DETERMINATION AND EFFECTS OF SOME PREPARED SUBSTRATES ON GROWTH AND NUTRITIONAL COMPOSITIONS OF *Pleurotus ostreatus* and *Pleurotus florida*

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GANA, Reuben
MTech/SLS/2018/8196

DEPARTMENT OF PLANT BIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY

MINNA

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ABSTRACT

The nutritional composition of mushroom is majorly affected by the growth substrate and the method of cultivation. This research evaluated the growth performance of *Pleurotus ostreatus* and *Pleurotus florida* on Agriculture Residues. Preparation of substrates were carried out by weighing 2 kg of each sterilized substrate homogenously mixed with calcium carbonate in a bag. The spawns were inoculated with hand on the substrates. The bags were then tied and left in a dark room at room temperature (20° C -22° C) and good ventilation for 21 days. Nutritional analysis was carried out following the Association of official analytical chemist. The following parameters were considerd from mushroom height, weight, length, number of fruiting body. Data collected were subjected to Analysis of Variance (ANOVA). The results on morphological studies on P. *florida* growth revealed that mushroom grown on sawdust has the highest value in height (7.50 cm) and weight (12.13 g) which were significantly different($P<0.05$) from other treatments. Mushroom grown on maize bran has the highest value in length of stipe (5.23 cm) and sawdust + yam peel has the highest maturity period (6.67days). Mushroom grown on sawdust + yam peel has the highest number of fruiting body (3.67) and highest number of days for pin head formation was recorded in sawdust (51.00). The results on *P. ostreatus* growth revealed that, mushroom grown in substrate sawdust + rice bran has the highest value in height (7.03 cm), Mushroom grown on maize bran has the highest value in weight (18.66 g). Sawdust has the (3.40 cm) value in length of stipe and number of days for pin head formation of mushroom growth. Mushroom grown in substrate maize bran has the highest number of mushroom fruiting body (7.00). The results on proximate composition of *P. florida* grown in sawdust was 16.15% moisture content followed by sawdust + rice bran (14.55 %). Sawdust + rice bran recorded 17.70 % fibre followed by maize bran (15.30%). Mushroom grown on rice bran, sawdust + rice bran was recorded highest in ash content (14.05 %) while sawdust + rice bran was 17.00 % in protein followed by maize bran (14.65 %). The highest percentage of carbohydrate was obtained from mushroom grown on yam peel (67.65 %) while the least was 21.7 % on sawdust + rice bran. In P. *ostreatus* highest percentage of moisture content and fibre was obtained (16.70 %) and (16.95 %) respectively. Mushroom grown on maize bran was having the highest percentage of lipids and protein (8.55 %) and (17.50 %) respectively. Percentage Ash was highest in mushroom grown on rice bran (14.05 %) followed by sawdust (13.65 %). Mushroom grown in corn flour + maize bran produced the highest percentage of carbohydrate (64.95 %). Mycochemical analysis revealed the presence of major bioactive compounds such as flavonoids, polyphenols, saponins, triterpenoids and steroids. Sawdust amended with rice bran, maize bran and sawdust waste seems to have greater influence on the growth of these species. The observed differences in the substrates yield may be due to the percentages make-up or content of cellulose materials and essential nutrients. More study is encouraged to study the effect of substrate supplementation on sustain yield of edible mushrooms in various harvests with regards to commercial production.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Mushroom has been defined as a macro-fungus with a distinctive fruiting body, which can be hypogeous or epigenous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 2014). Oyster mushroom (*Pleurotus* species) belongs to the family of Tricholomataceae and is usually found clustering naturally on dead trees at raining season (Lee, 2016). Among all species of mushroom, the oyster mushroom is the second widely cultivated mushroom worldwide following the *Agaricus bisporus* (Kües and Liu, 2012). *Pleurotus spp*. are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency (Mane *et al.,* 2016). Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. Several species of mushrooms are of great importance because of their medicinal properties, for example, they are active against hypercholesterolemic conditions, hypertension, diabetes, cancer and other infections (Alam *et al.*, 2014).

The nutritional and chemical compositions of mushroom are responsible for their medicinal values. However, nutritional composition of mushroom is affected by many factors among which the composition of growth substrate and the method of cultivation are of major importance (Benjamin, 2017). During an investigation of the cultivation of mushroom on agricultural residues, it was found that rice husk, sorghum stover, saw dust, cotton waste, cocoa bean shell, and saw dust such as Gliricidia mixture were suitable substrates for the cultivation of edible mushroom (Belewu, 2016). Various substrates

have different effects on the growth, yield and quality of mushrooms (Ponmurugan *et al.*, 2012). The genus *Pleurotus* is a heterogeneous group of economic importance. Several species are of nutritional and medicinal importance (Cohen *et al.,* 2012). *Pleurotus spp*. have the ability to absorb microelements from different cultivation media and thus they may present an excellent dietary source (Stajic *et al.*, 2015). Fungi of the *Pleurotus* genus have an important place among the commercially cultivated Basidiomycetes, because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates (Kumari and Achal, 2010). Mushrooming in Nigeria rainforest zones is often possible only during the rainy season and is usually inefficient in terms of time spent to collect sufficient mushroom. Most edible species rot quickly and collector must be at the right time at the right place. Hence, there is a need for a cultivation of mushroom for lasting availability all year round. Also, there is an inadequate food supply in most rural areas, diminishing quality of health and increasing environmental deterioration. Mushrooms can help to improve health and nutrition. When used as food, mushrooms promote good human health, being rich sources of protein and vitamins (Kinge *et al.*, 2014).

Also, there is a very high incidence of malnutrition, especially of protein deficiency in most developing countries. This study would help to provide the community with an additional vegetable of high quality and enrich the diet with high quality proteins, minerals and vitamins which can be of direct benefit to the human health and fitness. The extractable bioactive compounds from medicinal mushrooms would enhance human's immune systems and improve quality of life. This study will serve as means of generating employment, particularly for rural women and youths in order to raise their social status. The harvested fruiting bodies can be sold in local markets for additional family income or exported for an important source of foreign exchange that will definitely improve the

economic standards of the people in and around the study area. Hence, this study is very Important because it takes little time, less energy and the use of wastes to provide a good and nutritious mushroom that is also medicinal.

1.2 Statement of the Research Problem

One of the major components of oyster mushroom (*Pleurotus ostreatus and Pleurotus florida*) cultivation is the substrate. The oyster mushroom grows on a wide range of agro-industrial residues; of which different researchers had use various types of substrates readily available. The quality, yield, cultivation time and nutrient composition of mushroom produced varies according to the chemical structure and nutritional content of substrate. Despite, available agricultural waste in Niger state there has been no clear and precise information on the usage of different substrates for mushroom production. Unpredictable nutrients content produced are because of the use of unsuitable substrates has demoralized many researchers who are often, unable to keep on with mushroom production (Masevhe *et al.*, 2016). In order to obtain good nutrients, use of right substrate is very paramount. This study will compare the nutrient contents and vegetative growth of *P. ostreatus* and *P. florida* cultivated on the different substrates

1.3 Aim and Objectives

The aim of the study was

determine the growth and nutritional composition of *Pleurotus ostreatus* and *Pleurotus florida* from some prepared substrates (Agricultural Residues)

The specific objectives of this research were to determine the:

i. Effect of different substrates on the vegetative growth of *P. ostreatus* and P. *florida*

ii. Proximate composition of *P. ostreatus a*nd *P. florida* grown on different substrates

iii. Mycochemical component of *P. ostreatus* and *P. florida* grown on different substrates

1.4 Justification for the Study

Oyster mushroom (*P. ostreatus* and *P. florida*) are grown for their flavor, texture, nutritional value and higher productivity per unit area which have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran and Ramabadran, 2000). Currently, high biofuel prices have caused an increase in food prices and food scarcity in many countries. To alleviate hunger and malnutrition in a world of rising food prices, cultivation of mushrooms is a very and portable option (Das and Mukherjee, 2007). In Nigeria and most developing countries in Africa, residue generated from wood processing are regarded as waste and this has led to open burning practices, dumping in water bodies or dumping in an open area which constitutes environmental pollution (Aiyeloja *et al.,* 2013). Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem (Das and Mukherjee, 2007). Oyster mushroom cultivation can be a labour-intensive agro-industrial activity, thus can help generate income and employment, particularly for women and youth in developing countries (Godfrey *et al.,* 2010)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Oyster Mushrooms

