# INVESTIGATIONS OF VARIOUS AGRO-WASTES AS SUBSTRATES FOR CULTIVATION OF OYSTER MUSHROOMS (*Pleurotus ostreatus* (Jacq.:Fr) P. Kumm AND *Pleurotus pulmonarius* (Fr.) Quèl)

by

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## DECLARATION

I, Takalani Lorreta Matidza, certify that this research project, which I am submitting for the degree of Master of Science in Agriculture (Horticulture) at the University of Venda, is my original work and that I have not previously submitted it. All of the information sources used are clearly acknowledged.

Student signature: …… Date: 27 October 2022

## DEDICATIONS

I dedicate this work to the Lord Almighty who strengthen and empowered me to complete this work. Not forgetting my brothers, mom, and pastors through their support and courage.

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## ABSTRACT

This study was conducted to assess the effects of various agro-waste substrates on the production of oyster mushrooms (*P. ostreatus* and *P. pulmonarius*). The substrates utilised in this research were banana pseudo-stems (BP), macadamia husks (MH), macadamia nutshells (MnS) and maize stalks (MS) as control. The experimental design used in study 1 was a complete randomized design (CRD) for chemical constituents with 3 replications (rep) and 2 bags per rep. In contrast, the experimental design on study 2 was factorial in a complete randomized design comprised of mushroom growth and yield replicated thrice and bags duplicated per rep. Study 3 was nutritional compositions replicated three times, having 2 bags per rep. The maximum pH was observed on MS and the minimum was obtained on MnS. Severe infections by *Trichoderma* species were observed from PO *Pleurotus ostreatus* (PO) and *Pleurotus pulmonarius* (PP) on MnS, and as a result, no fruiting bodies were formed. The growth in terms of the number of days to colonization (DC) was delayed on MH when compared to others. The yield was significantly superior on MS 75.73 and 94.40 g during March to May 2019 in terms of fresh weight (FW). Moreover, the highest yield was observed on MS and BP at 120.19 and 92.63 g from *P. ostreatus* and *P. pulmonarius* respectively during June to August 2019. The superior biological efficiency) (BE) was attained from mushroom grown on MS during March to May 2019 whereas on MS and BP was during June to August 2019. The least FW and BE was observed from *P. ostreatus* and *P. pulmonarius* grown on MnS. The maximum crude protein (CP) was obtained from PO and PP on MH. Mushrooms are enriched in potassium (K), and the maximum amount was found on MS when compared to others from both *Pleurotus* species. Therefore, MS and MH were best selected to produce Oyster mushrooms.

**Keywords:** *Pleurotus ostreatus*, *Pleurotus pulmonarius.*

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ANOVA | : | Analysis of Variance |
| BP | : | Banana pseudo-stems |
| $Ca(OH)_2$ | : | Calcium hydroxide |
| $CaCO_3$ | : | Calcium carbonate |
| MEA | : | Malt extract agar |
| MH | : | Macadamia husks |
| MS | : | Maize stalks |
| MnS | : | Macadamia nutshells |
| C/N ratio | : | Carbon Nitrogen ratio |
| EC | : | Electrolyte conductivity |
| $HSO_4$ | : | Hydrogen sulphite |
| NaOH | : | Sodium hydroxide |
| HCl | : | Hydrogen chlorine |
| Rep | : | Replication |

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# CHAPTER 1

# BACKGROUND OF THE STUDY

## 1.1 BACKGROUND INFORMATION

Oyster mushrooms (*Pleurotus* species) belong to the family of Tricholomataceae and in the order Agaricales that belong to the phylum Basidiomycota. They are important in people's life since they are utilised a food and for medical (Zeng *et al*., 2022). They can be used to meat alternatives. According to Adejumo & Awosanya (2005) and Raman *et al*. (2020), important minerals in mushrooms are not limited to calcium, iron, manganese, potassium, iron, zinc, copper, and phosphorus. Mushrooms are high in carbohydrate, fiber, and protein, and they contribute little to fat accumulation in the human body (Sifat *et al*., 2020). Apart from providing basic nutrition, Oyster mushrooms have antioxidants components such as phenolic, flavonoid and tannin that can be used to reduce risks of heart failure and cancer diseases (Islam, 2015; Sifat *et al*. 2020). Thus, they boost the immune system in the human body. Niazi & Ghafoor (2021) showed that few mushrooms such as Paddy straw (*Volvariella volvacea*), Shiitake (*Lentinula edodes*), Enoki (*Flammulina velutipes*), Button (*Agaricus bisporus*) and oyster (*Pleurotus* species) mushrooms are most widely grown.

Oyster mushrooms (*Pleurotus* species) are widely cultivated in Asia, North America, and Europe because they are simple to produce with low-cost manufacturing technologies while yet providing great biological efficiency (Hoa *et al*., 2015). Oyster mushrooms are the second most widely cultivated edible fungus. *Pleurotus* species account for 16.3% of global edible mushroom production (Singh & Singh, 2021). There is currently no record of the market value of oyster mushrooms in South Africa.

*Pleurotus* is a genus of crop that may be grown in a variety of temperate and tropical climates. They are gaining popularity as a result of their ability to be grown in a variety of environments. *Pleurotus* species can thrive at temperatures ranging from 20 to 30 0 to 30 "degree Celsius and relative humidity levels of 60 to 80 percent, even in the winter (Rathod *et al*., 2021). *Pleurotus ostreatus* and *P. pulmonarius* are two *Pleurotus* species that thrive in warmer climates (OECD, 2013). *Pleurotus flabellatus* was

discovered to grow easily in cool conditions, in contrast to warmer ones (Stamets, 2000). *Pleurotus* species are thus not constrained by their surroundings.

Oyster mushrooms are main decomposers that develop naturally on dead plant pieces (Igile *et al*., 2020). Unlike *Agaricus* species that require composted substrates, they can thrive on undecomposed lignocellulosic waste materials. According to Quimio *et al*. (1990), *Pleurotus* can also be grown utilizing agricultural wastes, forest residues, and industrial processing wastes. Oyster mushrooms are usually produced on agricultural wastes such straw, coconut wastes, sugarcane bagasse and sawdust (Masevhe *et al*., 2016; Singh & Singh 2021). *Pleurotus* cultivation is becoming more popular as a result of its ability to utilize a variety of lignocellulosic components as well as its nutritional value (Singh & Singh, 2021).

In a prior study in South Africa, maize stalks were treated with varying degrees of supplement and fast production was also found when no supplement added (Mkhize *et al*., 2016). Masevhe *et al*. (2016) investigated the ability of *Pleurotus* species to grow on wheat straw, wood chips, and thatch grass, and found that wheat straw performed best. Wheat straw, on the other hand, is not widely available in all parts of South Africa. In most regions of Limpopo Province, large quantities of macadamia husks, macadamia nutshells, banana pseudo-stems, and maize stalks are accessible.

The goal of this study was to investigate the effects of different substrates on oyster mushroom production, namely *P. ostreatus* and *P. pulmonarius*. The outcomes of this study are critical for the province's resource communities and small-scale farmers.

## 1.2 PROBLEM STATEMENT

Vhembe District is located in South Africa's Limpopo Province. Due to market demand and profitability, it is one of the few areas where a large number of farmers produce horticulture crops such as banana and macadamia nut trees (Maselesele *et al*., 2020). Moreover, throughout the cropping season, maize is grown practically every yard and field. Although certain agrowastes are used for composting and mulching, the remainders pollute the environment because they are normally disposed of by dumping; as a result, they have an impact on the ecosystem (Edokpayi *et al*., 2018). Growing oyster mushrooms instead of dumping them can be a viable alternative to landfilling. As a

result, the current research focuses on turning these Vhembe District agrowastes into *P. ostreatus* and *P. pulmonarius* mushroom cultivation.

