems, communities can harness its ecological benefits while reaping its nutritional and potential medicinal advantages.

2.5 Utilization of Waste Extracts in Mushroom Growth.

In 2022, the global population reached 8 billion, leading to heightened consumption of essential basic commodities, including water and nutrition. Climate change has resulted in a scarcity of potable water in numerous locations, with projections indicating a further decline in the future (UN, 2020). This is concerning, as water is essential for sustaining human existence and is integral to all human activities and products creation. Food production utilizes substantial volumes of water (132–12,650 m^3 ton^{-1} depending on the product) (Mekonnen & Hoekstra, 2011), whilst generating significant amounts of wastewater (Asghearneja *et al.,* 2021). Subsequent stages in food production, including food processing, are notably water-intensive, resulting in wastewaters rich in carbohydrates that account for 45% of all industrial organic pollution. Food production and processing waste streams (FPWS) can contain elevated levels of inorganic nutrients and lipids, contingent upon the specific food product.

Worldwide, the nutrients in FPWS are inadequately utilized, since they are either released directly into the environment or, when treated, 80% is transformed into bacterial biomass, resulting in sludge that incurs treatment and disposal expenses (Sankaran *et al.,* 2010). The elevated Chemical Oxygen Demand (COD), inorganic nutrients, and fat content of FPWS collectively provide challenges for wastewater treatment facilities (WWTPs) by inducing processing complications (Sitharama *et al.,* 2017). Nonetheless, these "problematic" nutrient-dense wastes signify a potential for the extraction of important raw materials. Inorganic nutrients, particularly nitrogen and phosphorus—key components of fertilizers—are constrained due to excessive mining of phosphorus rock and the high industrial demand for nitrogen, leading to scarcity and escalating prices. There is a necessity for more sustainable solutions for FPWS to diminish the environmental impact of food production and enhance its circularity.

The primary strategies for enhancing water circularity in food processing include:

(1) the reuse of white wastewater (characterized by low organic content) and grey wastewater (which contains higher levels of organics, suspended solids, and nutrients) (Asghearneja *et al.,* 2021);

(2) the extraction of valuable compounds such as polyphenols, pectin, fibers, proteins, antioxidants, and starch (Moreno-Gonzalez and Ottens, 2021); and

(3) biological methods aimed at nutrient recycling into value-added biomass (Asghearneja *et al.,* 2021).

The reuse of grey wastewater and the recovery of compounds necessitate energy-intensive processes, including membrane filtration, flotation, or crystallization, and may face fouling issues with membrane filtration. Consequently, they represent the most intricate and least sustainable alternatives, necessitating material and energy inputs that inherently generate waste (Moreno-Gonzalez and Ottens, 2021). The utilization of biological technologies represents a more environmentally sustainable approach to resource recovery. This utilizes the proliferation of bacteria, algae, fungus, or yeast in FPWS for nutrient recycling through the generation of value-added biomass (Asghearneja *et al.,* 2021). The generated biomass can serve as feedstock for biogas and biofuel production, be transformed into animal feed or fertilizer, or be utilized for the synthesis of several economically significant biochemicals that enhance the process's value (Li *et al.,* 2016).

Fungi necessitate substantial amounts of organic carbon as an energy source for growth, making them suitable for converting FPWS rich in organic material into value-added biomass (Purchase, 2016). Moreover, fungi synthesize a diverse array of enzymes that facilitate the decomposition and utilization of various substrates (e.g., sugars, resistant organics, ammonia,

and lipids) for metabolic processes and biomass proliferation (Purchase, 2016; Li *et al.,* 2016). The bioconversion of FPWS into valuable fungal biomass may serve as a pre-treatment for FPWS, mitigating nutrient overload in wastewater treatment plants and lowering treatment expenses. Fungi and yeasts generate a diverse array of lipids, proteins, biochemicals, vitamins, and supplements. They also generate numerous industrially significant enzymes and possess a balanced amino acid profile, rendering them potentially nutritious (Moura *et al.,* 2022). Moreover, filamentous fungi can serve specialized functions such as flocculating microalgae, offering a cost-effective option for microalgal harvesting, which is now an expensive procedure. The cultivation of fungi in FPWS for diverse uses enhances sustainability and promotes the circular economy within food processing facilities, advancing the EU's Sustainable Development Goal 12.

Another primary benefits of utilizing waste extracts in mycology is the promotion of environmental sustainability. Traditional waste disposal methods often involve landfilling or incineration, both of which contribute to pollution and resource depletion. In contrast, employing fungi to break down organic waste offers a natural solution that aligns with circular economy principles. Fungi possess the enzymatic machinery necessary to degrade a wide range of substrates, including lignocellulosic materials and other complex organics (Wibowo *et al.,* 2024). This capability not only helps in recycling nutrients back into the ecosystem but also reduces the burden on waste management systems.

Moreover, the economic advantages of using waste extracts are significant. The cultivation of fungi on low-cost substrates can lower production costs for various fungal products. For example, studies have demonstrated that agricultural by-products such as fruit and vegetable wastes can be effectively used as culture media for fungal growth (Ancin-Murguzur *et al.,* 2018).

This not only provides a cost-effective solution for producers but also encourages the valorization of agricultural waste that would otherwise contribute to environmental degradation.

In addition to economic benefits, the use of waste extracts in mycology can lead to enhanced fungal biomass production. Fungi require substantial amounts of organic carbon for growth, which is abundantly available in food production waste streams (Bansfield *et al.,* 2023). The metabolic activity of fungi in these substrates can yield high-value biomass that can be utilized in various applications ranging from food supplements to biofuels. For instance, certain strains of fungi have shown remarkable growth in cheese whey and other food processing wastes, demonstrating their potential for industrial applications (Bansfield *et al.,* 2023).

Furthermore, the integration of fungi into bioremediation strategies highlights another critical aspect of their utility in managing waste. Fungi can effectively degrade pollutants present in contaminated environments through bioremediation techniques. This involves utilizing their natural detoxifying abilities to break down hazardous substances into less harmful forms (Sharma, 2020). The application of fungi in this context not only aids in cleaning up polluted sites but also contributes to sustainable practices by reducing reliance on chemical treatments that may produce secondary pollutants.

The exploration of mycelium-composite materials represents an innovative avenue within this field. Mycelium—the vegetative part of fungi—can be cultivated on various organic wastes to create biodegradable composites that serve as alternatives to plastics (Frontiers in Materials). This approach not only addresses plastic pollution but also promotes sustainable material science by utilizing renewable resources.

2.6 Types of Waste

The utilization of agricultural and industrial waste as substrates or supplements for fungal growth has garnered significant attention in recent years. This approach addresses two key global challenges: managing waste sustainably and promoting the circular bioeconomy. Among the wastes studied for their potential in mycological applications are banana leaves, cotton waste, paper waste, melon seed peels, and *Terminalia catappa* (Indian almond) leaves. These materials offer diverse nutrient profiles that can significantly enhance fungal vegetative growth while reducing environmental pollution.

2.6.1 Banana Leaves

Banana leaves are a rich source of cellulose, hemicellulose, and lignin, making them a valuable substrate for fungal cultivation. Studies have highlighted their potential in supporting fungal growth due to their high fiber content and the presence of bioactive compounds with antimicrobial and antioxidant properties. Banana leaves have been effectively utilized for producing enzymes such as cellulase and as a base material for fungal cultivation, owing to their ability to retain moisture and provide essential nutrients like potassium, calcium, and magnesium (Zou *et al.,* 2022; Putra *et al.,* 2022). Research has shown that banana leaves can be biodegraded by fungi into biofertilizers or bioethanol, highlighting their dual utility as a nutrient source and a solution for waste management (Bishnoi *et al.,* 2023). This underscores their relevance in sustainable agriculture and mycology.