The genus *Pleurotus* (oyster mushroom) comprises some of the most popular edible mushrooms belonging to the family Pleurotaceae. According to Kong (2016), approximately 70 species of *Pleurotus* have been recorded and new species are being discovered more or less frequently although some of these are considered identical with previously recognised species. Many *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide. The type species of genus *Pleurotus* (Fr.) Queli is *P. ostreatus* (Jacq. Et Fr.) Kummer. This mushroom has basidia each with four basidiospores and a tetrapolar mating system. Its hyphae have clamp connections and most members of the genus, except a small minority, have a monomitic hyphal system (Kong, 2016). Examples of other established biological species within *Pleurotus include P. pulmonarius, P. populinus, P. cornucopiae, P. djamor. P. eryngii, P. ostreatus, P. florida* and *P. tuberregium.*

The Oyster Mushroom is wide-spread in temperate and subtropical forests throughout the world. It is a saprotroph that acts as a primary decomposer on wood. Cultivated around the world for food and its medicinal value, it can also be used industrially for mycoremediation purposes. The mushroom is quite adaptable to a range of climates and substrate materials, making itself the second most common mushroom produced worldwide following botton mushroom (Kong, 2016).

2.1.1 History and distribution of *Pleurotus tuberregium*

Chen and Huang (2014) revealed that *Pleurotus tuberregium* (Fr.) Sing., a nematode-trapping mushroom is best known as "tiger milk mushroom" or "sclerotiaproducing *Pleurotus*" in China. It is also known as the "King Tuber Oyster Mushroom". It is a basidiomycete found in the tropical and subtropical regions of the world (Isikhuemhen and LeBauer, 2016). In Nigeria, it is known as "osu" or "ero nsu" in Ibo, "olu" in Yoruba and "katala" or "rumbagada" in Hausa Language. *P. tuberregium* is the only species that produces true sclerotia (Isikhuemhen *et al*., 2014). Isikhuehmen and LeBauer (2016) found the mushroom to be indigenous to tropical Africa and the Australasian-Pacific region of the world. *P. tuberregium* is of great economic importance in all parts of Nigeria. Both the sclerotium and the mushrooms grown from it are eaten. The outer brown portion of the sclerotium is peeled off and the inner white portion cut into small pieces, ground and used in making soup. In this form it may replace melon in okro or vegetable soup. Oso (1977) stated that the pileus and stipe of the mushroom are cut into pieces, boiled and added to okra or vegetable soup.

Consumption is widespread in Nigeria and across many tribes in sub-Saharan Africa. The local people who use this fungus for food and medicine usually collect the sclerotia from the wild. However, easy growing method of this fungus was established to produce sclerotia using many lignocellulose agricultural wastes as cultivation substrates (Isikhuehmen and LeBauer, 2016). The species with a tetra polar mating system (Chen and Huang, 2014) has been found in tropical and subtropical Africa (Nigeria, Ghana, Cameroon, Burundi, Tanzania, Ivory Coast, Chad Republic, Zaire Republic, Uganda, Zambia, Zimbabwe, Kenya, Samoa and Sierra Leone), the Australasian/Pacific region (Australia, Papua New Guinea, Malaysia, Burma, Indonesia, India, Sri Lanka and Hunan Province of China). *P. tuberregium* is only distributed in the Southern region of Hunan

Province, such as Zhanfeng and Tengchong of Ruili District, the Southern region of Gauligong Mountain. Today, this fungus has attained international recognition and is actively studied in laboratories in the US, Europe and Asia for its application in modern medicine (Akpaja *et al*., 2011).

2.1.2 Taxonomy

Pleurotus tuberregium (Fr.) Sing. (*Lintinus tuberregium*) is a tuberous basidiomycete, order Agaricales and family Pleurotaceae (Kong, 2016). The mushroom looks somewhat like *Pleurotus ostreatus* oyster mushroom except that, when mature, the cap of *P. tuberregium* sporophore curves upward to form a cup-like shape. The sclerotium is spherical to ovoid and can be quite large - up to 30 cm (11.8 inches) or larger in diameter. It is dark brown on the outside and white on the inside (Okhuoya and Okogbo, 2012). Petersen *et al*. (2009) stated that *P. tuberregium* is the only *Pleurotus* species in which basidiomata arise from a sclerotium, to which the epithet refers. *Lentinus tephroleucus* produces similar (but lamellate) basidiomata from a pseudosclerotium and an unidentified subtropical American *Lentinus* produces dark brown basidiomata with very deep purple lamellae from a true sclerotium. Unlike cultures of *Lentinus*, however, cultures of *P. tuberregium* produce microdroplets diagnostic for *Pleurotus*.

The taxonomic position of *P. tuberregium* has been very difficult to analyse because it produces leathery fruit bodies and possesses a dimitic hyphal system with intercalary skeletal hyphae. Previously some authorities placed it in either *Panus* or *Lentinus*. More recently, *P. tuberregium* was demonstrated to produce nematotoxic microdroplets in culture, supporting its classification in *Pleurotus* by Singer (Petersen *et al*., 2009). According to them, this genus can be divided into six species. Four of these (*lepiotarii*, *calyptrati*, *coremiopleurotus*, and *tuberregium*) are clearly differentiated from each other

on the basis of some morphological features, such as the presence of a veil on the basidiomata and the formation of chlamydospores, coremia, or sclerotia. However, in the remaining two species (*pleurotus* and *lentodiellum*), which include the most economically important species; there are serious ambiguities. According to Isikhuemhen *et al*. (2016) the placement of *tuberregium* species in *Pleurotus* genus is supported by molecular systematic studies.

2.1.3 Ecology

Oyster mushrooms are found wild in temperate forests and some species in tropical forests. Typically, they grow on dead logs; one relatively uncommon species attacks weak living trees. We would, therefore, expect logs to be the best substrate, but it has been found that straw and some other ligno-cellulosic wastes are better for cultivation (Chang, 1991). *Pleurotus tuberregium* is a white rot fungus which derives nutrients from the degradation of lignocellulose material (usually hardwood). Forests are generally moist places with dim light. Logs are sometimes buried by other forest debris.

The survival of the species depends on the ability of mushrooms to protrude above the surface. Relatives of the common commercial mushroom are found in more open places and are associated with manure and already rotting debris (Cho, 2014). In nature, dead wood is colonized either by mycelia in the soil or airborne spores discharged from individual fruit bodies. Once the substrate is fully colonized and has reached an advanced stage of decay, sclerotia will form at the end of the rainy season. Isikhuehmen *et al* (2016) revealed that the sclerotia survives the dry and hot season until the rain returns, at which point the sclerotia will either continue to enlarge or form sporophores.