## 1.3 JUSTIFICATION

*Pleurotus* may consume a variety of lignocellulosic materials. As a result, the variety of diverse substrates discovered in local places that do not require further treatment provides a wide range of options for growing *Pleurotus* species. Banana pseudo-stems are frequently thrown in the Vhembe District of Limpopo Province, while macadamia nutshells are used to manufacture plates in some businesses and macadamia husks are either mulched or composted. Oyster mushroom cultivation could potentially increase income for low-income people in rural areas by creating jobs. The use of oyster mushroom cultivation, on the other hand, has not been investigated. Farmers and end-users in the area will profit from this approach.

## 1.4 RESEARCH QUESTIONS

- What are the chemical constituents of the different substrates?
- What are the effects of different substrates on the growth and yield of oyster mushrooms?
- What are the nutritional compositions of oyster mushrooms cultivated on various substrates?

## 1.5 OBJECTIVES

### 1.5.1 Broad objective

To investigate the effects of different substrates on the production of Oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus pulmonarius*).

### 1.5.2 Specific objectives

- To determine the chemical constituents of substrates.
- To evaluate the effects of substrates on growth and yield of oyster mushrooms.
- To determine the nutritional composition of oyster mushrooms grown on various substrates.

## 1.6 HYPOTHESES ($H_0$)

- There are no significant variations in chemical contents between substrates.
- There are no significant differences in oyster mushroom growth and yield between substrates.
- There are no significant changes in the nutritional contents of oyster mushrooms on substrates.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 BACKGROUND INFORMATION

The total mushroom production in the world was predicted to be 8 939 060 tons (FAO, 2018). China is the greatest producer of edible mushrooms, followed by United States and Netherlands, with 6 675 364 tons, 416 050 tons, and 280 232 tons, respectively (FAO, 2018). According to (Potoˇcnik *et al*., 2015), button mushrooms (*A. bisporus*) are the most widely grown edible mushrooms, followed by oyster mushrooms (*Pleurotus* species), and Shiitake mushrooms (*L. edodes*). These three contributes 30 %, 27 %, and 17 % of global edible mushroom production, respectively (Royse, 2014). Thakur (2020) recently predicted a global edible mushroom market worth around 40 million tonnes with nations including China, United States, Netherlands, France, Spain, Ireland, Canada, United Kingdom, and Italy. Oyster mushrooms are estimated roughly 1.5 million tons of edible mushroom production every year around the world (Soldatenko *et al*., 2019). Africa contributes roughly 28 676 tons of edible mushroom production to the global total (FAO, 2018). According to the FAO (2018), South Africa produced around 21 116 tons of edible mushrooms in 2018, making it to the top producer in Africa. There is currently no available information on the state of mushroom production in Limpopo Province's Vhembe District. A market value of oyster mushrooms in South Africa has not been documented.

## 2.2 THE HISTORIC INFORMATION OF MUSHROOMS

Edible mushrooms were harvested during the rainy season when the ground was wet, and they were highly prized as human food. Mushroom cultivation is said to have begun in China about the year 600 (Chitamba, 2007). *Pleurotus* spp. cultivation, on the other hand, began about 1917 in Germany on wood logs and stumps (Carrera, 1998). The first record of mushroom cultivation in South Africa was in 1940 (Eicker, 1990). *Pleurotus ostreatus* was also developed on log cultivation in the early 1950s in Japan before being switched to bottle cultivation in the early 1960s (Yamanaka, 2011). Later, it was discovered that lignocelluloses, forest wastes, and farm crop residues may all be used to grow a variety of species (Carrera, 1998).

## 2.3 BOTANICAL NOMENCLATURE OF OYSTER MUSHROOMS

Because of the white mycelia that oyster mushrooms generate; they are known as white-rot fungus. Oyster mushrooms belong to the phylum Basidiomycota and the order Agaricales (Adebayo *et al*., 2021). According to CABI (2018), oyster mushrooms are members of the Pleurotaceae family. All the strains could be grouped into seven pedigrees, each identifying one morphological species, namely *P. eryngii, P. placentodes*, and *P. abieticola* (Li *et al*., 2017). *Pleurotus* species are known as abalone mushrooms in China (Quimio *et al*., 1990). According to Hemalatha (2016), *P*. *pulmonarius* is known as the Indian oyster while *Pleurotus ostreatus* is known as the grey oyster. *Pleurotus* species are primary decomposers, meaning they can colonize and fully breakdown lignin from dead plant tissue or lignocellulose materials (Grzegorz *et al*., 2017).

## 2.4 THE MORPHOLOGICAL CHARACTERISTICS OF OYSTER MUSHROOMS

Oyster mushrooms feature a shell-like cap that can range in size from 5 to 20 cm in diameter, is meaty and has an eccentric or lateral stipe (Carrera, 1998). *Pleurotus* produces spores in a variety of colours, from white to grey lilac (Tisdale, 2004). Oyster mushrooms are divided into three parts, namely: fleshy shell or spatula-shaped pileus (cap); stipe which is a short or long lateral or central stalk; and long ridges and furrows beneath the mushroom cap (pileus) which are referred to as gills or lamellae (Chaudhary & John, 2017). *Pleurotus* species differs with their internal and exterior features (Kumari *et al*., 2012).

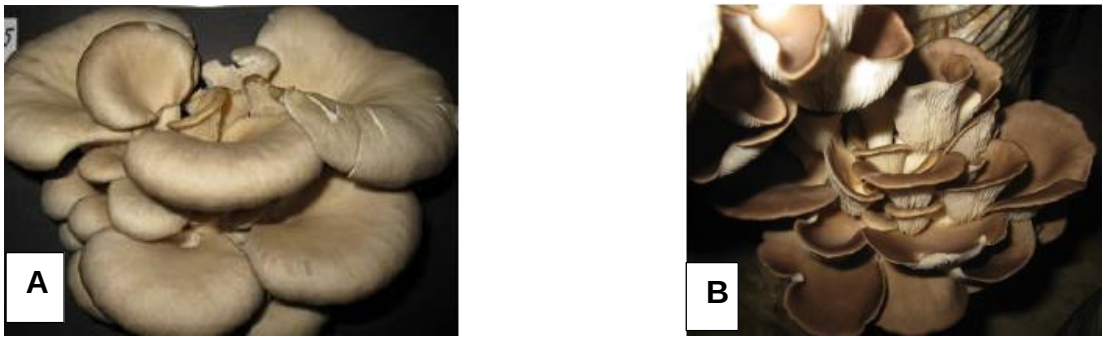


Figure 1. Morphology of oyster mushrooms: A - *Pleurotus ostreatus* and B - *Pleurotus pulmonarius* (Source of mushrooms: Myronycheva *et al*., 2017).

### 2.2.5 The importance of Oyster mushrooms

### 2.5.1 Oyster mushrooms as a source of food

Oyster mushrooms are eaten and prized throughout the world for their flavour. They are also beneficial to the economy and the environment, as well as having medical characteristics. They are inexpensive source of nutrients and minerals. According to Niazi & Ghafoor (2021), they are high in energy, protein, carbohydrate, fiber and minerals. The nutrient contents of oyster mushrooms have been found to be 17-47 percent sugar, 8-12 percent minerals, and 2-5 percent fat (Miles & Chang, 1997). According to Jackson *et al*. (2018), the amount of protein in oyster mushrooms range from 6.95 to 31.69 grams. Protein concentration is influenced by a variety of factors including substrate type, species, and nutrients supplementation. As a result, they can be useful addition to vegetables, fruits, and legumes. Furthermore, scientific research has found that oyster mushrooms are typically nutritionally similar to meat (Thongnaitham, 2012). As a result, oyster mushrooms can be used to replace meat in recipes.