2.6.2 Cotton Waste

Cotton waste, primarily composed of cellulose, is an excellent carbon source for fungal growth. It is abundantly available as a by-product of the textile industry and has been utilized in

cultivating edible mushrooms such as *Pleurotus* spp. Cotton waste is particularly effective due to its high lignocellulosic content, which fungi can metabolize to produce bioactive compounds. Studies have demonstrated that combining cotton waste with other organic residues can enhance fungal yield and nutrient bioavailability, offering a sustainable pathway for upcycling textile industry by-products (Musa and Ali 2019).

2.6.3 Paper Waste

Paper waste, a ubiquitous by-product of the paper and packaging industries, primarily consists of cellulose, a vital nutrient for fungi. The recycling of paper waste for fungal cultivation not only reduces environmental pollution but also transforms waste into value-added products such as fungal biomass, enzymes, and secondary metabolites. For example, *Marasmiellus inoderma*, known for its role in decomposing lignocellulosic materials, has shown potential in breaking down paper waste effectively (Tesfay *et al.,* 2020). The deinking and pulping processes of paper waste improve its digestibility for fungi, enabling higher yields. Moreover, combining paper waste with other organic residues can enhance its nutrient profile, making it more suitable for fungal growth.

2.6.4 Melon Seed Peels

Melon seed peels are rich in proteins, fats, and fibers, making them a promising substrate for fungal cultivation. These agricultural by-products have been investigated for their ability to support the growth of fungi used in bioremediation and biofertilizer production. The nutrient-rich profile of melon seed peels, including essential amino acids and fatty acids, supports robust fungal growth, particularly for species involved in soil health improvement and organic matter decomposition (Hasanin & Hashem, 2020). Research has also explored the potential of melon

seed peels in producing fungal enzymes like lipases and proteases, which have applications in the food and pharmaceutical industries.

2.6.5 Terminalia catappa Leaves

The leaves of *Terminalia catappa*, commonly known as Indian almond, are abundant in tannins, flavonoids, and polyphenols, which exhibit antimicrobial and antioxidant activities. These bioactive compounds make *Terminalia catappa* leaves a unique substrate for cultivating medicinal and industrial fungi. The decomposition of these leaves by fungi like *Marasmiellus inoderma* can release nutrients that promote vegetative growth and enzyme production. In addition to serving as a fungal substrate, *Terminalia catappa* leaves have been explored for their potential in wastewater treatment and soil conditioning, further emphasizing their versatility (Toghueo *et al.,* 2017).

2.7 Use of Waste Extract in Mycelial Growth.

Mycelial growth is fundamentally dependent on the ability of fungi to absorb nutrients from their environment. This process begins with the secretion of enzymes that catalyze the breakdown of complex organic materials into simpler compounds that can be absorbed by the fungal cells. In the case of *Marasmiellus inoderma*, studies have shown that its mycelium can effectively utilize lignocellulosic waste, such as that derived from Elaeis guineensis (oil palm), which is rich in cellulose and lignin (De Kesel *et al.,* 2008). The degradation of these components is essential for nutrient acquisition and involves several biochemical pathways.

The primary pathway for nutrient absorption in fungi involves the breakdown of polysaccharides into monosaccharides through enzymatic hydrolysis. Enzymes such as cellulases and hemicellulases play a crucial role in this process. Cellulases degrade cellulose into glucose

units, while hemicellulases target hemicellulose, yielding a variety of sugars including xylose and mannose (Haneef *et al.,* 2017). Once these sugars are released into the extracellular environment, they are transported into the fungal cells via specific transport proteins located in the cell membrane. This process is energy-dependent and is facilitated by proton gradients established by ATPase activity within the fungal cells (Islam *et al.,* 2017).

Moreover, *Marasmiellus inoderma* has been observed to produce a range of extracellular enzymes that enhance its capacity to degrade complex organic materials found in waste extracts. The enzymatic profile includes laccases, peroxidases, and various glycoside hydrolases, which are instrumental in breaking down lignin and other recalcitrant compounds present in plant biomass (Shi *et al.,* 2023). Laccases, for instance, are known for their ability to oxidize phenolic compounds and play a significant role in lignin degradation. The secretion of these enzymes is often spatially regulated within the mycelium; higher enzyme activities are typically observed at the growing tips of the mycelial network where nutrient uptake is most critical (Shi *et al.,* 2023).

At a cellular level, mycelium interacts with waste extracts through a combination of physical and biochemical mechanisms. The hyphal tips of *Marasmiellus inoderma* penetrate the substrate, increasing surface area contact with potential nutrients. This interaction not only facilitates enzyme secretion but also enhances nutrient absorption efficiency. The mycelium forms a symbiotic relationship with its substrate; as it breaks down complex organic materials, it simultaneously creates a more favorable environment for further growth by altering the chemical composition of the substrate (Nawawi *et al.,* 2020).

The role of enzymes produced by *M.* inoderma extends beyond mere degradation; they also participate in signaling pathways that regulate mycelial growth and development. For example, certain enzymes may trigger secondary metabolite production that can inhibit

competing microorganisms or promote further colonization of the substrate (Jones *et al.,* 2017). This dynamic interaction underscores the adaptability of *M.* inoderma to various waste environments, allowing it to thrive on substrates that would otherwise be considered unsuitable for growth.

Additionally, different types of waste extracts can influence enzymatic activity and mycelial growth patterns. For instance, substrates rich in nitrogen may enhance protein synthesis within the fungal cells, leading to increased enzyme production and improved growth rates (Pelletier *et al.,* 2013). Conversely, substrates with high lignin content may require more extensive enzymatic action before effective nutrient absorption can occur. This adaptability highlights *M*. inoderma's potential as a bioremediation agent capable of converting agricultural waste into valuable biomass while simultaneously contributing to environmental sustainability.

2.8 Influence of Environmental Factors on Fungi Mycelial Growth

2.8.1 Temperature

Temperature is one of the most critical environmental factors influencing mycelial growth. Fungal species exhibit specific temperature preferences that are essential for optimal growth. Generally, temperatures ranging from 20 °C to 30 °C (68 °F to 86 °F) are favorable for many fungi, including *Marasmiellus inoderma* (Tang *et al.,* 2015). Deviations from this range can lead to reduced metabolic activity and slower growth rates. For instance, excessively high temperatures can denature proteins and disrupt cellular functions, while low temperatures can slow down enzymatic reactions necessary for nutrient assimilation (Schoder *et al.,* 2023).

2.8.2 pH Level

Another crucial factor is the pH level of the substrate. Most fungi prefer a slightly acidic to neutral pH (around 5.5 to 7.0) for optimal growth. A study indicated that mycelial growth is significantly inhibited in highly alkaline or acidic conditions due to the detrimental effects on nutrient availability and enzyme activity (Adamez *et al.,* 2012). The pH affects the solubility of nutrients in the substrate; therefore, maintaining an appropriate pH is essential for maximizing mycelial colonization and subsequent fruiting body production (Soti *et al.,* 2015).

2.8.3 Humidity

Humidity levels also play a pivotal role in mycelial development. Fungi require specific moisture levels to thrive; too little moisture can lead to desiccation, while excessive moisture can create anaerobic conditions detrimental to mycelial health. Research has shown that maintaining relative humidity between 60% and 80% is ideal for many fungal species (Schoder *et al.,* 2024). In particular, white rot fungi like *Marasmiellus inoderma* flourish in high humidity environments as it promotes healthy mycelial expansion and nutrient uptake.

2.8.4 Aeration

Aeration is another critical aspect influencing mycelial growth. Proper gas exchange is essential for aerobic respiration; which fungi rely on for energy production. Insufficient oxygen levels can lead to anaerobic conditions that inhibit growth and may result in the production of toxic metabolites (Sahay *et al.,* 2019). Studies have demonstrated that increasing aeration significantly enhances mycelial growth rates by ensuring adequate oxygen supply and facilitating the removal of carbon dioxide produced during respiration (Sadh *et al.,* 2018).