2.1.4 Sclerotia

Sclerotium as a non-fertile or sterile mycelial structure, tightly woven. It is an organ for survival and resistant to adverse environment such as drought. Sclerotia are hard in nature and may be viable for 7 years or more after harvest. The sclerotium is often dark brown on the surface and white inside. The sizes and weights of the sclerotia could vary, and may be as large as 30 cm in length and weigh over 5 kg. It enjoys popular usage in Nigeria as a food condiment or in medicine. The sclerotia are usually harvested from decaying logs; the dark brown exterior is peeled off and the white compact mycelial tissue is used for food or medicine. The sclerotia can also be cut into pieces and buried in the soil, and then watered regularly to produce the sporophore (mushroom) which is consumed. One of the most common dietary applications of *P. tuberregium* in Nigeria is as a soup thickener. The white tissue is blended into fine powder and when added to soup, it swells and adds bulk to the soup (Iwuagwu and Onyekweli, 2014). Sclerotia give rise to fruiting bodies in most environments at high temperatures. Chiejina and Olufokunbi (2010) confirmed that basidiocarps can be easily induced by burying the sclerotia in soil. It is of economic importance in food and medicine preparations (Oso, 1977)

2.1.5 Substrates

Mushrooms can be classified into 3 categories by their tropic patterns: saprophytes, parasites or mycorrhizae. The most commonly grown mushrooms are saprophytes which decompose organic matters like wood, leaves and straw in nature. Raw materials can be used as substrates for primary decomposers such as oyster mushroom which have lignocellulosic enzymes. Mushroom requires carbon, nitrogen and inorganic compounds as its nutritional sources for the synthesis of cellulose, hemicelluloses and lignin. Thus most organic matter containing cellulose, hemicelluloses and lignin can be used as

mushroom substrate. Examples are cotton, cottonseed hull, corncob, sugarcane waste, sawdust and so on. However, required amount of each nutritional source differs according to mushroom species. Mushroom mycelia secrete digestive enzymes into the substrate and absorb the dissolved nutrients. Cellulose, the main nutritional source of mushroom is one of the most abundant organic matters on earth, but its digestive enzyme cellulose is owned by several microorganisms including fungi. Cho (2014) stated that mushroom as a saprophyte is the only way by which cellulose is dissolved and absorbed and transformed into food for mankind.

2.1.6 Spawn

What spawn is to mushroom is what seed is to plant. Unlike spore, spawn is already at its mycelial stage growing on its own substrate such as sorghum, barley or sawdust. The life cycle of mushroom starts from spores, but growers inoculate mycelia origin spawn into the substrates rather than using the spore directly because of possible variations and mutations. The quality of spawn according to Cho (2014) is one of the most decisive factors for successful crop. The various types of mushroom spawn include grain, sawdust, plug and liquid.

2.1.7 Environment

Mushroom cultivation requires an appropriate environment both for vegetative and reproductive growth. Not being protected by a skin layer, fungi are easily affected by their growing conditions. So it can be said that the success or failure of mushroom cultivation depends on the control of growing conditions. Environmental factors affecting mushroom cultivation include temperature, humidity, light and ventilation. Optimal levels of these at vegetative stage differ from those at reproductive stage.

Mushroom mycelia can survive between 5 and 40^0 C depending on the species. Mushroom mycelia grow well at a temperature range of 20-30^0 C. Pin form grows best at 10-20^0 C, which is lower than mycelial growth by 10^0 C. Over 80 percent of the fruit body is water. Substrate moisture content should be 60-75 percent and log moisture content 34-45 percent

During fruiting, different relative humidity levels, ranging from 80-95 percent are needed at the early, mid and later stages (Cho, 2014). Though mycelia can grow without light some species require light for fruit body formation. Being aerobic fungi, mushrooms need fresh air during growth, but ventilation is required more for reproductive stage. Creating the optimal conditions for transition from vegetative stage to reproductive stage is crucial to successful mushroom cultivation.

2.1.8 Nutritive value

Pleurotus tuberregium is of great economic importance in all parts of Nigeria. Both the sclerotium and the mushrooms growing from it are eaten. The outer brown portion of the sclerotium is peeled off, and the inner white portion cut into small pieces, ground, and used in making soup. In this form it may replace melon in okro or vegetable soup. The pileus and stipe of the mushroom are cut into pieces, boiled and added to okro or vegetable soup (Oso, 1977). Isikhuehmen *et al*. (2016) stated that analyses of both sclerotia and sporophores show that they are rich in carbohydrates, proteins, vitamins and minerals while low in fats. According to Ogundana and Fagade (1981); the mushroom has 16.5% dry matter and of the dry matter, 7.4 percent is crude fiber, 14.6 percent crude protein and 4.48% fats and oils. Protein levels of shiitake and *P. ostreatus* are higher than that of wheat. Shiitake is 18 percent, *P. ostreatus* is 30 percent while wheat is 13 percent and milk 25 percent (all based upon dry weight). Fat levels are comparable to those of

other mushroom species. Total sugar content is about 18.6% with high concentrations of galactose and low concentrations of glucose and maltose. Levels of oxalic acid, which can reduce the food value, were low as were levels of hydrocyanic acid which can be toxic. The mushrooms also contained low levels of vitamin C. Experiments conducted by Jonathan *et al*. (2015) showed that ethanol, soluble sugar and lipid content of the mushroom were generally low. This suggests that diabetics and those with heart or weight problems can consume this mushroom. *P. tuberregium* has the highest amount of crude fibre compared with other wild edible mushrooms (Jonathan *et al*., 2015). From the studies conducted by Jonathan *et al*. (2015), young fruit bodies of *P. tuberregium* were generally richer in protein than the matured fruit bodies. Therefore, it is suggested that the young sporocarps should be preferred to the matured fruit bodies. Hence, fruit bodies of the mushroom can be eaten as protein supplement or as an alternative to fish and meat in rural areas where these items are not affordable. Vegetarians also eat mushrooms because it served as alternative protein supplement in their diets. Mushroom proteins are generally higher than those of green vegetables and oranges (Jonathan *et al*., 2015). According to Isikhuehmen and LeBauer (2016), already research is going on to incorporate sclerotium powder into bread as a cheap source of protein supplement to bread.

2.2 Medicinal uses of *Pleurotus Tuberregium*

African tribesmen have used the tuber-like sclerotia from *Pleurotus tuberregium* to solve a variety of health problems, ranging from skin diseases, inflammation, childhood malnutrition, headache, stomach problem, cold, asthma, fever, high blood pressure, diabetes and small pox (Oso, 1977). Oso (1977) revealed that some Nigerian native doctors use various combinations of herbs and other ingredients in their medicine. *P. tuberregium* is used in some of these combinations that are intended to cure headache,

stomach ailments, colds and fever as well as asthma, smallpox and high blood pressure. In the South East of Nigeria, it is used to treat heart problem, while it is used to treat asthma, cough, and obesity among the people of Edo State of Nigeria (Isikhuehmen and Okhuoya, 2014; Isikhuehmen and LeBauer, 2016). In Ghana, they are used in medicine for illnesses that relate to malnutrition and anemia in children and in the rural areas as one of the ingredients in embalming dead bodies (Okhuoya *et al*., 1998).

Stated that various Pleurotus species have been shown to possess a number of medicinal properties, such as antitumour, immunomodulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antihyperglycaemic, antimicrobial and antiviral activities. These therapeutic activities are exhibited by the extracts or isolated compounds, fermentation broth, mycelia and fruiting bodies of *Pleurotus* spp. Gregori *et al.* (2007) In particular, polysaccharides appear to be potent antitumour and immuno-enhancing substances, besides possessing other beneficial properties. However, the biochemical mechanisms of these therapeutic activities still remain largely unknown.

According to Isikhuehmen and LeBauer (2016), recent scientific studies have shown that sclerotia of *Pleurotus tuberregium* contain polysaccharides and other compounds with positive medicinal benefits. Publications from Asia have shown that fresh sclerotia of this fungus have high contents of useful compounds like β-glucan and lectin that have promising medicinal properties. Iwuagwu and Onyekweli (2014) showed that the powder obtained from the mycelia of the edible giant mushroom, *Pleurotus tuberregium*, may be used as an alternative to maize starch BP as a tablet disintegrate. Its disintegrate ability in comparison with maize starch BP was investigated in paracetamol tablets prepared. The *Pleurotus* powder, however, showed superior flow, swelling capacity as well as high water retention capacity compared to maize starch BP. Tablets prepared with *P*.

tuberregium powder disintegrated faster than those prepared with maize starch BP at concentrations below 10 % w/w. It is believed that the ability of *Pleurotus* powder to swell by over three times its volume in the presence of water may explain its ability to function as a tablet disintegrate. Equally, Badalyan *et al*. (2017) reported that the antagonistic/antifungal activity of *P. tuberregium* against filamentous fungi can be used as new and effective antimycotic drugs, which are very important tools for preventing and treating widely spread resistant human and animal infections (e.g. mycoses) caused by opportunistic fungi in an immunocompromised host.