### 2.5.2 Oyster mushrooms in medicine

Given the persistent threat of multi-drug resistance, oyster mushrooms can be used as a source of natural antioxidants and in the production of pharmaceuticals (Fakoya *et al*. 2020). They also have important therapeutic benefits; they have been used to treat diabetes and cancer (Randive, 2013). According to Piska *et al*. (2017), lovastatin is one of the most important chemicals found in *P. ostreatus* and is used to treat dyslipidemia. As a result, this species has become a valuable therapeutic mushroom. Oysters have the ability to decrease cholesterol in the human body, hence reducing cardiovascular problems (Patel *et al*., 2012; Piska *et al*., 2017). According to Nguyen *et al*. (2016), *P. pulmonarius* can also be used to boost immune system because of its antioxidant, anti-cholinesterase, and anti-inflammation properties. As a result, oyster mushroom is a desirable crop that is gaining popularity due to its human health benefits.

### 2.5.3 The ecological importance of Oyster mushrooms

Oyster mushrooms play an important function in the environment. They are well-known for being one of the most efficient natural organisms at producing lignin

peroxidases and cellulases, both of which have incredibly powerful degradative capabilities (Stamets, 2000). Luz *et al*. (2012) discovered that during *P. ostreatus* colonization, considerable ecological ligninolytic activities of enzymes (cellulase, xylanase, manganese peroxidase, and laccase) for cellulose, hemicellulose, and lignin degradation increased. Furthermore, this occurred when rice bran was added to the substrate. This could imply that adding supplements to the substrates result in an increase in enzyme activity. *Pleurotus* mushrooms receive nutrients from degraded organic elements into their cells after they have been broken down. According to Kusrini *et al*. (2019), one of the strategies used to prevent the production of dangerous gases, and thus, create a pleasant environment is the growth of oyster mushrooms. Adedokum (2014) discovered that *P. pulmonarius* can grow on a variety of agricultural wastes including corn husk, cotton, sawdust, rice straw, and corn cob; demonstrating the efficacy of bioconversion of these waste materials. As a result of its ability to digest diverse lignocellulosic materials into fruiting bodies, the oyster mushroom is excellent for cultivation.

## 2.6 THE INFLUENCES OF ENVIRONMENTAL FACTORS ON THE PRODUCTION OF OYSTER MUSHROOMS.

Several environmental conditions influence the development of *Pleurotus* mushrooms including temperature, light intensity, gaseous exchange, and relative humidity (Reynders & Reynders, 2013; Bellettini *et al*., 2017). Ogbu *et al*. (2021) observed that oyster mushrooms may be produced at temperatures ranging from 22.3 to 30.4 $^{o}$C and relative humidity levels of 50.0 to 79.9%. However, it has been found that the ideal temperature range for fruiting bodies is between 10 and 21 degrees Celsius (Reynders & Reynders, 2013). According to Stamests (2000), light ranging from 500 to 2000 lux/hour is required for primordial creation; however, light ranging from 500 to 1500 lux/hour is required for fruiting body growth. Nonetheless, light intensity more than 2000 lux/hour causes the primordial development to fail (Stamets, 2000). Furthermore, the intensity of light influences the colour of oyster mushrooms (Zawadzka *et al*., 2022). According to Reynders & Reynders (2013), plenty of fresh air (oxygen) is needed to avoid carbon dioxide build-up and encourage the optimal fruiting body growth. The metabolic system in oyster mushrooms can be harmed by lack of oxygen. As a result, having enough oxygen results in a wider diameter (Rambey *et al*., 2019).

The relative humidity can be maintained between 50.3 to 80.70 percent, according to Chitra *et al*. (2019). Oyster mushrooms can be cultivated almost anywhere if the right environmental conditions are in place.

## 2.7 THE INFLUENCE OF SUBSTRATE COMPOSITION ON THE PRODUCTION OF OYSTER MUSHROOMS.

### 2.7.1 The pH of substrate

Each *Pleurotus* species has a different pH range that is suitable for the growth of oyster mushrooms. Mycelium, for example, requires a pH of less than 7.2, whereas the pH necessary for the production of the fruiting body is between 5.5 and 6.4 (Khan *et al*., 2013; Sultana *et al*., 2018). According to Jha and Gotame (2020), the ideal pH for mycelia establishment and subsequent fruiting body expansion and yield is between 6.5 and 7.13. According to Jha and Gotame (2020), *Pleurotus ostreatus* planted on *Aspergillus* flavus treated compost at a pH of 7.33 resulted in fast mycelia colonization, pinhead formation, and fruiting. Several investigations have been carried out, and it has been discovered that different Oyster mushrooms grown on different lignocellulosic substances prefer varied pHs (Prabhakar & Kumar, 2018; Jha & Gotame 2020).

### 2.7.2 The Carbon - Nitrogen (C/N) ratio of substrates

Nutrients are obtained from carbon compounds like as cellulose and lignin, nitrogen, and inorganic compounds (Besufekad *et al*., 2020). One of the factors that influences oyster mushroom production is the C/N ratio (Belletini *et al*., 2017). According to Sardar *et al*. (2020), the C/N ratio is a critical component in determining mycelial colonization and fruiting body formation. According to Belletini *et al*. (2019), the manifestation of an insufficient C/N ratio in the cultivation substrate is more favorable during the fruit body growth stage. According to Belletini *et al*. (2019), the manifestation of an insufficient C/N ratio in the cultivation substrate is more favorable during the fruit body growth stage. In addition to the C/N ratio influencing the development of fruit bodies, an excess of nitrogen can influence the breakdown of lignin, which may limit mycelium development (Belletini *et al*., 2019). Belletini *et al*. (2019) support Rizki & Tamai (2011), who observed a lower C/N ratio from palm oil trunk which was the most rapidly colonized by mycelium and had fewer days until primordial initiation. This

demonstrates that lower C/N substrates can be used to reduce production time. According to Zanetti & Ranal (1997), when nitrogen exceeds carbon, the mushrooms' mycelial development is inhibited more effectively. A number of investigations have revealed that different lignocellulose materials have varied C/N ratios. As a result, maintaining an acceptable C/N ratio during oyster mushroom cultivation is critical.

### 2.7.3 The electrolyte conductivity (EC) of substrates

On lignocellulose materials, oyster mushrooms respond differently at varying levels of electrolyte conductivity. Hoa *et al*. (2015) found that low EC promotes fast mycelia colonization. High EC levels, on the other hand, enhance mushroom fruiting body weight (Hoa *et al*., 2015)., Prasad *et al*. (2021) agrees with Hoa *et al*. (2015) who found higher yield on the substrates used to cultivate *A. bisporus* than *P. ostreatus* due to high amount of EC. According to Hoa *et al*. (2015), increasing the C/N ratio and pH value on substrates increases the EC of the substrates. Depending on the amount of EC accessible within a substrate, different lignocellulosic materials affect the generation of *Pleurotus* species in different ways.

## 2.8 THE EFFECTS OF SUBSTRATES ON THE METABOLIZABLE ENERGY (ME), (KCAL/G) OF OYSTER MUSHROOMS

According to Hoa *et al*. (2015), the substrate has an impact on the protein, carbohydrate, fat, and total energy of oyster mushrooms (*Pleurotus ostreatus*). Elattar *et al*. (2020) employed various lignocellulose sources when cultivating oyster mushrooms and reported ME ranging from 296.23 to 327.69 Kcal/100g. This revealed that substrates were influenced differently depending on the species. Elattar *et al*. (2020) concurs with Igile *et al*. (2020), who found that *P. ostreatus* cultivated on Rubber Wood Sawdust had the greatest ME (316.01 ± 4.75). As a result, testing the ME of substrates is critical for identifying substrates that can stimulate oyster mushroom formation.