2.8.5 Light Exposure

Light exposure during the colonization phase also affects mycelial development. While some fungi require light for fruiting body formation, excessive light during the colonization phase can hinder mycelial growth by inducing stress responses (Mwandira *et al.,* 2024). It is generally recommended to keep mycelium in dark or low-light conditions until sufficient colonization occurs before introducing light to stimulate fruiting.

2.8.6 Substrate Composition

The composition of the substrate itself is perhaps one of the most significant factors affecting mycelial growth. Different substrates provide varying levels of nutrients essential for fungal metabolism. Substrates rich in lignin and cellulose are particularly beneficial as they provide structural components that fungi can degrade and utilize for energy (Mwandira *et al.,* 2023). Moreover, agro-industrial wastes such as fruit and vegetable peels have been shown to support robust mycelial growth due to their high nutrient content (Adámez *et al.,* 2012). The interaction between fungal hyphae and substrate particles is crucial; thus, selecting an appropriate substrate is fundamental for successful cultivation

2.9 Applications of Waste Extract on Fungal Growth

Fungi play a crucial role in nutrient cycling within ecosystems, and their capacity to decompose organic matter makes them ideal candidates for bioconversion processes. The use of agricultural and food production waste streams as substrates for fungal cultivation not only addresses waste management issues but also enhances sustainability by transforming these materials into valuable biomass. According to De Kesel et al. (2008), *Marasmiellus inoderma* can be cultivated on waste materials from *Elaeis guineensis* (oil palm), demonstrating its

adaptability and potential for large-scale cultivation using low-cost substrates. This method not only provides a sustainable means of utilizing agricultural by-products but also contributes to local economies by creating opportunities for small-scale mushroom farming (De Kesel *et al.,* 2008).

One of the most significant applications of fungal growth on waste extracts is in the field of bioremediation. Fungi have been shown to possess remarkable capabilities in degrading various pollutants, including hydrocarbons and heavy metals, through enzymatic processes such as laccase production. Laccases are oxidoreductase enzymes produced by fungi that can catalyze the oxidation of phenolic compounds and other substrates, making them valuable in the treatment of industrial effluents and contaminated soils (Sahu *et al.,* 2023). The ability of *M*. inoderma to thrive on lignocellulosic agricultural wastes positions it as a potential agent for bioremediation efforts, as these wastes often contain phenolic compounds that can stimulate laccase production (Sahu *et al.,* 2023). By employing *M*. inoderma in bioremediation strategies, we can harness its enzymatic capabilities to detoxify polluted environments while simultaneously producing edible mushrooms.

Moreover, the circular economy concept is significantly advanced through the integration of fungal cultivation with waste management practices. Food production generates substantial nutrient-rich waste streams that are often underutilized or discarded, leading to environmental degradation and nutrient loss (Bansfield *et al.,* 2023). By utilizing these waste streams as substrates for fungal growth, valuable nutrients can be recycled back into the food system or converted into high-value products such as biofuels, animal feed, or biochemicals (Bansfield *et al.,* 2023). This approach not only mitigates the environmental impact of food waste but also enhances resource efficiency within food processing plants.

The economic implications of utilizing waste extracts for fungal cultivation are profound. The cost-effectiveness of using agricultural and food production wastes as substrates can significantly lower production costs for mushroom farmers while providing a sustainable source of income (Frontiers in Materials, 2024). Additionally, the production of value-added products from fungal biomass can open new markets and enhance profitability. For instance, fungi like M. inoderma can be processed into nutraceuticals or functional foods due to their rich nutritional profile and potential health benefits (De Kesel *et al.,* 2008).

However, while the applications of fungal growth on waste extracts are promising, several challenges must be addressed to fully realize their potential. One major concern is the variability in substrate quality and composition, which can affect fungal growth rates and enzyme production (Sahu *et al.,* 2023). The presence of inhibitory compounds in certain waste materials may hinder mycelial development or enzymatic activity. Therefore, thorough characterization and pre-treatment of waste substrates may be necessary to optimize conditions for fungal cultivation. Additionally, scaling up biotechnological processes involving fungi poses logistical challenges. While laboratory-scale studies have demonstrated the feasibility of using waste extracts for cultivating M. inoderma, translating these findings into commercial applications requires careful consideration of factors such as substrate availability, processing techniques, and market demand (Bansfield *et al.,* 2023). Developing standardized protocols for substrate preparation and fungal inoculation will be essential for ensuring consistent results across different production settings.

Furthermore, public perception and acceptance of using waste materials for food production must be addressed. Consumers may have concerns regarding food safety and quality when products are derived from agricultural wastes. Therefore, education and outreach efforts

are crucial to inform stakeholders about the safety measures implemented during the cultivation process and the nutritional benefits associated with consuming mushrooms grown on waste substrates.

2.10 Previous Studies on Waste Extract Utilization in Fungi

Ram and Kumar (2010) examined *A. bisporus* fruiting body morphology (pin head initiation, flush harvesting, fruit body diameter and weight), total yield on agricultural waste. Studies employed six combination formulations

Several authors have studied *P. ostreatus* growth, yield, biological efficiency (BE), and mushroom size (Nwokoye *et al.,* 2010; Samuel and Eugene, 2012; Oseni *et al.,* 2012; Govindaraju *et al.,* 2013; Yang *et al.,* 2013; Ashrafi *et al.,* 2014; Mohammed *et al.,* 2014). Rice straw was used to cultivate two P. ostreatus crops. At spawning, crop I rice straw was mixed with 0%, 25%, 50%, 75%, and 100% banana leaves or Leucaena leucocephala, maize bran, or maize cobs. At spawning, crop II rice straw was supplemented with 0%, 1%, 2%, 3%, 4%, and 5% sunflower or cotton seed cake. Mushroom output (1,040.0 g) and BE (98.5%) were higher on 50/50 rice straw and banana leaves. Rice straw treated with 2% sunflower seed hulls (yield =1,087.5 g, BE =103.3 %) yielded 1,073.8 g, BE =101.8 %) and was much higher than other supplement ratios. The largest mushrooms (21.0 g) came from non-supplemented rice straw (Mamiro & Mamiro, 2011). Three strains of P. eryngii—Pe-1 (native to Bangladesh), Pe-2 (from China), and Pe-3 (from Japan)—were grown on sawdust and rice straw to study their growth and yield (Moonmoon *et al.,* 2010). Pe-1 on sawdust had the highest biological production and BE (73.5%). Pe-1 had more fruiting bodies and mycelium run rate than the other two strains. The quality of mushroom strains was comparable. Sawdust produced better yields and efficiency than rice straw, but rice straw mushroom fruiting bodies were larger.

Mondal et al. (2010) studied P. florida cultivation on various substrates. The highest mycelium running rate was on banana leaves and rice straw (1:1), the lowest in control. Running time for mycelium was lowest on banana leaves and rice straw (1:3 and 3:1). Total and effective primordia were highest in control, although rice straw had the thickest pileus. Rice straw produced the highest biological and economic yield (164.4 g and 151.1 g) compared to control.

Several papers have examined *P. pulmonarius* cultivation on cotton waste and cassava peel (Adebayo et al. 2009)

Mane et al. (2007) examined the yield and morphological features of P. sajor-caju fruiting bodies grown on cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves, and wheat straw.

Rice bran supplemented mycelial development and yield best with all substrates (Pokhrel *et al.,* 2013). Dehariya and Vyas (2013) examined the yield and BE of soybean straw, wheat straw, paddy straw, sugarcane bagasses, sun flower stalks, maize stalks, domestic waste, used tea leaves, fruit waste, semal flowers, newspaper, bamboo leaves, sawdust, and their 1:1 combination in P. sajor-caju cultivation.