2.2.1 Bioremediation

Atlas and Bartha (2010) defined bioremediation as the enhancement of live soil organisms such as fungi, bacteria and plant to break down hydrocarbons and organic contaminants. It involves the application of organisms and nutrients such as phosphate and nitrogen to the contaminated soil for breakdown processes. Nitrate and phosphate supplements enhance biodegradation of oil. According to Adenipekun (2016), bioremediation involves the transformation of complex or simple chemical compounds into non-hazardous forms by biological agents resulting in materials of a higher nutritive value or simply reducing the final bulk of the product. This then gives rise to a variety of products most of which will be much more water-soluble than the parent hydrocarbon.

White-rot fungi have been known for their ability to degrade lignin, a non-repeating structural polymer found in woody plants and this ability enables them to degrade xenobiotic pollutants (Adenipekun, 2016). Many studies have reported the use of *Pleurotus* species in bioremediation exercises (Isikhuemhen *et al*., 2016; Adenipekun, 2016). *Pleurotus tuberregium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soil and the resulting soil sample supported germination and seedling growth of *Vigna unguiculata* (Isikhuemhen *et al*., 2016).

2.2.2 Nematode trapping

Most major groups of fungi are known to contain species that attack and consume nematodes by means of adhesive or ingested spores or various structures in vegetative hyphae. Hyphal modifications for nematode attack or capture include adhesive knobs, nets, stephanocysts, and filaments, constricting and non-constricting rings, cells that secrete paralyzing toxin droplets and mycelia that contain cytoplasmic toxins (Hibbett and Thorn, 2009). Sultana *et al*. (2016) revealed that the extra cellular enzyme of the oyster mushroom acts as an anaesthetic and stings these nematodes and allows the mycelium to grow directly into their immobilised bodies. Aerial hyphae of *P. tuberregium* cultures on agar by Hibbett and Thorn (2009) produced droplets of toxin on stalked secretory processes. Nematodes that came in contact with the toxin droplets were paralysed and then colonized by hyphae.

2.2.3 Mycofilteration and waste water purification

The mycelium is thread made up of interconnected or interwoven strands of cells. According to Sultana *et al*. (2016), one colony can range from a few centimetres to many acres e.g. *Armillaria*. The exquisite Lattice-like structure of the mushroom mycelium often referred to as mycelia network is perfectly designed as filtration membrane. Each colony extends long, complex chains of cells that fork repeatedly in matrix like fashion, spreading to geographically defined borders. The mushroom mycelium, being a voracious forager for carbon (C), and nitrogen (N), secretes enzymes that unlock organic complexes. The use of mycelium for mycofilteration, is shown by placing sawdust, implanted with mushroom mycelium in drainage basins down streams from farms raising livestock, the mycelia act as sieve and trap biological contaminants like fecal bacteria from the surface of water passing directly into sensitive water sheds (Sultana *et al*., 2016).

The study of Yongabi (2012) confirmed that the sclerotium of *Pleurotus tuberregium* is a good coagulant and disinfectant which can be used in natural water and waste water purification.

2.2.4 Pests and diseases

Mushrooms, like any other cultivated crop, are attacked by pests and competitors. In the study conducted by Okhuoya and Okogbo (2012), *Sclerotium rolfsii* was the only fungus that caused stipe rot while others caused crop failure or disfigurement of *P. tuberregium* mushroom. The stipe rot appeared as a yellowish brown rot on the stipe, which prevented the formation of a cap. The stipe gradually deteriorated and disintegrated into the substrate. *S. rolfsii* is a soil-borne pathogen, especially in subtropical and tropical countries, causing diseases ranging from root rot to fruit rot. Most fungi encountered in oyster mushroom production grow and develop on the substrate and are very rarely parasitic. The most frequently encountered genera include *Aspergillus, Botrytis, Coprinus, Fusarium, Monilia, Mucor, Penicillium, Trichoderma,* and *Trichothecium*.

Fungal infestation may be more of a problem when substrates are supplemented with nitrogen-rich nutrients, especially if the supplements are not commercial delayed-release nutrients. Infesting fungi may also be more of a problem when substrate temperatures rise above 35^0 C. Higher substrate temperatures may injure mushroom spawn, reduce mycelial growth rates, and leave the substrate vulnerable to competitors such as *Coprinus* spp. (ink caps) and *Trichoderma* spp. (green mold) (Royse, 2017). Royse (2017) showed that the most common bacterial problem encountered by growers is *Pseudomonas tolaasii*. Symptoms of the disease include reduced yield and orange discoloration and brittleness of the basidiocarps. Infected mushrooms have a reduced shelf life.

Insects infesting mushroom tissues cause the greatest losses for growers, particularly during summer months. The most important insect pests associated with oyster mushroom tissues include members of the families Cecidomyiidae *(Mycophila speyeri),* Scatopsidae,Sciaridae *(Lycoriella solani),* and Phoridae *(Megaselia halterata, M. nigra)*. Oyster mushroom primordia are very sensitive to chemical vapors, so using pesticides to control insects is difficult. Use of various flytraps and adherence to strict hygiene practices, particularly during spawning and spawn run, help keep fly populations below economic threshold levels. According to Royse (2017), deformed mushrooms may result from several causes, many of them still unknown. However, most deformed mushrooms may be traced to insufficient ventilation, smoke, chemical vapours, overheated substrate during spawn run, extreme low fruiting temperature (below 10^0 C), and insufficient light.

2.2.5 Marketing and prospects

Total mushroom production worldwide has increased more than 18-fold in the last 32 years, from about 350,000 metric tons in 1965 to about 6,160,800 metric tons in 1997. The bulk of this increase has occurred during the last 15 years (Royse, 2017). A considerable shift has occurred in the composite of genera that constitute the mushroom supply. The People's Republic of China is the major producer of edible mushrooms, producing about 3,918,300 tons each year or about 64 percent of the world's total. China also produces more than 85 percent of all oyster mushrooms (*Pleurotus spp*.) grown worldwide (Royse, 2017). In Africa, women and children collect and sell sclerotia of *Pleurotus tuberregium* at roadsides or in the market stands for seasonal income and financial autonomy. The sclerotium of *Pleurotus tuberregium* is an essential commodity for local consumption, especially in the West African sub-region and has also become a commodity for export to developed countries, since they are sold in shops across the

many continents. According to Isikhuehmen and LeBauer (2016), people from the regions of the world where the sclerotia is used for food and medicine, who now live in Europe and America actively search for this material in African food shops in those continents. Royse (2017) stated that the value of the 2001–2002 specialty mushroom crops in the United States amounted to $37 million, down 12 percent for the 2000–2001 seasons. The average price per pound for specialty mushrooms received by growers, at $2.77, was down to $0.27 for the previous season. Sales volume of oyster mushrooms, at 4.03 million pounds, was up by 11 percent for the 2000–2001 seasons, with a total of 51 growers producing 4.27 million pounds of the mushrooms in the 2001–2002 seasons. Total production includes all fresh market and processing sales plus amount harvested but not sold (shrinkage, cullage, dumped, etc.). Average oyster mushroom output per farm increased by 249 pounds (18.3 percent) per week, from 1,359 pounds in 2001 to 1,608 pounds in 2002. Oyster mushrooms accounted for 14.2 percent (875,600 tons) of the total world production (6,161,000 tons) of edible mushrooms in 1997, the most recent year for which statistics were available (Royse 2017). The higher price received for fresh oyster mushroom reflects, in part, the less developed and less-reliable technology available to growers for cultivating these species. Thus, growers need potentially higher income to help offset the increased risks associated with producing *Pleurotus* spp.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

The spawn of *P. ostreatus* and *P. florida* were collected from University of Jos Plateau State at Food and Nutrition Department. The following substrates were used for the cultivation of the mushroom; corn flour, sawdust, rice bran, yam pill, maize bran, sawdust + rice bran, corn flour + maize bran, yam peel + sawdust. Sawdust (Mahogany tree) was collected from Maitumbi saw mill, Minna Niger State. Rice bran and maize bran were collected from Dutsen kura milling machine, Minna Niger State. Corn flour, was collected from household while yam peel was prepared in the Lab by peeling the back of the yam.