## 2.9 THE INFLUENCES OF PESTS AND DISEASES ON THE YIELD OF OYSTER MUSHROOMS

### 2.9.1 The influences of insect pests on the yield of *Pleurotus* species

Mites and flies such as scarid and phorid flies are the most common insects that attack oyster mushrooms (Bellettini *et al*., 2018). It has been reported that these insects become pests when substrates are not properly pre-treated according to the tightest quality control requirements (Mignucci *et al*., 2000). Mites feed on mycelia and fruiting bodies cause yield loss and lower the value of fruiting bodies, according to reports (Bellettini *et al*., 2017). Bellettini *et al*. (2018) also observed that *Tarsonemus* spp. and *Histiostoma* spp. are harmful mites that can cause significant losses if not controlled. Minor pests such as beetles, molluscs, rats, and termites, as well as termites, inflict damage to the fruiting bodies (Bellettini *et al*., 2017). According to Bellettini *et al*. (2017), houses with inadequate hygienic conditions of oyster mushroom production might provide a welcoming setting for a variety of pests. Furthermore, the existence of insect pests could potentially reduce oyster mushroom production.

### 2.9.2 The effect of diseases on the yield of *Pleurotus* species

*Pleurotus* mushrooms are susceptible to diseases such as green mould (*Trichoderma* spp.), brown blotch (*Pseudomonas* spp.), and cobweb (*Cladobotryum* spp.) that reduce production and result in large yield losses (Jongman *et al*., 2018). *Pseudomonas tolaasii* produces yellowing (bacterial illness) of fruiting bodies in *P. ostreatus*, resulting in a yellowish colour and sticky fruiting bodies (Sante, 2011). According to Mignucci *et al*. (2000), bacterial illness causes 10 to 30% loss whereas *Trichoderma harzianum* causes a 30 to 50% loss, reducing the yield of *Pleurotus* spp. Green mould outbreaks have also been documented in commercial oyster mushroom production (Bellettini *et al*., 2017). According to a study conducted by Allaga *et al*. (2021), a wide range of other species belonging to the *Trichoderma harzianum* species complex can likewise cause severe symptoms on other edible mushrooms. According to Jongman *et al*. (2018), a popular approach for controlling diseases on mushroom cultivation around the world is to use pesticides like prochloraz, which are effective. Diseases that affect oyster mushrooms compete with mycelia for space and nutrition making it difficult for mycelia to colonize.

## 2.10 THE EFFECTS OF DIFFERENT TYPES OF GRAIN SPAWN ON THE OYSTER MUSHROOMS PRODUCTION

Inoculating sterilised grains or organic materials with the desired strain using a pure culture of the fungus cultivated on an agar media produces oyster mushroom spawn or inoculum. Millet, rye, sorghum, barley, wheat, and rice are the most commonly used cereal grains for spawn production (Maurya *et al*., 2019). Furthermore, because of its high performance, sorghum spawn is the best grain spawn (Jongman *et al*., 2013). According to Jongman *et al*. (2013) found that mycelium development on sorghum was superior when compared to other grains from a hybrid *P. ostreatus* x *P. florida*. This suggests that mycelium colonization of sorghum takes a short time, which can lead to early flushing. Maurya *et al*. (2019) found pearl millet to be the best when compared to other grain spawning, which opposes other scientists. According to a study conducted by Sofi *et al*. (2014), millet is an ideal grain for spawn production since it takes less time to colonize than other grains. This is a sign of healthy mycelial growth. When compared to other grain spawns, grain spawn (sugarcane bagasse) colonized quickly with mycelium (Besufekad *et al*., 2020). Sugarcane bagasse (grain) could be an excellent source of carbon and energy for mycelia colonization. Despite the fact that sorghum grain has been suggested the most, various studies have revealed that pearl millet is becoming a vital.

## 2.11 THE EFFECTS OF SUBSTRATE PRE-TREATMENTS ON THE PRODUCTION OF OYSTER MUSHROOMS

One of the elements that influences oyster mushroom productivity is substrate pre-heat treatment. The use of an effective method in the cultivation of *Pleurotus* spp. could be beneficial. According to Gowda & Manvi (2020), substrate's pasteurization (steam or hot water) is recommended for small-scale mushroom producers since the substrates are more stable and less susceptible to contamination. Oseni *et al*. (2012) found that compost horse manure pasteurized at 60 $^{o}$C for 2 and 3 hours had greater contaminations when compared to the sterilised substrates, and as a result, no fruits developed. This could be due to the substrate's pre-treatment procedure being ineffective. As a result, it promotes the growth of competitors and causes infections. According to Kumari & Kudada *et al*. (2018), untreated substrates had the shortest stipe length, stipe diameter, pileus diameter, and fresh weight when compared to treated

substrates. Contaminants such as green moulds that release poisonous compounds or metabolites that impede mycelium growth could be to blame for poor performance on untreated substrates (Hemalatha, 2016). In comparison to chemically treated substrates, steam treatment resulted in a shorter incubation time and a higher yield in terms of BE (Hemalatha, 2016). According to Gowda and Manvi (2020), the type of chemical used for substrate pre-treatment can result in the development of mould resistance and chronic diseases. As a result, choosing a substrate pre-treatment approach should be taken into account regardless of its effectiveness, but also the consequences on humans and the environment.

## 2.12 THE EFFECT OF SUBSTRATE ON THE PRODUCTION OF OYSTER MUSHROOMS

### 2.12.1 The effects of type of substrates on the production of oyster mushrooms

Varied lignocellulose materials may have different effects on *Pleurotus* spp. development and yield. When compared to other substrates, cotton waste had superior growth performance in terms of cap diameter, fresh weight, and biological efficiency (Muswati *et al*., 2021). The substrate (a mixture of 100g rice straw, 100g rice bran, 50g chalk, and 750g sawdust) needed less time to colonize and reach harvesting (Rambey *et al*., 2019). This could imply that combining valuable substrates is also a good way to cut down on production time. Garuba *et al*. (2017) found that *P. ostreatus* and *P. pulmonarius* cultivated on banana leaves grew faster in terms of colonization and days to pinhead initiation. According to this study, *Pleurotus ostreatus* and *P. pulmonarius* are excellent white-rot fungi that may use any waste materials containing hemicellulose and lignin to recycle nutrients. The output of oyster mushrooms is largely dependent on the nutrition and composition of the substrate (Hemalatha, 2016). Senghie *et al*. (2021) discovered that harvesting 100 sawdust took the least amount of time when compared to other substrates. As a result, this substrate may result in a quick cropping phase. however, this is dependent on the type of agro-waste materials used. *Pleurotus* spp. growth and yield can be boosted by the availability of nutrients, and their life cycle can be theoretically shortened.

### 2.12.2 The effects of supplements on the production of oyster mushrooms

Some substrates when used alone may not be successful in promoting oyster mushroom development and yield unless they are supplemented. According to Singh & Singh (2021), the dietary value of mushrooms as an outcome strongly dependent on the chemical compositions of the substrate which is a mixture of straw or hay, corn cobs, water, cottonseed meal and nitrogen supplement. According to Adenipekun & Omolaso (2015), rice straw supplemented with 30% rice bran gave the highest yield, followed by un-supplemented substrate whereas banana leaves supplemented with 10% and 20% wheat bran gave the highest yield and BE, respectively. These differences could be attributable to the chemical composition of the substrate and the type of supplements used. Supplementation typically increases yield; however, if supplementation hits a peak, yield may be reduced. Pal *et al*. (2017) found that wheat straw combined with cottonseed meal produced higher yield and BE. This could indicate that a nutrient supplement based on cottonseed meal has the potential to obstruct nutrient access. As a result, competitors are kept at bay allowing mycelium to gradually get access to nutritional material as it gains dominance on the substrates (Carrasco *et al*., 2018). Several researchers discovered that when 30% of the supplement was added, the best yield was obtained; however, this did not apply to all substrates (e.g Adenipekun & Omolaso, 2015; Biswas *et al*., 2016).