Philippoussis et al. (2004) studied the effects of seven oak-wood sawdust substrates (OS) enriched with wheat straw (WS) or corn-cobs (CC) on *Lentinula edodes* (a popular edible mushroom) mycelium growth and sporophore generation. Hassan (2011) studied L. edodes cultivation on hard wood sawdust, rice straw, crushed corn cobs, and crushed bagasse supplemented with 20% wheat bran, 1% soy bean flour, and 2% gypsum. Incubation, early harvesting, yield, BE, and fruit body drying parameters were estimated. Incubation and first harvesting day times were shortest for sawdust and longest for bagasse. Sawdust had the largest yield (297 g/kg wet media) and BE, while bagasse had the lowest.

Pani (2012) tested cotton wastes and sunflower stalks alone or with paddy straw (1:3, 1:1, 3:1) for milky mushroom sporophore development. BE was higher with paddy straw mixtures than cotton wastes and sunflower stalks alone. There were more fruiting bodies, faster substrate colonization, and primordial initiation. Cotton wastes + paddy straw (1:3) yielded the most mushrooms (73.2 % BE), which was statistically comparable to paddy straw (71.3 %). Akavia et al. (2009) grew five *Hypsizygus marmoreus* strains on 24 substrates (Table 1). During one month, average daily colonization particles, BE, mushroom quantity, and weight were evaluated. The best BE substrate was corn cob with bran and olive press cake with 85.6%. The identical composition without olive press cake has 67.5% BE.

Gizaw (2010) examined Pholiota nameko harvesting yield and BE on Eucalyptus, Cordia, coffee husk, Pinus, cotton seed, and teff straw. Wheat bran was added 100:10 and 100:30 w:w to the primary substance. Eucalyptus shaving with 30% wheat bran yielded the most (797.33 g, BE = 53.27 %).

Akinyele and Adetuyi (2005) examined how pH and temperature affect *Volvariella volvacea* development on agricultural wastes singly and in combinations. Mycelia yield was highest at pH 5.5–8.5 and weight was highest at pH 6.5. The mushroom had high mycelia growth between 25°C and 30°C, with the greatest DW of 80.0 mg at 30°C. The effect of different substrates on *Volvariella* diplasia and *V. volvacea* mycelial growth and yield was also examined (Tripathy *et al.,* 2011). Paddy straw, oil palm fibre, sawdust, and a combination were tested for V. *volvacea* culture. As this mushroom's traditional substrate, paddy straw was the control. Straw naturally encouraged mycelial growth and fruit body production. Fruit bodies grew on oil palm fibre like paddy straw. The oil palm fiber-sawdust blend produced few fruit bodies. Few tiny fruit bodies were formed on sawdust alone (Onuoha *et al.,* 2009). V. *volvacea* biodegrades agro

waste, not just paddy straw. First, *Pleurotus* and *Volvariella* fruit bodies are grown on cheap substrates, but researchers also want to grow famous and tasty edible mushrooms like *Agaricus*, *Lentinus*, and *Hypsizygus marmoreus*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Materials

Six common wastes were collected from Ugbowo and around the University of Benin community in Benin city, Edo state. The mushroom mycelium culture was kindly provided from the culture collection of mushrooms of the African Centre for Mushroom Research and Technology Innovations (ACMRTI), University of Benin, Benin city, Edo State, Nigeria. Irish potatoes and Petri dishes were purchased from the market and scientific stores located in Benin City. The six waste materials used for this study includes: Leaves of Banana plant (*Musa sapientum*), leaves of *Terminalia catappa,* Oil palm (*Elaeis guineensis*) fruit fibre, husk of Melon (*Citrullus lanatus*) seeds locally known as Egusi, Cotton (*Gossypium* spp) waste and disposed paper waste.

3.2 Media Preparation

100g (dry weight) of each waste was boiled separately for 10 minutes in 500ml of water. 200 g of Irish potato was also weighed and boiled and then filtered with a sieve cloth. The filtrate from each of the extracts was collected and used for the media preparation. Ordinary Dextrose Agar was made by adding 20 g of Dextrose and 20g of Agar to 1 liter of water in a conical flask.

Each extract was made up to 1000ml by adding distilled water. The various treatments were labelled as follows: Banana leaves extract (Banana), *Terminalia catappa* leaves extract (TC), Oil palm fruit fiber extract (OPFF), Melon (Egusi) husk extract (Melon), Cotton waste extract (Cotton), Disposed paper waste extract (Paper), Banana extract+PDA, *Terminalia catappa* leaves extracts+PDA (TC+PDA), OPFF extract +PDA, Melon (Egusi) husk extracts

+PDA, Cotton waste extract+PDA, Paper waste+PDA. PDA and pure Agar media were used as standard media and control respectively and were labelled as such.

Each media composed of 20g of Dextrose sugar and 20g of Agar. The different media were poured into different conical flasks which have been labelled, covered with foil paper and then autoclaved at 121 degrees Celsius for 15minutes. After cooling, each media was poured into 90mm petri dishes in three replications.

3.3 Inoculation and Incubation\

Mycelium discs (10mm in diameter) from a 7day old pure culture of *Marasmiellus inoderma* was inoculated into each Petri dish and placed in the center of the plate. This process was carried out in a laminar flow chamber. Thereafter the inoculated plates were kept in the incubator at room temperature for mycelial growth.

3.4 Measurement of Mycelia Growth

The vegetative growth of the mushroom mycelia on the different waste extracts media were assessed by measuring the diameter of the mycelium in the Petri dishes. The diameter and growth rate of the mycelium was measured daily and recorded in Millimeters(mm). Average mycelia extension and growth rate were recorded. The morphology and growth characteristics of the mycelia of the mushroom was noted. The biomass of the fungus was harvested after 12 days of inoculation by melting the media. The mycelia were dried at 60ºC in an oven for 24 hours to constant weight. The average weight of the dried mycelia was then recorded.

Statistical analysis was done using averages, means and standard deviations. The results were presented in tables, illustrations and graphs.