3.2 Preparation of Substrates

Two kilogram (2 kg) of each substrate was measure using a measuring scale balance. The measured substrate was added to the 30 g of calcium carbonate. The components were homogenously mixed using a spade. The mixing was done on a cemented floor that has been well cleaned. 5000 mls of Water was then added to the mixture to about 65 % moisture content. The moisture content was tested by squeezing the mixture in the palms of the hands. After testing the moisture content, calcium carbonate was then applying on the hand to avoid microbial contamination and substrates was then fill in black polythene bags (25 ×18 cm) using the hand. The substrate was pressed in the bag while filling. The bag will fill such that each bag weight as 2 kg. The bag was tight with a knob so that it could be easily untied later. These bags were then transfer to the sterilization unit (Zhang *et al.,* 2017).

3.3 Sterilization of Substrates

A tripod was place in the pot such that it occupied 1/5 of the height of the pot. This was done to prevent direct contact of substrates and water. Water was then poured d into the drum and jute bags were placed on top of the tripod and around the walls of the drum. The polythene bags containing the substrates was then pace in the drum. Substrates was place round the pot and three each in the middle up till the drum got full. The pot was then cover on the top with jute bags to prevent heat from escaping. Wood was used to set up heavy flames on which the substrate is sterilized (at about 100° C) for 4 hours. Sterilization of substrate bags were done under high temperature using a pot. The substrate had enough heat and started boiling after 30 min. From the boiling point, the substrate was sterilized (at about 100° C) for 4 hours in order that all other microorganisms were destroyed and the substrate is well sterilized (Stamets, 2011).

3.4 Spawning

The substrates bag was remove from the pot and transferred to the spawning room. The bags were then untying and allowed to cool. The spawns were introduced using a knife to avoid contamination. The substrates were planted at a ratio of 1 bottle to 3 substrates of 2 kg (1:3). The spawn was introduced deep into the substrates and was well mixed with the help of the knife such that the spawn covered almost all the whole substrates. The bags were then tie and transferred to a room with shelves. The mushroom substrates were stored in a dark room for colonization to take place. The substrates were placed on shelves that had been painted with calcium carbonate and the window was covered with black polythene bag to reduce the light in the room. The substrates were left in dark room for 21 days at 25° C. Spawn run for mushroom differ from species to species, After the incubation period of 21 days, the windows were open for proper ventilation. At the beginning of fruiting, the substrates were transfer to a fruiting room. The number of days

taken for the initiating of primordial and harvesting were noted for the different substrate mixture and species. To maintain proper temperature, moisture and humidity, the room was watered daily and by watering the bags (Dlamini *et al.*, 2016).

Bagging of the substrates

After sterilization of substrates

Plate I: Bagging and after Sterilization of Substrates

3.5 Harvesting

Harvesting was carried out when the fruiting bodies is matured. Harvesting was made three times from each bag of substrate. The process of harvesting involves the removal with the hand of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The mature mushroom was held on their stipe below the pileus and close to the substrate level and was gradually pulled out. All fruiting bodies of a particular substrate bag was harvested at the same time since each bag had to be watered after harvest. Watering was done by immersing the bags in a bowl of water for 5 second. This is to enable the substrate to have moisture that enables fruiting to occur again for harvest (Ayodele and Okhuoya 2007).

3.6 Experimental Layout

Treatment for the experiment were laid out using a Completely Randomized Design (CRD) and all treatment was replicated five times. The measurements from the various replicates were added and their mean value calculated.

3.7 Morphological Data Collection

The growth and yield of *P. ostreatus* and *P. florida* on the different substrates were determined by recording the number, weight and size of the fruit bodies after sprouting following method adopted by Ayodele and Okhuoya (2007) as detailed below:

3.7.1 Time required for primordial initiation

Each bags for a particular substrate was sampled at random after 21days incubation process completion. The bags were observed on daily basis to note the number of days it took after incubation to the formation of the first primordial.

3.7.2 Time required for harvest

Time taken for harvest was done for the three sample bags from initiation stage to the time of maturation of fruiting body. The average of the time taken for primordial initiation and the time taken for harvesting were calculated and recorded.

3.7.3 Number of total primordial

The number of primordial was counted for each of the sample bags and the average for the eight bags were calculated and recorded.

3.7.4 Number of total effective fruiting body

The number of effective mature fruiting body was counted just before harvesting was done for each bags. The average number of fruiting bodies for the bags were calculated and recorded

3.7.5 Weight of individual fruiting body

The weight of the individual fruiting bodies per bag for was measured using a scale balance. The average bags were calculated and recorded. The weight taken will be the fresh weight.

3.7.6 Height of fruit bodies

This entails measuring the distance from the substrate where the stalk starts growing. The height was measured in centimetres using transparent ruler from the base of the stipe to the pileus. The height of the stalk was carried out for at least five fruiting bodies for each of the bags of the particular treatment. The average height was recorded and calculated.

3.8 Nutritional Analysis

Nutritional analysis was carried out followed the Association of official analytical chemist (AOAC, 2019) to compare the composition of nutrient in *P. ostreatus* and *P. florida* cultivated on the substrates. The data was recorded on moisture, crude protein, crude fibre, crude fat, ash, carbohydrate

3.8.1 Moisture analysis

Twenty gram of fresh mushroom was weighed into a weighed moisture and dried in an oven at 100 to 105°C and cooled in a desiccator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content of mushroom was also expressed in percent and calculated by the formula;

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100 \qquad (3.1)$$

3.8.2 Determination of total protein

Five grams of ground mushroom will be taken with 50 ml of 0.1 N NaOH and boiled for 30 min. The solution was cooled at room temperature and centrifuged at 1000 × g by a DSC-200T table top centrifuge. The supernatant was collected and total protein content

3.8.3 Determination of total lipid

Total lipid will be determined by slightly modifying the method of Five grams of ground mushroom was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution will be filtrated and centrifuged at 1000 g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

3.8.4 Determination of crude fibre

Ten grams of moisture and fat-free sample was take in a beaker and 200 ml of boiling 0.255 N H2SO4 was added. The mixture was boiled for 30 min keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transfer to the same beaker, and 200 ml of boiling 0.313 N NaOH will be added after boiling for 30 min.

3.9 Mycochemical Analysis

3.9.1 Test for alkaloids

0.5 g of the five extracts were dissolved individually with 10ml of dilute hydrochloric acids inside test tubes. They were filtered and filtrates were used to test for the presence of alkaloids. The filtrates were subjected to addition of Mayer's reagent and changes were carefully observed. Formation of yellow creaming precipitate indicated the presence of alkaloids (Santhi and Sengottuvel, 2016).

3.9.2 Test for flavonoids

Portions 0.5 g of plant extract from each of the extracts were heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered, 4ml of the filtrates were shake with 1ml of diluted ammonia solution and colour change was observed. The formation of bluish black colour reveals the presence of flavonoids (Akinyeye *et al*., 2014).

3.9.3 Test for tannins

From each of the dried extracts, 0.5 g was taken, mixed with 20ml of distilled water in test tubes and heated on a water bath. The mixtures were filtered and 0.1% Ferric chloride

($FeCl_3$) solution was added to each extracts change was observed. Dark green or blue-black colour reveals the presence of Tannins (Sneh *et al*., 2013).

3.9.4 Test for phenols

About ten (10) ml of distilled were used to dissolve 0.01g of each of the extracts. They were treated with addition of few drops of lead acetate solution. Noticeable yellow colour precipitates indicate the presence of phenols (Manickan and Veerababu, 2014).

3.9.5 Test for saponins

About 0.5 g of dried extracts from each plant were separately poured into test tubes containing five (5) ml of distilled water, shaked vigorously and observed. The appearance of creamy miss of small bubbles (frothing) showeds that saponin is present (Sneh *et al*., 2013).

3.9.6 Test for steroids

A total of 0.5 g from each of the plant extracts were mixed with two acetic anhydrous, additional two (2) ml of chloroform and three (3) ml of concentrated surfuric acid (H_2SO_4) was carefully add and colour change was observed. Colour change from its original (violent)to blue or green indicated the presences of steroids (Santhi and Sengottuvel, 2016).