### 2.12.3 The effects of substrates on the nutritional compositions of oyster mushrooms

One of the most important elements in the number of nutrients that contribute to the development of *Pleurotus* species is the substrate. According to Hoa *et al*. (2015), oyster mushrooms require carbon, inorganic, and nitrogen molecules as sources of nutrients. Oyster mushrooms grown on *Astragalus membranaceus* var. *mongolicus* performed best in terms of protein and fresh weight (Zeng *et al*., 2022). Beans are a legume crop that can fix nitrogen from the atmosphere in the soil. As a result, the nitrogen concentration of bean straw is important for mushroom growth, and *P. ostreatus* produced the highest protein content when cultivated on bean straw rather than wheat straw (Michael *et al*., 2011). The compositions of the substrates may be to blame for this variation. Dinssa *et al*. (2020) discovered that *P. ostreatus* grown on 100 percent sugarcane bagasse had the maximum nutrient content, such as protein, while *P. ostreatus* cultivated on 75 percent wastepaper + 25 percent leaves of

*Prosopis Julia flora* had the lowest fat content. Garuba *et al*. (2017) found high carbohydrate, followed by protein, moisture content, ash, fibre, and least fat on varied substrates such as banana leaves, cassava peels, and sawdust. As a result, fruiting bodies made from these agricultural resources make great low-calorie snacks. *Pleurotus* species are often low in calories due to their low-fat content (Sifat *et al*., 2020). *P. ostreatus* is affected not only by crude protein, but also by moisture content, fiber, starch, and fat, as well as ash concentration from diverse substrates (Garuba *et al*., 2017). Cassava and altered sawdust from *P. pulmonarius* and *P. ostreatus* had higher levels of important elements like potassium (Garuba *et al*., 2017). Several research employing diverse organic materials have revealed variations in performance based on the substrates and species used.

## 2.13 THE SUBSTRATE'S OVERVIEW

Oyster mushrooms can be grown using a variety of lignocellulosic materials such as corn stalks, wheat, rice and rye straws as well as a variety of lignocellulosic substances such as compost, plant coverings, papers and pulp wastes (Operamolla, 2019; Kacprzak *et al*., 2021). Microorganisms such as moulds, fungi (oyster mushrooms) and bacteria can breakdown cellulose using extracellular enzymes such as cellulases. These findings show that lignocellulosic decomposition is dependent not only on environmental factors, but also on the degradative activity of microbes like *Pleurotus* spp. Enzymes including manganese peroxidases, laccases, lignin peroxidases, manganese peroxidase, and versatile peroxidase have been studied extensively, notably in white-rot fungi like *P. ostreatus* and *P. chrysosporium* (Wang *et al*., 2018; Kumar & Chandra, 2020). These enzymes' activities serve to speed up the breakdown and detoxification of lignocellulosic material in the environment. Due to the accessible enzymes that oyster mushrooms can produce for degradation; they can directly break down cellulose and lignin containing compounds without chemical or biological preparation such as fermentation. It was discovered that the activity of lignocellulolytic enzymes is controlled by substrate composition and colonization time (Luz *et al*., 2012).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 STUDY LOCATIONS

This research project consisted of 3 various studies, the determination of chemical contents in investigations 1 and 2 was carried out in the laboratory at the University of Venda, Thohoyandou. Furthermore, the proximate tests in study 3 were carried out in the laboratories of the University of Venda. The mineral determination in Study 3 was done by Soil Fertility and Analytical Services laboratory at Cedara (Pietermaritzburg).

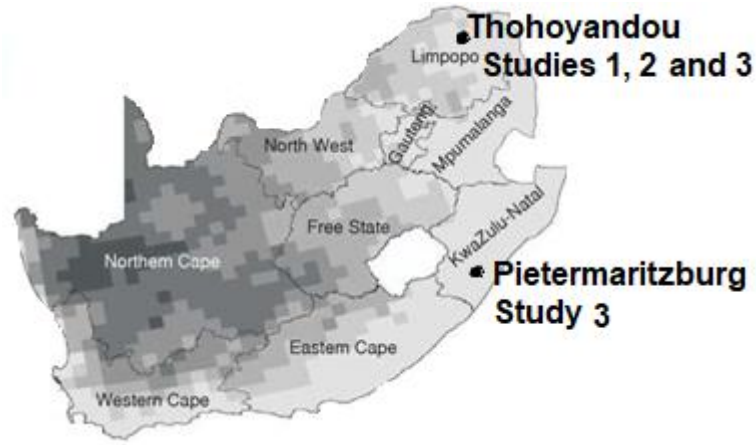


Figure 2. Location map (Source of the map: Adeala *et al*., 2015)

## 3.2 STUDY 1: CHEMICAL CONSTITUENTS OF THE EXPERIMENTAL SUBSTRATES

### 3.2.1 Experimental site

The study was carried in the postgraduate laboratory at the University of Venda, which is situated 22º58' S; 30º26.4' E at 595 m above sea level.

### 3.2.2 Experimental design

The experiment was laid in a Completely Randomized Design (CRD) with 4 treatments replicated 3 times. Treatments used were Maize stalk-control (MS), banana pseudo-stem (BP) and Macadamia nutshells (MnS) as well as Macadamia husks (MH), each substrate bag duplicated per replication.

### 3.2.3 Analysis of chemical constituents of various experimental substrates

The substrate samples were dried into constant temperature of 40°C using oven before it was ground into powder. Nelson and Sommers (1982)'s approach was used to calculate total carbon (C). Total nitrogen (N) was evaluated using dry substrates (2 g) powder following 98.6% $H_2SO_4$ hot digestion using the Kjehldal process (Ezeibekwe *et al*., 2009). The C/N ratio of each substrate was calculated. To determine pH and EC contents, a pH and EC meters were utilized (Cavins *et al*., 2000). For the sample to be saturated, 20 grams of substrate powder were combined with 200 mL water in a beaker (ratio 1:10). All substrates were subjected to the same procedure. After that, the solution was shaken for at least 15 minutes and then for 60 minutes. After that, the solution was filtered before the measurements were taken.

### 3.2.4 Data collection

i) Total carbon, C (%) was calculated as follows:

Total carbon (%) = [(weight of substrate powder + crucible) – (weight of ash + crucible)/ weight of substrate powder] x 100

ii) Total nitrogen, N (mg/kg) was calculated using the formula below:

% Nitrogen of the sample = (T × M × 0.014 × D.F × 100)/G

Where T is the Titre value, M is the Molarity of standard HCl, D.F is the Dilution factor, 100 = conversion to %, 0.014 = is a constant which means that 0.014 is liberated by 1 ml of 0.01 HCl, and G = Weight of a sample used.

iii) Carbon nitrogen (C/N) ratio (1:10) was determined as follows:

Carbon/Nitrogen ratio = Carbon/Nitrogen x 100

iv) Electrolyte conductivity (EC) value (mS/cm) was recorded by reading a value from EC meter.

v) The pH value.

### 3.2.5 Data analysis

STATISTICA version 8 was used to do an analysis of variance (ANOVA) on all of the data. On pH, EC, C, N, and C/N ratio, the mean differences between the substrates were further separated using Least Significant Difference (LSD) tests at a probability level of 0.05.

## 3.3 STUDY 2: THE CULTIVATION OF *Pleurotus ostreatus* AND *Pleurotus pulmonarius* ON VARIOUS SUBSTRATES

### 3.3.1 Experimental site

The investigation was conducted in the laboratory at the University of Venda, which is located at 595 meters above sea level at 22°58' S; 30°26.4' E. The experiments were conducted during the period from March to May 2019 and repeated from June to August 2019.

### 3.3.2 Experimental design

The experiment was set up as a 2 x 4 Factorial Completely Randomized Design replicated three times, substrate bag duplicated per replication. Two species (*P. ostreatus* and *P. pulmonarius*) and four substrates were factors (MS as a control, MH, MnS and BP).