CHAPTER FOUR

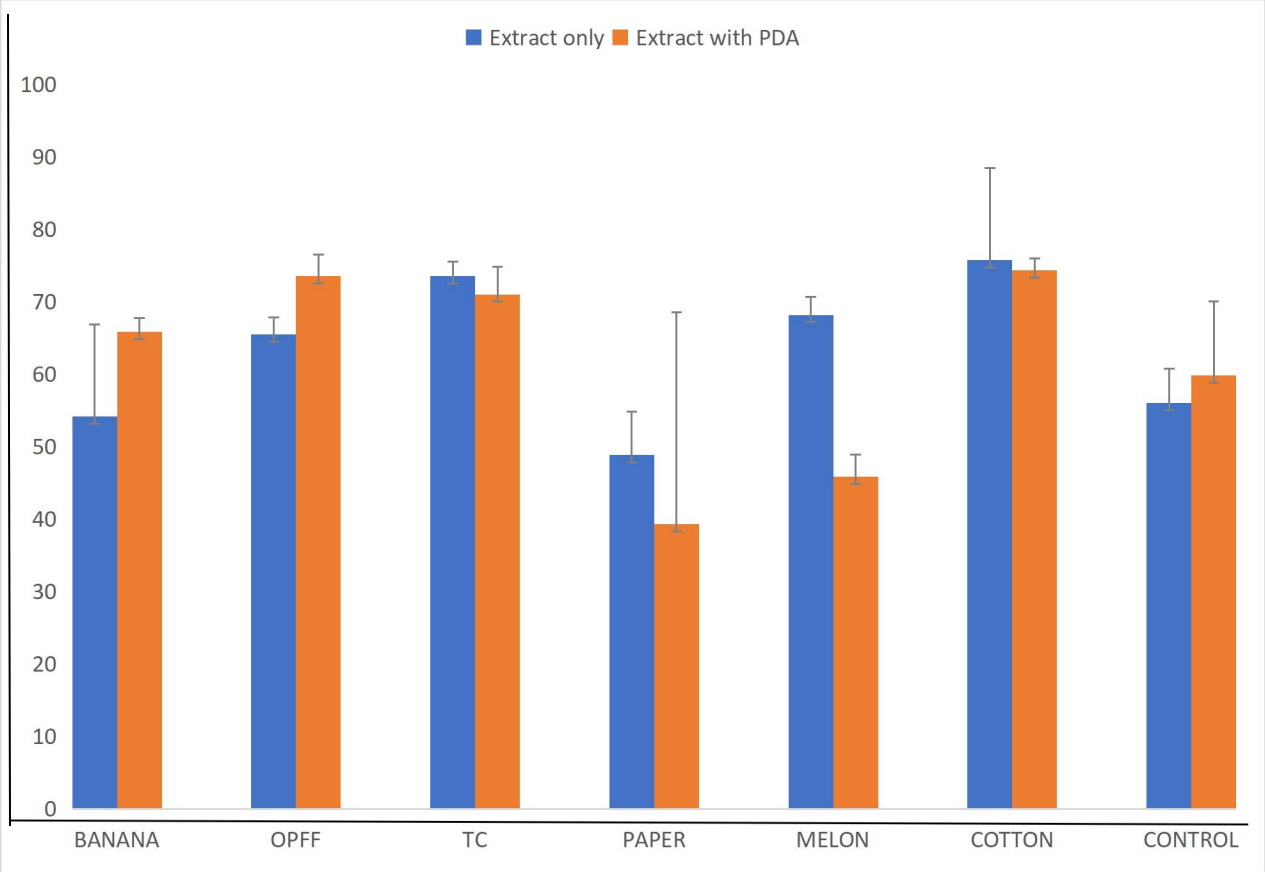
RESULTS

The results obtained in this study shows that *Marasmiellus inoderma* from Nigeria was able to utilize common wastes for its vegetative growth to varying degrees. It was observed that the mushroom was able to completely colonize the petri dish after 4 days of inoculation. The mycelia extension was highest for Cotton amended with PDA (29.5 ± 0.5) and least for PDA in day 2. In day 3, the highest extension was observed for Cotton amended PDA (56.67 ± 0.58) and the lowest was observed on Melon with PDA (31.00 ± 4.09). The highest and lowest mycelia extensions in day 4 were recorded for Cotton (75.75 ± 12.71) and Paper with PDA (39.33 ± 29.17) respectively.

Table 1: Daily mycelia extension of *Marasmiellus inoderma* on selected waste extracts.

	EXTENSION (mm)		
WASTE EXTRACTS	**DAY 2**	**DAY 3**	**DAY 4**
BANANA LEAF	20.17 ± 1.04	40.33 ± 6.89	54.17 ± 12.71
BANANA+PDA	23.50 ± 1.00	47.17 ± 1.26	65.83 ± 1.89
OPFF	21.67 ± 0.76	47.83 ± 2.51	65.50 ± 2.29
OPFF+PDA	27.00 ± 3.12	53.50 ± 1.00	73.50 ± 3.04
TC	24.00 ± 0.50	51.33 ± 0.76	73.50 ± 2.00
TC+PDA	24.33 ± 2.25	51.17 ± 1.76	71.00 ± 3.79
PAPER	17.33 ± 2.47	34.5 ±3.91	48.83 ± 6.01
PAPER+PDA	19.33 ±17.48	34.33 ± 27.9	39.33 ± 29.17
MELON HUSK	23.00 ± 2.29	46.50 ± 1.73	68.17 ± 2.47
MELON+PDA	16.33 ± 1.61	31.00 ± 4.09	45. 83 ± 3.05
COTTON	24.75 ± 2.47	54.75 ± 3.18	75.75 ± 12.71
COTTON+PDA	29.50 ± 0.50	56.67 ± 0.58	74.33 ± 1.61
CONTROL	22.33 ± 1.04	43.50 ± 1.00	56.00 ± 4.77
PDA	11.83 ± 3.81	31.17 ± 8.5	59.83 ± 10.25

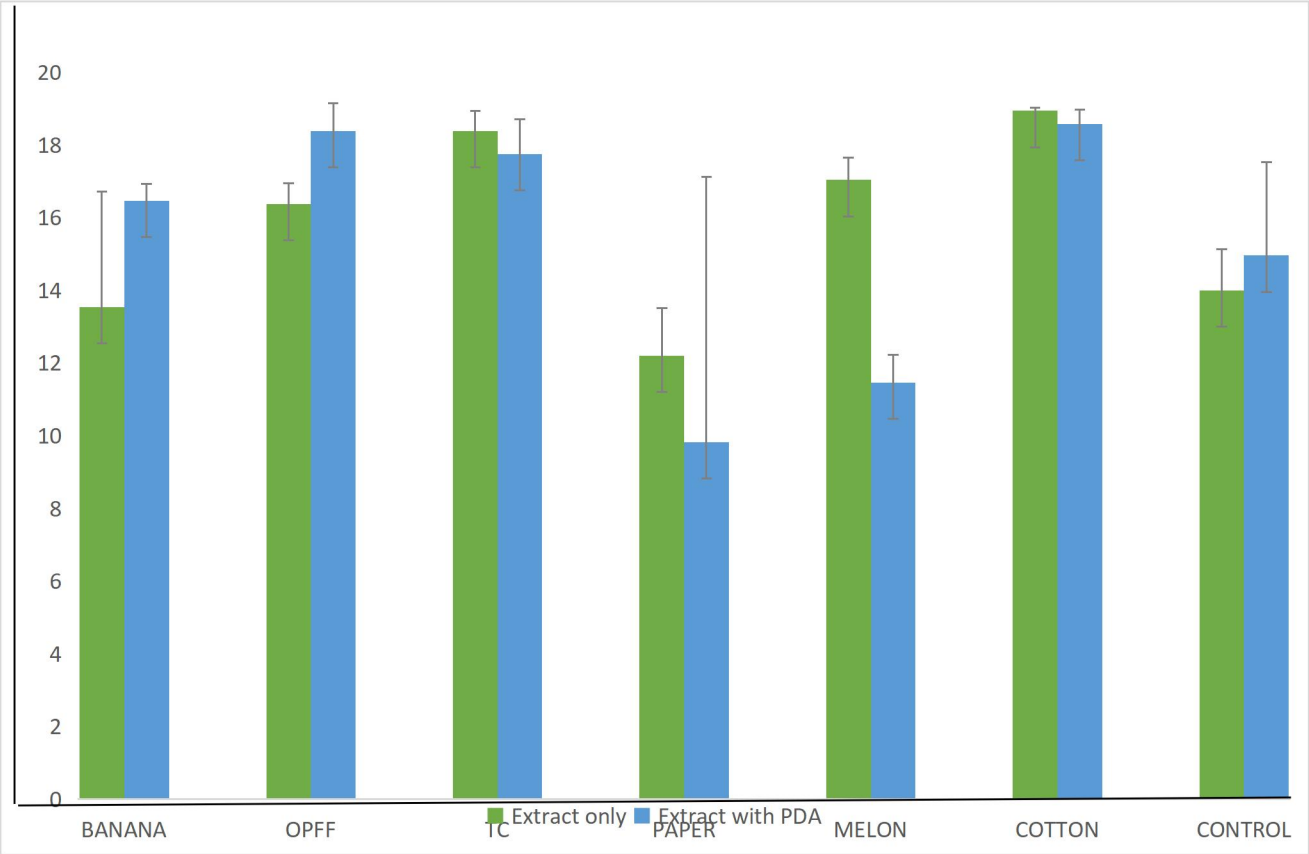
KEY: TC- *Terminalia catappa* leaf extract, OPFF- Oil palm fruit fiber extract



KEY: TC- *Terminalia catappa* leaf extract, OPFF- Oil palm fruit fiber extract

Figure 1: Mycelia extension of *Marasmiellus inoderma* on selected waste extracts after 4 days of inoculation.