3.9.7 Test for terpenoids

About 0.5 g each from the extracts were dissolve in five (5) ml of distilled water. They were mixed with two (2) ml of chloroform and three (3) ml of concentrated surfuric acid (H_2SO_4) was carefully added to form a layer and the appearance of reddish brown colour in the inner layer face or interface indicated the presence of terpenoids (Akinyeye *et al*., 2014).

3.9.8 Test for cardiac-glycosides

Five (5) g from each of the plant extracts were separately mix with (2) ml of glacial acetic acid containing one drop of Ferric chloride ($Fecl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring formed at the interface indicates deoxysugar characteristics of cardenloides. A violet ring may appear beneath the Brown ring, while the acetic acid layer, formed a greenish ring gradually throughout the thin layer revealing the presence of cardiacglycosides (Akinyeye *et al*., 2014).

3.10 Data Analysis

Data of collected on the morphological and yield parameters of the mushroom were subjected to analysis of variance (ANOVA). Where significant difference existing, mean will be separated using Dunca Multiple Range Test.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Result

4.1.1 Effects of different substrate on the growth parameter of *P. ostreatus.*

The result of morphological growth of *P. ostreatus* on different substrate are presented in Table 4.1 below. Significant highest height (7.03cm) of *P. ostreatus* was recorded on sawdust amended with rice bran while the least of (2.06cm) obtained on yam peel. Significantly highest weight (18.66 g) of *P. ostreatus* was recorded on maize followed by 14.00 g on sawdust while the least of 4.36 g was recorded on yam peel. There is significant different across the substrate on the length of stipe of *P. ostreatus*, Highest length of (3.40 cm) of *P. ostreatus* was recorded on sawdust while the least of (1.61 cm) was obtained on yam peel.

4.1.2 Effects of different substrate on the growth parameter of *P. florida*

The result of morphological growth of *P. florida* on different substrate are presented in Table 4.2 below. Significant highest (7.50 cm) *P. florida* was recorded on sawdust while the least of 2.06 cm was obtained on rice bran. The highest weight of (12.13 g) was obtained from sawdust while the least was recorded on maize bran (1.76 g). Highest length of stipe of (5.23 cm) *P. florida* was recorded on maize bran while the least of (1.78 cm) was obtained from yam peel.

Table 4.1: Morphological growth of P. *ostreatus* on different substrates

Name of substrates	Height of P. *ostreatus* (cm)	Weight of P. *ostreatus* (g)	Length of stipe of P. *ostreatus* (cm)
Rice bran	3.06±0.07^{b}	13.76±0.09^{f}	2.53±0.15^{bc}
Yam Peel	2.06±0.07^{a}	4.36±0.12^{a}	1.61±0.01^{a}
Corn flour	4.10±0.10^{cd}	6.70±0.12^{b}	2.09±0.10^{ab}
Maize bran	6.06±0.07^{e}	18.66±0.15^{g}	2.13±0.13^{ab}
Sawdust	3.06±0.07^{b}	14.00±0.23^{f}	3.40±0.15^{d}
Corn flour+Maize bran	4.53±0.48^{d}	12.90±0.15^{e}	2.67±0.30^{bc}
Sawdust+Rice bran	7.03±0.12^{f}	11.73±0.32^{d}	3.13±0.20^{cd}
Yam peel+Sawdust	3.43±0.37^{bc}	10.53±0.43^{c}	2.67±0.30^{bc}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$ as tested by DMRT.

Source: Author's work, 2023

Table 4.2: Morphological growth of P. *florida* on different substrates

Name of substrates	Height of *P. florida* (cm)	Weight of *P. florida* (g)	Length of stipe of *P. florida* (cm)
Rice bran	2.06±0.70[a]	11.17±0.15[e]	1.79±0.02[a]
Yam Peel	2.16±0.94[a]	3.30±0.15[a]	1.78±0.12[a]
Corn flour	3.49±0.12[bc]	2.53±0.89[ab]	2.12±0.14[ab]
Maize bran	2.50±0.11[a]	1.76±0.24[a]	5.23±0.18[c]
Sawdust	7.50±0.11[d]	12.13±0.89[e]	2.14±0.02[ab]
Corn flour+Maize bran	3.63±0.43[bc]	7.83±0.32[d]	2.63±0.27[b]
Sawdust+Rice bran	4.27±0.58[c]	6.70±0.91[d]	2.57±0.24[b]
Yam peel+Sawdust	2.77±0.38[ab]	4.90±0.45[c]	2.20±0.15[ab]

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$ as tested by DMRT.

Source: Author's work, 2023

4.1.3 Effects of different substrates on the yield of *P.ostreatus* and *P. florida*

The results (Table 4.3) obtained indicates that the highest value for maturity period of *P. ostreatus* was on maize bran (5.67 days) while the lowest was on corn flour (4.33 days). The highest value for maturity period of *P. florida* was recorded on sawdust amended with yam peel (6.67 days) while the lowest was recorded on corn flour amended with maize bran (4.00days). The highest number of fruiting body was recorded in maize bran (7.00) for *P. ostreatus* while the lowest was on rice bran (3.67). The highest number of fruiting body for *P. florida* on maize bran was (3.67) while the least was on corn flour (2.00). Maize bran recorded highest in pin head formation of *P. ostreatus* (40.33 days) while the least was on corn flour amended with maize bran (25.33days) for *P. florida*, sawdust recorded the pin head formation (51.00days) with the least obtained from sawdust amended with rice bran (27.00days).

Table 4.3: Yield Result of Fruiting Bodies of *P. florida* and *P. ostreatus* on Different Substrates

Name of substrates	MPD		NFB		PHF	
	P. ostreatus	***P. florida***	***P. ostreatus***	***P. florida***	***P. ostreatus***	***P. florida***
Yam peel	4.67±1.20^{a}	5.00±1.53^{a}	4.33±1.20^{ab}	3.00±0.58^{a}	33.33±0.33^{d}	34.00±0.58^{c}
Rice bran	5.00±0.58^{a}	4.33±0.33^{a}	3.67±0.88^{a}	2.00±0.58^{a}	44.00±0.58^{f}	31.00±0.58^{bc}
Maize bran	5.67±0.33^{a}	4.67±1.20^{a}	7.00±1.53^{b}	3.67±1.20^{a}	40.33±0.88^{e}	42.33±1.45^{d}
corn flour	4.33±0.88^{a}	4.67±0.88^{a}	5.00±1.53^{ab}	2.00±0.58^{a}	31.00±0.58^{cd}	33.67±0.88^{c}
Sawdust	5.00±0.58^{a}	4.33±0.33^{a}	5.00±0.58^{ab}	2.33±0.33^{a}	47.00±1.20^{g}	51.00±0.58^{e}
Sawdust +Yam peel	5.33±0.66^{a}	6.67±0.88^{a}	4.67±0.33^{ab}	3.67±0.68^{a}	29.67±0.88^{bc}	30.00±1.15^{b}
Corn flour+Maize bran	4.67±0.33^{a}	4.00±0.58^{a}	5.67±0.33^{ab}	3.67±0.33^{a}	25.33±1.20^{a}	27.00±0.58^{a}
Sawdust + Rice bran	5.00±0.58^{a}	5.33±1.20^{a}	6.33±0.33^{ab}	3.33±0.88^{a}	27.33±1.20^{ab}	26.00±1.53^{a}

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at $P<0.05$ (DMRT.)