### 3.3.3 The preparation of planting materials

A working environment was disinfected with chlorine bleach diluted water to generate 10% bleach (a solution of 5% sodium hypochlorite) or 70% ethanol before the cultures were grown. Afterwards, 1000 ml glass bottles were filled with distilled water and fifty grams of MEA (malt extract agar) which was autoclaved for 20 minutes at 121°C. Its slants were placed at 4 degrees Celsius. Then, each pure culture of *Pleurotus* spp. was isolated into Petri dishes filled with MEA. The Petri dishes were labelled and wrapped in parafilm. The Petri dishes were placed upside down in a sterile container and placed in an incubator at a temperature of 25 ± 2 °C for seventeen days until mycelium had colonized the agar.

### 3.3.4 The preparation of grain spawn

The inoculum was made using a modified process (Fritsche, 1978). Sorghum grain (1.8 kg) was soaked in water all night (1.5 litres). The excess water was then allowed to drain from the sorghum grain. Approximately 900 grams of sorghum grain were mixed with 12 grams of gypsum ($CaSO_4$) and 3 grams of calcium carbonate ($CaCO_3$) were added into 900 g sorghum grain after soaking. Afterwards, 1000 ml bottles were filled halfway and sterilised for 15 minutes at 121˚C in an autoclave (Labtech, LAC5060S). The sterilised grain was cooled in laminar flow hood to room temperature. After cooling, three mycelium discs from each *Pleurotus* species were inoculated in a sterile grain bottle. The bottles were kept in the incubator for three weeks at temperature of 25 ± 2 ˚C until mycelia had colonized the grain completely.

### 3.3.5 The preparations of substrates and spawning

The banana BP came from the University of Venda's experimental farm, and the substrate MS came from Ngovhela in Thohoyandou. Macadamia husks were collected from a Barotha farm in Levubu, while MnS was obtained from a Royal macadamia plantation. Substrates such as MS and BP substrates were chopped into lengths ranging from 3 - 5 cm using a blade. Five hundred grams of substrates were packed into each 55 cm long white plastic tube (400 mm Flat). The bags had 20 holes punched in them and were attached at the bottom with a cable tire and top with a rubber bands. The substrates were pre-treated with calcium hydroxide (Hernandez and Sánchez, 2013). Total of 150 g Ca $(OH)_2$ and 50 g of $CaCO_3$ were poured into water to produce a milky white solution. For 16 hours, the bags filled with substrate were submerged in solution. Following that, the run-off water was drained for two to three hours. According to Islam *et al*. (2009), the moisture level of a handful mixture was evaluated by running it through a hand, if the material stayed in shape with no surplus water it meant the moisture content was roughly 65 percent. Sorghum spawn was inoculated into each bag (at 10 percent rate). Afterwards, the bags were zipped and placed on top of the benches. The bags were covered with black plastic sheet to encourage mycelia to colonize surfaces (15 microns). The black plastic sheet was removed after colonization to aid in the production of fruiting bodies.

### 3.3.6 Data collection

Data collected based on the following:

i. The number of contaminated bags for substrates (NBC).
ii. The number of days for substrates from inoculation to colonization (DC).
iii. Number of days from colonization to Primordia initiation (DP).
iv. The number of days from initiation of fruiting bodies to flushing (DF).
v. Mushroom pileus/cap diameter (PD) and thickness (PT) of the fruiting body was measured using Vernier calliper in mm.
vi. Mushroom stipe length (SL) and diameter (SD) were measured using the Vernier calliper. Measurement starts from the underneath of the cap to the basal portion of the stem before the root in cm and mm, respectively.
vii. The number of fruiting per flush (FB) from each replication was counted.
viii. The total number of flushes (F) from the harvesting of the first flush up to the last flush was also recorded.
ix. Time taken between flushes (PBF) was calculated by using the following formula:

The period between flushes = Days taken from the first harvest to last/ Total number of flushes (harvesting).

x. Fresh weight (FW) was measured by weighing mushroom fruits. Fruiting bodies were harvested when pileus was completely matured and earlier the curl started to twist. The fresh oyster mushroom was measured utilising a weighing balance (g).

xi. Biological efficiency biological efficiencies (BE in %) of substrates was calculated as follows:

Biological efficiency (%) = total fresh weight (g) of mushroom yield across all flushes / the total weight of the dry substrate X 100 (Royse *et al*., 2004)

### 3.3.7 Data analysis

Data collected was subjected to analysis of variance (ANOVA) using STATISTICA version 8. At a probability level of 0.05, the Least Significant Difference (LSD) was

utilised to assess the means of significant variations across the substrates, species, and interactions between the species and substrates.

## 3.4 STUDY 3: DETERMINATION OF NUTRITIONAL COMPOSITION OF OYSTER MUSHROOMS

### 3.4.1 Study site

Moisture content (M) and ash content (AC) were determined in the postgraduate laboratory. Crude fiber (CF), crude protein (CP), total carbohydrate estimation (C), and metabolizable energy content (ME) Fat was determined in the food science laboratory whereas fat content was done in animal science laboratory at University of Venda. Mineral analyses (Ca, Mg, Fe, Zn, Cu, and K) were carried out in the Soil Fertility and Analytical Services Laboratory at Cedara (Pietermaritzburg).

### 3.4.2 The experimental design

The experiment was laid in as 2 x 4 Factorial in Completely Randomized (CRD) design replicated thrice. Each sample of oyster mushrooms harvested from MS, BP and MH were duplicated per replication.

### 3.4.3 Determination of moisture content

A moisture analyser was set at a temperature of 105 $^{o}$C. afterwards, about 4 g of fresh mushroom was weighed and analysed. After detection of moisture percentage, the moisture analyser stops, and the values were recorded.

### 3.4.4 Determination of crude protein

The standard Micro-Kjeldahl method was utilised to determine the crude protein content of the oyster mushroom (AOAC, 2011). Each substrate received two grams of oven dried oyster mushrooms which were weighed and placed in a Kjeldahl digestion flask (Model FK500/31, Barloworld UK) with 25 millilitres of pure $H_2SO_4$. Two potassium sulphate catalyst pills were added. The mixture was gently cooked in a gas cupboard on a digesting rack until it became a bluish-green transparent emulsion. The digest was chilled and solidified for 24 hours, till it became white in colour. Sixty

millilitres (60 ml) of deionised water were added to the hardened sample to prevent caking. The distilled water was added into a digest multiple washing. A ten millilitres aliquot of the digest was collected and deposited in the Micro-Kjeldahl distillation section's Erlenmeyer's flask. A 100 mL receiver flask containing 3 drops of methyl red indicator solution was placed beneath the distillation machine's condenser with a 2 cm tip remaining inward the indicator. In a distillation unit, the digested sample was distilled. The 0.01 M standard HCl was titrated into the accumulated distillate in the receiver flask until the colour changed to pink.

**Calculation**

% Nitrogen of the sample = (T × M × 0.014 × D.F × 100)/G

Where, T = Titre value, M = Molarity of standard HCl, D.F. = Dilution factor, 100 = conversion to %, 0.014 = a constant which means that 0.014 is liberated by 1 ml. of 0.01 HCl and G = Weight of a sample used

Therefore, Percentage Protein = % Nitrogen × 6.25

Where 6.25 = is a protein constant according to Kjeldahl's method.

### 3.4.5 Determination of fat

Using the Soxhlet extraction device, the amount of fat was determined (AOAC, 2007). The mushroom sample was measured and placed in the thimble at three grams. The Soxhlet beakers were washed with water and heated in the oven for ten minutes at 100 ˚C to eliminate superfluous water. The initial weight of the beaker was recorded after cooling. Three grams of dried fungus were placed in a beaker and thimble. The Soxhlet beaker was then filled with petroleum ether, and the thimble was placed inside the Soxhlet machine for three hours and thirty minutes to complete the fat extraction procedure. The Soxhlet beakers containing fat were separated and weighed after the extraction procedure was completed to record the amount of fat (AOAC, 2007).