The mycelia growth or extension of *Marasmiellus inoderma* on the different waste extracts after four days are displayed in the graph above. The highest extension was observed in pure Cotton waste extract (75.75 ± 12.71) followed immediately by Cotton with PDA media (74.33 ± 1.61), OPFF amended PDA media (73.5 ± 3.04) and TC extracts (73.5 ± 2). The least mycelia extension after four days was recorded to be in Paper amended PDA media (39.33 ± 29.17).



KEY: TC- *Terminalia catappa* leaf extract, OPFF- Oil palm fruit fiber extract

Figure 2: Average growth rate of the mycelia of *Marasmiellus inoderma* on selected waste extracts.

The above figure indicates the average growth rate of the mycelium of *Marasmiellus inoderma* on the different media used in this study, as earlier stated, the highest growth rate was recorded on Cotton waste extract (18.94±0.08) immediately followed by the Cotton with PDA media (18.58±0.40), *T. catappa* leaf extract (18.38±0.56), Oil palm fruit fiber amended with PDA (18.38±0.76) and so on. The least mycelia growth rate was recorded on Paper with PDA media (9.83±7.29

Table 2: Biomass of *Marasmiellus inoderma* cultured on selected waste extract media after 12 days.

EXTRACTS	BIOMASS(g)
BANANA LEAF	0.43±0.21
BANANA+PDA	0.70±0.07
OPFF	0.47±0.09
OPFF+PDA	0.59±0.16
TC	0.30±0.04
TC+PDA	0.47±0.02
PAPER	0.32±0.27
PAPER+PDA	0.36±0.13
MELON HUSK	0.67±0.09
MELON+PDA	0.27±0.04
COTTON	0.82±0.02
COTTON+PDA	0.41±0.11
CONTROL	0.20±0.09
PDA	0.27±0.08

KEY: TC- *Terminalia catappa* leaf extract, OPFF- Oil palm fruit fiber extract

In Table 2 above, the Biomass(weight) of the mycelium of *Marasmiellus inoderma* cultured on each waste extract was recorded. A comparison in the biomass of *Marasmiellus inoderma* mycelia on different waste media is made in this table. The highest biomass (in grams) was recorded to be on Cotton waste extract (0.82±0.02) and the lowest biomass was the mycelium of the Control media (0.20±0.09).

Table 3: Density of *Marasmiellus inoderma* mycelia on selected waste extracts after 12 days.

EXTRACTS	DENSITY
BANANA LEAF	+ +
BANANA+PDA	+ +
OPFF	+
OPFF+PDA	+ +
TC	+
TC+PDA	+ +
PAPER	+ +
PAPER+PDA	+ +
MELON HUSK	+ + +
MELON+PDA	+ + +
COTTON	+ +
COTTON+PDA	+ + +
CONTROL	+
PDA	+ + +

KEY: + Slightly dense, + + Dense, + + + Very Dense, TC- *Terminalia catappa* leaf extract, OPFF- Oil palm fruit fiber extract

The table above denotes the density and of the mycelium of *Marasmiellus inoderma* on different waste extracts used in this study. Most of the mycelia cultured on only waste extracts were noticed to be dense and flat(appressed) with melon being the only exception. And the mycelia on some of their PDA supplemented counterparts was seen to have a very dense and wooly or fluffy form.

Plate 1: Slightly dense mycelia of *Marasmiellus inoderma* on fully colonized petri dishes

Plate 2: Dense mycelia of *Marasmiellus inoderma* on fully colonized perti dishes

Plate 3: Very dense mycelia of *Marasmiellus inoderma* on fully colonized plates

Table 4: Morphology of *Marasmiellus inoderma* cultured on different waste extracts after 12 days.

EXTRACTS	MORPHOLOGY
BANANA LEAF	Appressed
BANANA+PDA	Appressed
OPFF	Appressed
OPFF+PDA	Appressed
TC	Appressed
TC+PDA	Appressed
PAPER	Appressed
PAPER+PDA	Fluffy
MELON HUSK	Wooly
MELON+PDA	Wooly
COTTON	Appressed
COTTON+PDA	Fluffy
CONTROL	Appressed
PDA	Wooly

KEY: TC- *Terminalia catappa* leaf extract OPFF- Oil palm fruit fiber extract

The table above displays the physical form of *Marasmiellus inoderma* mycelia cultured on waste extracts. Most of the mycelia cultured on only waste extracts were noticed to be appressed (i.e they appear flat as shown in plate 2) with melon being the only exception. And the mycelia on some of their PDA supplemented counterparts was seen to have a wooly or fluffy form**.**

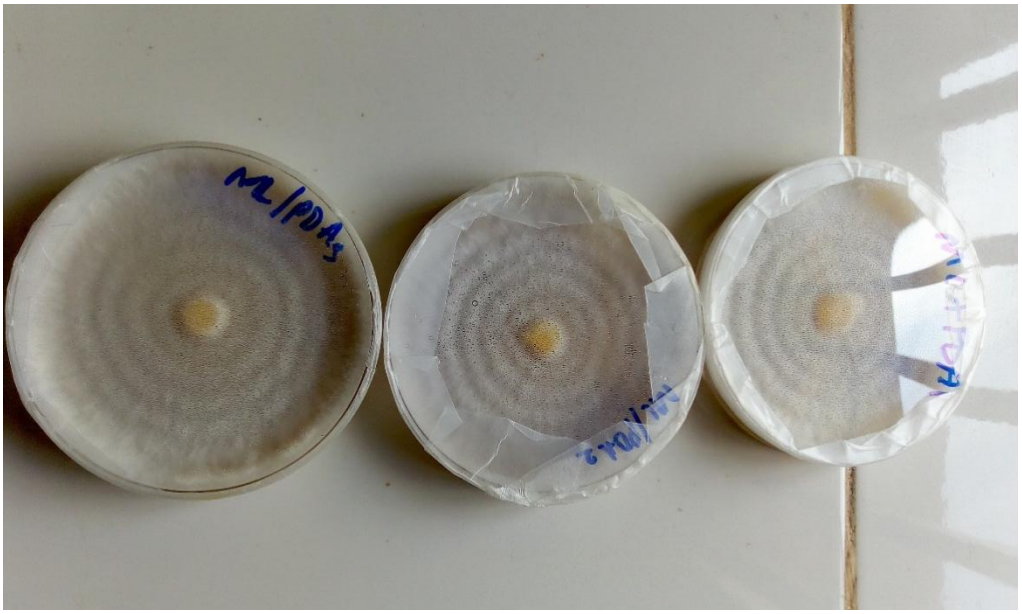


Plate 4: Wooly form of *Marasmiellus inoderma* on fully colonized petri dishes.

Plate 5: Appressed appearance of *Marasmiellus inoderma* on fully colonized plates.

Plate 6: Fluffy morphology of *Marasmiellus inoderma* on fully colonized waste extract plate.

CHAPTER FIVE

DISCUSSION

Substrate is one of the most important factors in mushroom cultivation as mushrooms depend on substrate for nutrition to support mycelia growth and development into mushroom fruiting bodies (Lakshmi and Sornaraj., 2014). In this study, it took an average of four days for the mycelium of *Marasmiellus inoderma* species to completely colonize the waste extracts tested. All the media made from waste materials recorded higher mycelia growth rate when compared with the control and Potato Dextrose Agar. This result compares favorably with the report given by Ukoima *et al*., (2009) from the growth of *Volvariella volvacea, Pleurotus tuber-regium* and *Pleurotus sajor-caju* on oil palm fiber, cassava peels, rice bran supernatants. It is further supported by the findings of Jenison (1948) and Kaul (1981) who obtained excellent mycelia growth when they incorporated legumes, meals of grain, extracts of banana, oranges, etc. into agar. A study carried out by Quimio (1981) reported accelerated mycelia growth of *Auricularia* spp when malt and rice bran extracts were incorporated into agar. The reason for this accelerated growth of the mycelia on waste extract media is suspected to be because of the presence of certain substances that induces faster growth of the fungus. The chemicals are amino acids, vitamins and essential nutrients, which combine to influence the growth of the mycelium on these culture media (Ukoima *et al*., 2009). This implies that these wastes do contain some vitamins and nutrients that are useful for the growth of this fungus. These wastes may also contain some complex nutrients and growth elicitors which helps in the growth of the mushroom mycelia. These compounds help in the release of carbon and nitrogen for metabolism (Crognale *et al*, 2003).