Keys MPD = maturity period, NPH = Number of fruiting body PHF = pin head formation

Source: Author's work, 2023

4.1.4 Effects of different substrates on the proximate composition of *P. florida*

The result (Table 4.4) shows significantly different, the highest lipid percentage was recorded on sawdust amended with rice bran (15.00 %) on *P. florida* while the lowest was recorded seen in yam peel (7.10 %). Highest moisture content was obtained in sawdust (16.15 %) and the lowest was on yam peel (7.10 %). The Ash content was significantly higher in rice bran amended with sawdust (14.05 %) and least on sawdust (4.05 %). Crude fibre was higher in sawdust amended with rice bran and lower in corn flour respectively (17.70 and 4.45 %). Protein was higher in sawdust (17.00 %) and lower in sawdust (5.95 %). The carbohydrate content was higher in yam peel amended with sawdust (63.25 %) and lower in sawdust amended with rice bran (21.7 %)

Table 4.4: Nutritional Analysis of *P. florida* on Different Substrates

Substrates	Moisture	Lipids	Ash	Fibre	Protein	CHO
Sawdust	16.15±0.25^{d}	4.20±1.00^{ab}	4.05±0.25^{a}	12.05±0.50^{d}	5.95±1.85^{a}	57.06±0.50^{d}
Yam peel	7.10±1.00^{a}	2.45±0.35^{a}	7.70±0.40^{c}	6.55±0.55^{b}	8.55±0.45^{ab}	67.65±0.50^{a}
corn flour	9.95±2.15^{abc}	3.10±1.00^{a}	9.60±0.20^{d}	4.45±0.35^{a}	6.45±0.35^{a}	66.45±0.30^{bc}
Maize bran	11.90±1.00^{bcd}	4.65±0.55^{abc}	13.35±0.45^{e}	15.30±0.30^{e}	14.65±1.45^{c}	40.15±0.85^{d}
Rice bran	11.95±0.15^{bcd}	6.95±0.15^{cd}	14.05±0.05^{e}	12.15±0.50^{d}	16.05±0.50^{c}	38.85±0.05^{ab}
Sawdust + Rice	14.55±2.25^{d}	15.00±1.20^{e}	14.05±0.05^{e}	17.70±0.40^{f}	17.00±0.20^{c}	21.7±0.90^{d}
Corn + Maize	14.05±0.25^{cd}	5.95±0.15^{bcd}	10.35±0.45^{d}	9.15±1.00^{c}	11.10±1.00^{b}	49.4±0.80^{c}
Yam peel + sawdust	9.65±0.55^{ab}	7.65±0.45^{e}	5.70±0.70^{b}	7.65±0.45^{bc}	6.10±0.60^{a}	63.25±0.90^{ab}

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at P<0.05 (DMRT.) **Source:** Author's work, 2023

4.1.5 Effects of different substrates on the proximate composition of *P. ostreatus*.

The result (Table 4.5) indicate that the moisture content was higher in sawdust and lower in corn flour amended with maize bran respectively (16.70 % and 5.70 %). Sawdust amended with rice bran showed the highest value in lipid content (7.60 %) and the lowest was on yam peel (2.05 %). The Ash content was significantly higher on rice bran (14.05 %) and lower in maize bran (8.15 %). The fibre and carbohydrate contents were significantly higher on sawdust and corn flour amended with maize bran at (16.95 % and 64.95 %) respectively. The highest percentage of protein was observed on maize bran (17.50 %) while the lowest was on peel + sawdust (6.40 %).

Table 4.5: Nutritional Analysis of *P. ostreatus* on Different Substrates.

Name of substrates	Moisture	Lipids	Ash	Fibre	Protein	CHO
Sawdust	16.70±2.20^{c}	7.10±1.00^{bc}	13.65±0.55^{c}	16.95±0.85^{d}	9.00±0.80^{cd}	36.62±0.40^{e}
Yam peel	11.95±0.15^{b}	2.05±0.05^{a}	8.65±0.55^{a}	7.95±0.15^{c}	10.00±0.00^{d}	33.3±0.45^{b}
corn flour	12.40±1.20^{c}	3.65±0.45^{a}	10.60±0.50^{b}	6.05±0.15^{abc}	7.65±0.50^{b}	53.7.±0.15^{a}
Maize bran	6.20±2.10^{a}	8.55±0.55^{c}	8.15±0.05^{a}	5.45±1.35^{ab}	17.50±0.30^{f}	54.15±0.05^{e}
Rice bran	11.95±0.15^{b}	6.95±0.15^{bc}	14.05±0.05^{c}	6.95±0.85^{abc}	16.05±0.05^{e}	44.04±0.05^{d}
Sawdust + Rice bran	11.05±1.05^{b}	7.60±0.40^{bc}	12.95±0.15^{c}	5.20±0.50^{ab}	10.15±0.05^{d}	53.05±0.15^{ab}
Corn flour + Maize	5.70±0.10^{a}	4.80±1.70^{ab}	11.60±0.20^{b}	4.90±0.20^{a}	8.05±0.05a^{b}	64.95±0.95^{d}
yam peel+ sawdust	8.45±0.45^{ab}	8.10±1.00^{c}	8.20±0.00^{a}	7.40±0.40^{bc}	6.40±0.30^{a}	61.45±0.45^{c}

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at P<0.05(DMRT). **Source:** Author's work, 2023

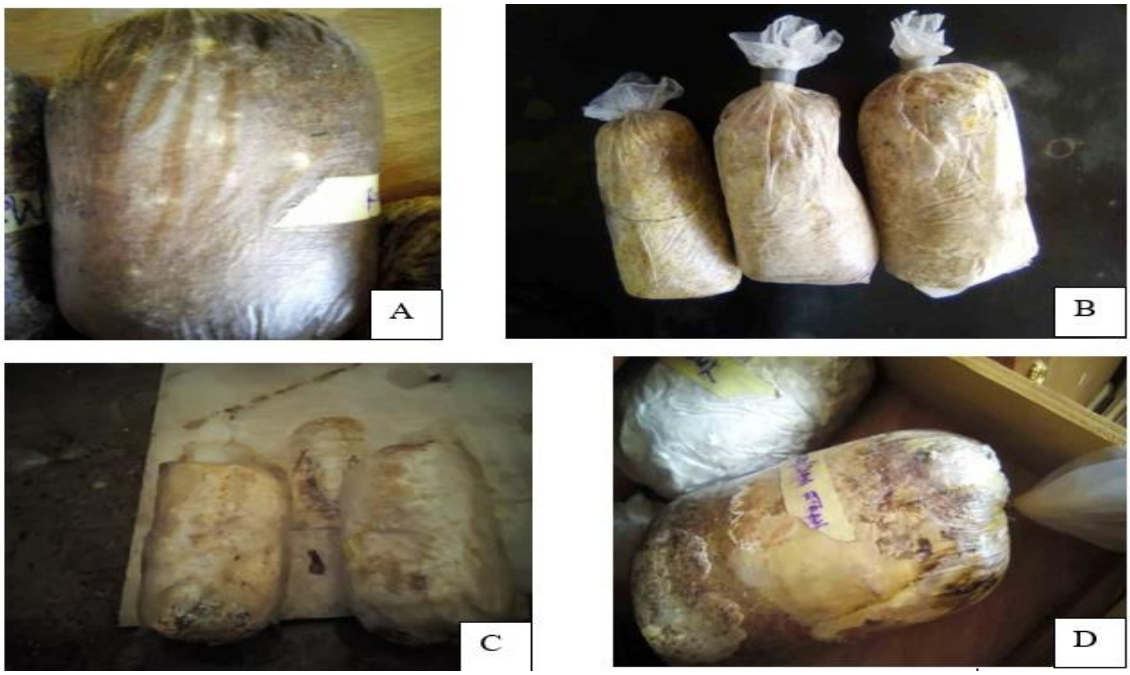
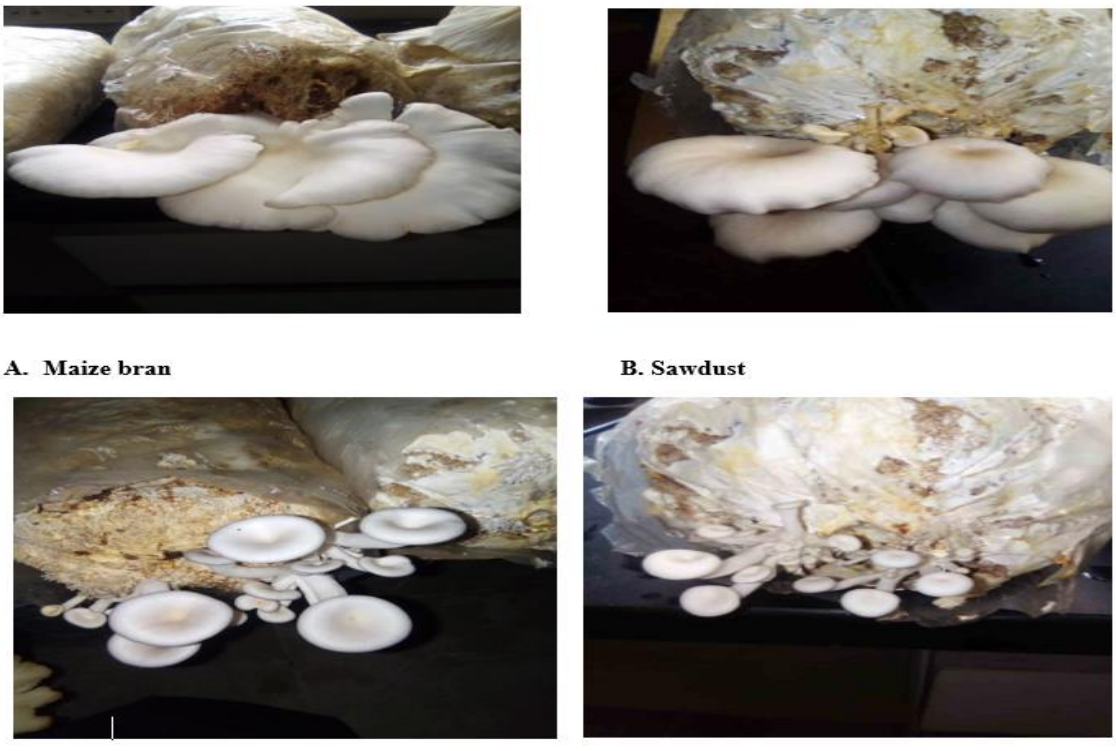