Fat percentage = (Weight of a beaker and oil) -Weight of blank beaker/weight of mushroom used x 100

### 3.4.6 Determination of ash content (AC)

One gram of pulverized oyster mushroom was put in a crucible and heated for 360 minutes at 550 °C in a muffle furnace. A sample was heated and then cooled in a desiccator before being weighed again. After that, the crucible was put in a muffle furnace and heated for an hour before cooling and recording the mass in grams. This method was continued until the ash had changed colour to greyish white or white, and the weights were the same. The following are the ash content calculations:

AC (g/l00g dry matter) = ash weight x dried mushroom percentage /weight of a dried mushroom taken.

### 3.4.7 Crude fibre

In a beaker, 2 g of dried oyster mushroom was weighed. A total of 200 millilitres of boiling Sulphuric acid (0.255 N) was supplemented. As a result, the solution was allowed to boil for half an hour while the volume was maintained by adding distilled water at regular times. The solution was allowed to pass through a filter using a muslin cloth. Afterwards, the remainder was cleansed using hot water until no more acid was present. The item was then taken out and placed back into the same beaker. Afterwards, 200 millilitres of boiling sodium hydroxide (0.313 N) was supplemented. Afterwards, the solution was boiled for half an hour while retaining the same volume. The solution was then allowed to flow across a muslin cloth filter and the remainder was thoroughly washed with hot water until no alkali remained. Following that, the remainder was cleaned with ether and alcohol. Thereafter, the residual was placed in the crucible to dry for an entire night at a temperature of a hundred degrees Fahrenheit. The sample mass was measured (We) using electric balance after drying (KEY: JY-2003; China). After that, the crucible was placed inside a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) and heated for six hours at 600 degrees Celsius. After heating, the sample was allowed to cool before being weighed once more (Wa). The mass in grams of crude fibre was expressed in the difference in grams (We-Wa).

% Crude fibre = {100- (moisture + fat)} x We -Wa/weight of oyster mushroom.

### 3.4.8 Total carbohydrate estimations

Available carbohydrate estimates in the sample were estimated using the method described by Ashraf *et al*. (2013). This was calculated as the difference obtained after subtracting the crude protein, fat, ash, and crude fibre values from the total dry matter using the formula below:

% Carbohydrate = 100 – (amount of crude protein + Fat + amount of ash content + amount of crude fibre)

### 3.4.9 Determination of metabolizable energy content (kcal/g)

Crude protein, fat and carbohydrate estimates give metabolizable energy. Metabolizable energy was determined utilising beneath method:

Metabolic Energy, ME (Kcal /100 g) = [(3.5 X % Crude Protein) + (8.5 X % Crude Fat) + (3.5 X carbohydrate)]

### 3.4.10 Determination of minerals

Twenty-four samples of oyster mushrooms were dried by a Moisture Analyser (105$^{o}$C) and delivered to soil fertility and analytical services laboratory at Cedara (Pietermaritzburg) for mineral analysis. Each tray held 11 PVC cups with a capacity of 70 ml, which were excavated from the samples. As a result, a tray was utilized to hold 9 unknown samples, one of which served as a quality control sample and the other as a blank. Batches of three trays containing 24 samples, 3 unknowns, and 3 blanks were used for operations like dispensing and mixing, as well as quality control. To distribute aliquots of reagent or extractant to three samples at a time, several dispensers and diluters were used. Total of 5 g subsamples were ashed at 450 °C for 24 hours and then dissolved in 25 ml M HCl. Elements such as Ca, Fe, Zn, Mg, Cu, and K were determined utilising inductively coupled plasma optical emission spectroscopy after being diluted four times with deionized water. By atomic absorption, the amount of calcium (Ca), magnesium (mg), iron (Fe), copper (Cu), zinc (Zn), and potassium (K) in the solution was determined.

### 3.4.11 Data collection

Data were collected as follows:

i. A final percentage value of Moisture content (%) was detected at the Moisture Analyser and thereafter recorded.
ii. Crude protein (%) was calculated by first calculating the value of Nitrogen and then multiplying a value by 6.25.
iii. Fat (%) was calculated using a formula:
%Fat = weight of a beaker - the weight of an empty beaker divided by the weight of a sample used and then multiply by 100.
iv. Ash content (%) was calculated according to the formula below:
Ash content (g/l00g sample) = weight of ash x percentage of dried sample/weight of a dried sample taken.
v. Crude fibre (%) was calculated as follows:
Crude fibre (g/l00g sample) = [100- (moisture + fat)] x weight of sample + crucible - weight of ash + crucible / weight of sample.
vi. Carbohydrate estimates (C) was estimated using the formula below:
% Carbohydrate = 100 – (amount of crude protein + amount of fat + amount of ash content + amount of crude fibre).
vii. Metabolizable energy content (kcal/g) was calculated as follows:
ME (Kcal /100g) = [(3.5 X crude protein) + (8.5 X fat) + (3.5 X carbohydrate)].
viii. Minerals such as calcium (Ca), magnesium (Mg), Iron (Fe), zinc (Zn) and copper (Cu) and potassium (K) were recorded.

### 3.4.12 Data analysis

Data collected were subjected to statistically analysis (ANOVA) using STATISTICA version 8. Variations amongst the treatments, species and the interactions between substrates and species were further separated utilising Fisher's Least Significant Difference test at a probability level of 0.05.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 STUDY 1: CHEMICAL CONSTITUENT OF THE SUBSTRATES

There are significant differences among the four substrates in terms of all the chemical constituents investigated.

**Table 1. Results of analysis of variance for the chemical constituents of substrates**

| | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **pH** | | | | | |
| *Substrates* | 3 | 1.0711 | 59.48 | 0.0000 | Sig |
| *Error* | 8 | 0.0180 | | | |
| **EC** | | | | | |
| *Substrates* | 3 | 1659149 | 49.8014 | 0.0000 | Sig |
| *Error* | 8 | 33315 | | | |
| **C** | | | | | |
| *Substrates* | 3 | 39.86 | 459.9 | 0.0000 | Sig |
| *Error* | 8 | 0.09 | | | |
| **N** | | | | | |
| *Substrates* | 3 | 0.3001 | 5.6618 | 0.0223 | Sig |
| *Error* | 8 | 0.0530 | | | |
| **C/N** | | | | | |
| *Substrates* | 3 | 956.67 | 8.0666 | 0.0084 | Sig |
| *Error* | 8 | 118.60 | | | |

Sig = significant difference, No-Sig = No-significant difference

The pH of substrates varied significantly from 5.46 to 6.84, according to the data in Table 2. The maximum pH was on MS. Despite this, the greatest EC was found on MH at 1454.33 mS/cm while the lowest EC was found on MS at 3.76 mS/cm. MnS (89.47 %) had the highest total C and the least was on BP (80.80 %). The total nitrogen content of the substrates varied greatly, ranging from 0.96 to 1.67%. The C/N ratio varied greatly amongst substrates ranging from 51.13 to 87.24. It was discovered that the agrowaste with a greater C/N ratio had a minimum EC, and vice versa. A greater

C/N ratio favoured the expansion of mycelium while a minimum C/N ratio favoured mushroom development (Yang (2000). However, mycelium colonization is hampered by high nitrogen levels. As a result, MS was an excellent substrate for mycelium colonization. Maize stalks, On the other hand, was the most effective at promoting fruiting body growth. The pH, EC, total N, total C and C/N ratio of the substrates are important in oyster mushroom cultivation because they affect colonization, fruit formation, flushing days, pileus and stipe diameter, and pileus thickness (Kalmis *et al*., 2008; Hoa *et al*., 2015 & Urben, 2004).