However, when some of these waste extracts were supplemented with PDA, they recorded higher growth than when only the extracts were used. Extracts of Melon husk, Cotton waste and

Terminalia catappa leaf were able to support the growth of the mushroom better than when amended with PDA. These extracts have been reported to contain high cellulosic components which highly favor the growth of this mushroom. The addition of PDA to these extracts may possibly lower or adjusted the pH of the media to favor the growth of the mushroom. A biochemical analysis of paddy straw, cotton waste, banana leaves, corn husks, sugar cane bagasse which were used to cultivate *Volvoriella volvaceae* was carried out by UI Haq *et al.,* 2011. The mean values of Nitrogen percentage of the substrates showed that nitrogen was highest in cotton wastes and the minimum was recorded in banana leaves. The highest crude fiber and ash content was also high in cotton waste. It can also be inferred that the reason for better growth of the mycelia of *Marasmiellus inoderma* on the other wastes extracts when they were supplemented with PDA was because these extracts did not possess enough nutrient concentration to support fast growth of this fungi and so the PDA served to supply extra nutrient to the media. From these results, it can be inferred that media made from the extracts of banana leaves, paper and oil palm fruit fiber contain nutrients suitable for the gowth of *Marasmius* species. These extracts when supplemented with PDA can increase the growth or mycelial development of this mushroom. This result is supported by Okhouya and Osewengie (2009) who observed the ability of this mushroom to grow on the fronds and trunks of Oil palm trees in Edo state and also on dry decaying leaves. The slow growth rate of the mycelia on paper extract could be attributed to poor nutrient composition of the paper used. However, if paper media has to be used to grow this mushroom, it should be supplemented with PDA or other nutrients to boost the vigor of the mycelium.

In general, the best media for the cultivation of this mushroom is cotton waste media as it provides better nutrient for this mushroom even without being supplemented with PDA. Extracts

from oil palm fruit fiber, banana leaves, *Terminalia catappa* leaves, melon seed peels are also suitable for culturing this mushroom when the conventional media is not available as also supported by Dede and Okungbowa (2007) for culturing *Ceratocystis paradoxa*.

CONCLUSION

This study shows that these wastes do not have to always be discarded or burnt but can be transformed or recycled for producing more food for man in the form of mushrooms. Mushrooms have the ability to degrade these wastes and make them available for farmers again as compost. The extracts used in this study are available, show better support for culturing *Marasmiellus inoderma* and continuous use of these wastes for mushroom cultivation can help reduce environmental pollution. It has been observed from various studies that when a defined medium is used for cultivation of mushroom mycelia, the mushroom fruit bodies may also perform well on substrate of such medium. Therefore, these wastes can be used to produce good yield of fruiting bodies of this mushroom. More research should be carried out to find more wastes that can be used to cultivate this mushroom for its numerous benefits to the health of man and to investigate the fruiting body yield of this mushroom on solid substrates made from these wastes.

REFERENCES

Abubakar, A., Suberu, H. A., & Bello, I. M. (2013). Effect of pH on mycelial growth and sporulation of Aspergillus parasiticus. *Journal of Plant*, 4, 64–67.

Adámez, J. D., Samino, E. G., Sánchez, E. V., & González, G. D. (2012). In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (Vitis vinifera L.). *Food Control*, 24, 136–141.

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). Introductory Mycology. Wiley.

Ancin-Murguzur, F. J., Barbero-López, A., Kontunen-Soppela, S., & Haapala, A. (2018). Automated image analysis tool to measure microbial growth on solid cultures. *Computers and Electronics in Agriculture*, 151, 426–430.

Asgharnejad, H., Nazloo, E. K. L. M. M., Hajinajaf, N., & Rashidi, H. (2021). Comprehensive review of water management and wastewater treatment in food processing industries in the framework of water-food-environment nexus. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 1–37.

Ashrafi, R., Mian, M. H., Rahman, M. M., & Jahiruddin, M. (2014). Recycling of spent mushroom substrate for the production of oyster mushroom. *Research in Biotechnology*, **5**(2), 13–21.

Bansfield, D., Spilling, K., Mikola, A., & Piiparinen, J. (2023). Growth of fungi and yeasts in food production waste streams: A feasibility study. *BMC Microbiology*, 23(1), 328.

Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2007). Chemical composition and biological properties of Portuguese wild mushrooms. *Journal of Agricultural and Food Chemistry*, **55**(12), 4656–4662.

Bastos, C., Liberal, Â., Moldão, M., Catarino, L., & Barros, L. (2023). Ethnomycological prospect of wild edible and medicinal mushrooms from Central and Southern Africa—A review. *Food Frontiers*, **4**(2), 549–575.

Bishnoi, S., Sharma, S., & Agrawal, H. (2023). Exploration of the potential application of banana peel for its effective valorization: A review. *Indian Journal of Microbiology*, 63(4), 398–409.

Carhart-Harris, R. L., et al. (2018). Psilocybin with psychological support for treatment-resistant depression. *The Lancet Psychiatry*, **5**(7), 597–609.

Chang, S.-T., & Miles, P. G. (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact. *CRC Press*.

Dyer, P. S., Inderbitzin, P., & Debuchy, R. (2016). Mating-type structure, function, regulation, and evolution in the Pezizomycotina. In Growth, Differentiation and Sexuality (pp. 351–385). *Cham*: Springer.

Govindaraju, S., Sangeetha, S., & Indra Arulselvi, P. (2013). Effect of different agro-wastes on mass production of edible mushroom *Pleurotus* ostreatus. *Indian Journal of Applied Research*, 3(6), 33–35.

Haneef, M., Ceseracciu, L., Canale, C., Bayer, I. S., Heredia-Guerrero, J. A., & Athanassiou, A. (2017). Advanced materials from fungal mycelium: Fabrication and tuning of physical properties. *Scientific Reports*, 7, 41292.

Hasanin, M. S., & Hashem, A. H. (2020). Eco-friendly, economic fungal universal medium from watermelon peel waste. *Journal of Microbiological Methods*, 168, 105802.

Hnain, A. K., Cockburn, L. M., & Lefebvre, D. D. (2011). Microbiological processes for waste conversion to bioenergy products: *Approaches and directions. Environmental Reviews*, **19**(4), 214–237.

Islam, M. R., Tudryn, G., Bucinell, R., Schadler, L., & Picu, R. C. (2017). Morphology and mechanics of fungal mycelium. *Scientific Reports*, 7, 13070.

Jones, M., Huynh, T., Dekiwadia, C., Daver, F., & John, S. (2017). Mycelium composites: A review of engineering characteristics and growth kinetics. *Journal of Bionanoscience*, 11, 241–257. https://doi.org/10.1166/jbns.2017.1440

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, **113**(1), 9–16.

Kesel, A. D., Guelly, A. K., Yorou, N. S., & Codjia, J. C. (2008). Ethnomycological notes on *Marasmiellus inoderma* from Benin and Togo (West Africa). *Cryptogamie, Mycologie*, 29(4), 313–319.

Kidd, P. M. (2000). The use of mushroom glucans and proteoglycans in cancer treatment. *Alternative Medicine Review*, **5**(1), 4–27.

Lee, S. C., & Heitman, J. (2014). Sex in the Mucoralean fungi. Mycoses, 57, 18–24.

Li, D., Tang, Y., Lin, J., & Cai, W. (2017). Methods for genetic transformation of filamentous fungi. *Microbial Cell Factories*, **16**(1), 1–13.

Mekonnen, M. M., & Hoekstra, A. Y. (2011). The green, blue and grey water footprint of crops and derived crop products. *Hydrology and Earth System Sciences*, 15, 1577–1600.