Plate II: Emergence of Mycelia of *P. ostreatus* and *P. florida* on Different Substrates



A. Maize bran **B. Sawdust**

C. Sawdust amended with rice bran **D. Rice bran**

Plate III: Full Grown Bodies (*P. ostreatus* and *P. florida*) Mushroom on Different Substrates.

E. Yam peel amended with sawdust

F. Yam peel

G. Corn flour amended with maize Bran

H. Corn flour

Plate IV: Full grown bodies (*P. ostreatus* and *P. florida*) mushroom on different substrate.

4.1.6 Mycochemical component of *Pleurotus ostreatus* and *Pleurotus florida*

The result of mycochemical component of *P. ostreatus* and *P. florida* on different substrate are presented in Table 4.6 below. Bioactive component analysis of edible mushrooms *P. ostreatus* and *P. florida* revealed the presence of major bioactive components such as flavonoid, polyphenols, saponins, triterpenoids and steroids.

Table 4.6: mycochemical component of *Pleurotus ostreatus* and *Pleurotus florida*

mychoochemical components	*Pleurotus florida*	*Pleurotus ostreatus*
Alkaloids	-	-
Flavonoids	+	+
Polyphenoids	+	+
Triterpenoids	+	+
Steriods	+	+
Saponins	+	+
Glycosides	-	-
Resins	-	-
Tannins	-	-

The (-) sign indicates the absence of the compounds while the (+) sign indicates the presence of the component. **Source:** Author's work, 2023

4.2 Discussion

4.2.1 Effects of different substrate on the growth parameter of *P. ostreatus*

The species of oyster mushroom cultivated on different substrate indicated that highest height was recorded on sawdust amended with rice bran, highest weight was recorded on maize bran and the length of stipe recorded highest on sawdust. This study is in line with the study of Shah *et al* (2017) who reported that sawdust and maize bran gave maximum yield of *P. ostreatus.*

4.2.2 Effects of different substrate on the growth parameter of *P. florida*

The result obtained from the growth parameter of *P. florida* show the specie was better in sawdust than in other substrates as it had the highest height, weight and maize bran had the highest stipe length and weight. This study is in line with the study of Shah *et al* (2017).

4.2.3 Effects of different substrate on the yield of *P. ostreatus and P. florida*

From the result obtained from yield of species it show that *P.ostreatus* on flour amended maize bran gave lesser days for pin head formation, maturity period and number of fruiting body. The least results were indicated in *P. florida* on rice bran, corn flour and corn flour amended with maize bran. The variability in pin head formation, maturity period and number of fruiting body is due to presence of different composition of substrate. This result is in line with Bughio (2014) which reported that maturity of fruiting bodies took 5 to 6 days after pin head formation as in the same line with this study.

4.2.4 Effects of different substrate on the proximate composition on *P. florida*

The nutritional composition of edible mushroom is affected by many factors among which composition of substrate is of major importance also mentioned by Belewu (2016). Nutritional properties also differ according to species, but this difference also depends on the substrates. These results also indicate that the studies oyster mushroom species have good nutritive value for human. Protein is an important nutritional component and protein deficiency is the world most serious human nutritional problem, especially a country like Nigeria. So oyster mushroom is a promising food that may overcome protein energy malnutrition problem and mineral deficiency in the Nigeria. While the protein content is lower than that found in the eggs, meat and fish, it is adequate to be used as a substitute in the diet of the general public. Concern nutritional properties of *P. florida* cultivated on sawdust amended with rice bran recorded highest amount of fibre (17.70 %) which helps in digestion, weight management and has highest amount of lipids that protect the vital organ. Highest percentage of protein record on sawdust amended with rice bran (17.00 %), high percentage of carbohydrate was recorded on yam peel (67.65 %) the result is in line with (Wang *et al.,* 2017).

4.2.5 Effects of different substrate on the proximate composition of *P. ostreatus*

The result indicates that *P, ostreatus* grown on maize bran which had the highest amount of protein of 17.50 %. This protein is of great importance to health as they help the body to build, repair and maintain body tissue. The study is in line with (Wang *et al.,* 2017).

4.2.6 Mycochemical component of *P. ostreatus* and *P. florida*

The mycochemical results is similar to that of (Iwalokun *et al.,* 2015). The compound alkaloids, glycosides, resins and tannins were absent in the extracts. Bioactive compounds

found in edible mushroom are known to play a vital role in promoting health. The absence of alkaloids and glycosides confirms the report of (Hamzah *et al*.,2014)

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study concluded that vegetative growth on sawdust amended with rice bran, maize bran and sawdust waste seems to have greater influence on the growth and yield of these species due to the nutritional composition in the sawdust such as cellulose, carbohydrate, protein, fibre, and ash content in the rice and maize bran. Furthermore, proximate composition and mycochemical compounds found in Oyster mushroom species have good nutritive value for humans.

5. 1.2 Recommendations

i. Mushroom growing should be integrated into our farming system, due to low lipid and higher fibre content which is of health benefit to mankind especially against heart disease and diabetes.
ii. Sawdust, maize bran and sawdust amended with rice bran should be used for growth and fructification oyster mushroom.
iii. More study is encouraged to study the effect of substrate supplementation on sustained yield of edible mushrooms in various harvests with regards to commercial production.

5.3 Contribution to Knowledge

The thesis established that *Pleurotus florid*a grown on sawdust from *Khaya senegalensis* (Mahogany) had the highest height (7.50 cm) and weight (12.14 g). But *P. ostreatus* grown on sawdust Mahogany plus rice bran recorded the highest height (7.03 cm); the highest weight in *P. ostreatus* was recorded in maize bran (18.66 g). Meanwhile, *P. florida* grown on maize bran had the highest stipe length (5.25 cm) while those grown on the sawdust plus yam peel recorded earlier maturity period of (6.67 days). Mushrooms grown on sawdust plus yam peel recorded the highest number of fruiting bodies (6.67). The highest proximate compositions of *P. florida* grown on sawdust from Mahogany was 16.15% for moisture content and sawdust plus rice bran recorded (14.55 %) moisture content. Highest protein and carbohydrate contents were recorded from maize bran (14.65 %) and (67.65 %) yam peel respectively. The highest protein content in *P. ostreatus* (17.5 %) was obtained in mushrooms raised in maize bran while the highest fibre contents (16,95 %) were obtained on those grown on sawdust only.

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