Mohammed, S., Muleta, D., & Abate, D. (2014). Bioconversion of coffee husk for oyster mushroom (*Pleurotus* ostreatus) cultivation in Jimma. *International Journal of Microbiology and Immunology Research*, **2**(6), 75–91.

Moreno-González, M., & Ottens, M. (2021). A structured approach to recover valuable compounds from agri-food side streams. *Food and Bioprocess Technology*, 14(8), 1387–1406.

Moura, M. A. F. E., Martins, B. A., Oliveira, G. P., & Takahashi, J. A. (2022). Alternative protein sources of plant, algal, fungal and insect origins for dietary diversification in search of nutrition and health. *Critical Reviews in Food Science and Nutrition*, **14**(1), 1–18.

Musa, H., & Ali, B. D. (2019). Studies on use of cotton waste substrate and some additives for the production of edible mushroom *Pleurotus* ostreatus (Florida). *FUW Trends in Science & Technology Journal*, 4, 225–227.

Mwandira, W., Mavroulidou, M., Gunn, M., & Gray, C. (2023, August). Use of fruit and vegetable waste as growth media in bacterial biocementation for ground improvement applications. *In 18th International Conference on Environmental Science and Technology* (CEST2023).

Nawawi, W. M. F. B. W., Jones, M., Murphy, R. J., Lee, K.-Y., Kontturi, E., & Bismarck, A. (2020). Nanomaterials derived from fungal sources: Is it the new hype? *Biomacromolecules*, 21, 30–55.

Ni, M., Feretzaki, M., Sun, S., Wang, X., & Heitman, J. (2011). Sex in fungi. *Annual Review of Genetics*, 45, 405–430.

Nieuwenhuis, B. P., Billiard, S., Vuilleumier, S., Petit, E., Hood, M. E., & Giraud, T. (2013). Evolution of uni- and bifactorial sexual compatibility systems in fungi. *Heredity*, **111**(6), 445–455.

Nwokoye, A. I., Kuforiji, O. O., & Oni, P. I. (2010). Studies on mycelial growth requirements of *Pleurotus* ostreatus (Fr.) *Singer. International Journal of Basic & Applied Sciences*, **10**(2), 47–53.

Oseni, T. O., Dube, S. S., Wahome, P. K., Masarirambi, M. T., & Earnshaw, D. M. (2012). Effect of wheat bran supplement on growth and yield of oyster mushroom (*Pleurotus* ostreatus) on fermented pine sawdust substrate. *Experimental Agriculture & Horticulture*, **1**(2), 30–40.

Pelletier, M. G., Holt, G. A., Wanjura, J. D., Bayer, E., & McIntyre, G. (2013). An evaluation study of mycelium-based acoustic absorbers grown on agricultural by-product substrates. Industrial Crops and Products, 51, 480–485.

Peraza-Reyes, L., & Malagnac, F. (2016). Sexual development in fungi. In Growth, Differentiation and Sexuality (pp. 407–455). *Cham*: Springer.

Putra, N. R., Aziz, A. H. A., Faizal, A. N. M., & Che Yunus, M. A. (2022). Methods and potential in valorization of banana peels waste by various extraction processes: In review. *Sustainability*, 14(17), 10571.

Ram, R. C., & Kumar, D. S. (2010). Agricultural wastes used as casing mixtures for production of button mushroom. *Indian Journal of Scientific Research*, **1**(1), 21–25.

Rousk, J., Brookes, P. C., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*, **75**(6), 1589–1596.

Sadh, P. K., Duhan, S., & Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: A review. *Bioresources and Bioprocessing*, 5(1), 1–15.

Samuel, A. A., & Eugene, T. L. (2012). Growth performance and yield of oyster mushroom (*Pleurotus* ostreatus) on different substrate compositions in Buea, South West Cameroon. *Science Journal of Biochemistry*. 2012

Sankaran, S., Khanal, S. K., Jasti, N., Jin, B., Pometto, A. L. I., & Van Leeuwen, H. J. (2010). Use of filamentous fungi for wastewater treatment and production of high-value fungal byproducts: *A review. Critical Reviews in Environmental Science and Technology*, 40(5), 400–449.

Schoder, K. A., Krümpel, J., Müller, J., & Lemmer, A. (2024). Effects of environmental and nutritional conditions on mycelium growth of three Basidiomycota. *Mycobiology*, **52**(2), 124–134.

Sharma, I. (2020). Bioremediation techniques for polluted environment: Concept, advantages, limitations, and prospects. *In Trace metals in the environment—New approaches and recent advances*. Primorsko-Goranska, Croatia: IntechOpen.

Shi, Y., Ji, M., Dong, J., Shi, D., Wang, Y., Liu, L., ... & Liu, L. (2024). New bioactive secondary metabolites from fungi: 2023. *Mycology*, 1–39.

Sitharama, A., Manatunge, J., Ratnayake, N., Nanayakkara, C. M., & Jayaweera, M. (2017). Rapid degradation of FOG discharged from food industry wastewater by lipolytic fungi as a bioaugmentation application. *Environmental Technology*, **39**(16), 2062–2072.

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. *Academic Press*.

Soti, P. G., Jayachandran, K., Koptur, S., & Volin, J. C. (2015). Effect of soil pH on growth, nutrient uptake, and mycorrhizal colonization in exotic invasive Lygodium microphyllum. *Plant Ecology*, 216, 989–998.

Stamets, P. (2005). Mycelium Running: How Mushrooms Can Help Save the World. *Ten Speed Press*.

Tang, W., Kuehn, T. H., & Simcik, M. F. (2015). Effects of temperature, humidity, and airflow on fungal growth rate on loaded ventilation filters. *Journal of Occupational and Environmental Hygiene*, **12**(8), 525–537.

Tesfay, T., Godifey, T., Mesfin, R., & Kalayu, G. (2020). Evaluation of waste paper for cultivation of oyster mushroom (*Pleurotus* ostreatus) with some added supplementary materials. *AMB Express*, **10**(1), 15.

The United Nations. (2020). *The United Nations world water development report 2020: Water and climate change*. Retrieved from http://www.unwater.org/news/un-world-water-development-report-2020-%E2%80%98water-and-climate-change%E2%80%99

Thiruchchelvan, N., Thirukkumaran, G., & Mikunthan, G. (2012). In vitro biological control of Marasmiellus sp., the causal agent of stem rot in bananas grown in Jaffna Peninsula, Sri Lanka. *Academic Journal of Plant Sciences*, **5**(3), 94–101.

Toghueo, R. M., Zabalgogeazcoa, I., de Aldana, B. V., & Boyom, F. F. (2017). Enzymatic activity of endophytic fungi from the medicinal plants Terminalia catappa, Terminalia mantaly, and Cananga odorata. *South African Journal of Botany*, 109, 146–153.

Tzianabos, A. O. (2000). Polysaccharide immunomodulators as therapeutic agents: Structural aspects and biologic function. *Clinical Microbiology Reviews*, **13**(4), 523–533.

Wallen, R. M., & Perlin, M. H. (2018). An overview of the function and maintenance of sexual reproduction in dikaryotic fungi. *Frontiers in Microbiology*, 9, 503.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, **60**(3), 258–274.

Wibowo, Y. G., Safitri, H., Khairurrijal, K., Taher, T., Ode Arham, L., & Jarwinda, et al. (2024). Recent advances in acid mine drainage treatment through hybrid technology: *Comprehensive review of scientific literature. Environmental Nanotechnology, Monitoring & Management*, 21, 100945.

Yang, W. J., Guo, F. L., & Wan, Z. J. (2013). Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi Journal of Biological Sciences*, 20(4), 333–338.

Zou, F., Tan, C., Zhang, B., Wu, W., & Shang, N. (2022). The valorization of banana by-products: Nutritional composition, bioactivities, applications, and future development. *Foods*, **11**(20), 3170.