**Table 2. Comparison of the different substrates under different chemical constituents**

| Substrate | Chemical constituents | | | | |
|---|---|---|---|---|---|
| | pH | EC mS/cm | C % | N % | C/N (1:10 ratio) |
| MS | 6.84d | 3.76a | 83.47b | 0.9600a | 87.24b |
| BP | 6.26c | 1207.33b | 80.80a | 1.5167bc | 53.53a |
| MnS | 5.46a | 109.97a | 89.47c | 1.2067ab | 77.70b |
| MH | 5.82b | 1454.33b | 83.80a | 1.6667c | 51.13a |

Means in the same column followed by the same letters are not significantly different at $p \leq 0.05$. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nutshells, MH = macadamia husks.

## 4.2 STUDY 2: THE EFFECT OF SUBSTRATES ON GROWTH AND YIELD OF OYSTER MUSHROOMS

There are significant differences in the number of days to colonization and days to flushing among substrates. However, there were no significant differences in the main effect of species and the interaction effects of species and substrates. The substrates, species, and substrates*species interactions all had significant effects on DP and IF.

**Table 3. Results of Analysis of variance for the growth and yield of oyster mushrooms during the period from March to May 2019**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of days to colonization (DC)** | | | | | |
| *Substrates* | 3 | 185.153 | 277.729 | 0.0000 | Sig |
| *Species* | 1 | 0.042 | 0.062 | 0.8058 | No-Sig |
| *Substrates*Species* | 3 | 0.264 | 0.396 | 0.7578 | No-Sig |
| *Error* | 16 | 0.667 | | | |
| **Number of days to primordia initiation (DP)** | | | | | |
| *Substrates* | 3 | 2217.15 | 400.088 | 0.0000 | Sig |
| *Species* | 1 | 315.38 | 56.910 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 128.82 | 23.246 | 0.0000 | Sig |
| *Error* | 16 | 5.54 | | | |
| **Number of days to fruiting (IF)** | | | | | |
| *Substrates* | 3 | 108.7083 | 163.062 | 0.0000 | Sig |
| *Species* | 1 | 35.0417 | 52.562 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 15.2639 | 22.896 | 0.0000 | Sig |
| *Error* | 16 | 0.6667 | | | |
| **Number of days to flushing (DF)** | | | | | |
| *Substrates* | 3 | 46.7083 | 50.9545 | 0.0000 | Sig |
| *Species* | 1 | 3.3750 | 3.6818 | 0.0730 | No-Sig |
| *Substrates*Species* | 3 | 2.1528 | 2.3485 | 0.1110 | No-Sig |
| *Error* | 16 | 0.9167 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 4 shows that the substrates, species, and interactions between substrates and species all had significant differences on FB. On SL, SD, and PT, there were significant differences between substrates; however, there were no significant variations between species and species*substrates relationships. Substrates, and substrates*species' interactions had effect on the attribute PD.

**Table 4. Results of analysis of variance for the growth and yield of Oyster mushrooms during the period from March to May 2019 cont'd**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of fruiting bodies (FB)** | | | | | |
| *Substrates* | 3 | 86.7778 | 17.5014 | 0.0000 | Sig |
| *Species* | 1 | 88.1667 | 17.7815 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 18.7222 | 3.7759 | 0.0000 | Sig |
| *Error* | 16 | 4.9583 | | | |
| **Stipe length (SL)** | | | | | |
| *Substrates* | 3 | 13.8333 | 15.0909 | 0.0000 | Sig |
| *Species* | 1 | 1.5000 | 1.6364 | 0.2191 | No-Sig |
| *Substrates*Species* | 3 | 2.2778 | 2.4848 | 0.0978 | No-Sig |
| *Error* | 16 | 0.9167 | | | |
| **Stipe diameter (SD)** | | | | | |
| *Substrates* | 3 | 447.246 | 50.1802 | 0.0000 | Sig |
| *Species* | 1 | 10.814 | 1.2133 | 0.2870 | No-Sig |
| *Substrates*Species* | 3 | 12.423 | 1.3938 | 0.2809 | No-Sig |
| *Error* | 16 | 8.913 | | | |
| **Pileus diameter (PD)** | | | | | |
| *Substrates* | 3 | 4904.59 | 118.849 | 0.0000 | Sig |
| *Species* | 1 | 17.51 | 0.424 | 0.5240 | No-Sig |
| *Substrates*Species* | 3 | 169.22 | 4.101 | 0.0245 | Sig |
| *Error* | 16 | 41.27 | | | |
| **Pileus thickness (PT)** | | | | | |
| *Substrates* | 3 | 2219.91 | 125.482 | 0.0000 | Sig |
| *Species* | 1 | 5.42 | 0.306 | 0.5877 | No-Sig |
| *Substrates*Species* | 3 | 5.76 | 0.325 | 0.8071 | No-Sig |
| *Error* | 16 | 17.69 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 5 revealed that there were significant changes on F between substrates only. Furthermore, on PBF there were differences across substrates and substrates*species interactions, but no significant changes between species. There were significant changes in parameter FW and BE between substrates, species, and the relationships between substrates and species.

**Table 5. Results of analysis of variance for the growth and yield of oyster mushrooms during the period from March to May 2019 cont'd.**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of flushes (F)** | | | | | |
| *Substrates* | 3 | 7.3750 | 44.2500 | 0.0000 | Sig |
| *Species* | 1 | 0.3750 | 2.2500 | 0.1531 | No-Sig |
| *Substrates*Species* | 3 | 0.1528 | 0.9167 | 0.4551 | No-Sig |
| *Error* | 16 | 0.1667 | | | |
| **The period between flushing (PBF)** | | | | | |
| *Substrates* | 3 | 38.6667 | 23.2000 | 0.0000 | Sig |
| *Species* | 1 | 0.6667 | 0.4000 | 0.5360 | No-Sig |
| *Substrates*Species* | 3 | 7.3333 | 4.4000 | 0.0194 | Sig |
| *Error* | 16 | 1.6667 | | | |
| **Fresh weight (FW)** | | | | | |
| *Substrates* | 3 | 6477.75 | 84.2265 | 0.0000 | Sig |
| *Species* | 1 | 5215.60 | 67.8155 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 748.86 | 9.7369 | 0.0007 | Sig |
| *Error* | 16 | 76.91 | | | |
| **Biological efficiency (BE)** | | | | | |
| *Substrates* | 3 | 259.110 | 84.2265 | 0.0000 | Sig |
| *Species* | 1 | 208.624 | 67.8155 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 29.954 | 9.7369 | 0.0000 | Sig |
| *Error* | 16 | 3.076 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 6 revealed that the number of days to colonization (DC) differed significantly across the substrates used on *Pleurotus* species cultivation. The mean values that were not significant among the substrates, species, or substrates*species interactions were denoted by a minus sign (-) in the table. On MS (10.33 days), colonization took few days to complete but due to green mould attack there were no DC on MnS (0.00). These findings are similar to those of Onyeka *et al*. (2018), who found that sawdust + banana leaves needed at least 120.10 days to totally invade the substrate. These findings are like those of Garuba *et al*. (2017), who found that mycelia colonization required 17.00 ± 0.00 and 15.33 ± 0.67 days on banana leaves used to produce *P. pulmonarius* and *P. ostreatus*, respectively. Senghie *et al*. (2021) reported maximum days of 28.78 ± 2.14 and minimum days of 24.44 ± 0.84 to complete colonization on 100 Sawdust and 100 Sago Bark, however the findings contradict current study. As a result, the type of substrates had the greatest impact on colonization.

The number of days to primordia initiation (DP) differed significantly between substrates, species, and substrate-species interactions. On the substrate, BP (42.83), and from the species, *P. pulmonarius*, PP, the DP was prolonged (31.83 days). The total DP found in the interactions between substrates and species ranged from 31.67 to 33.33 days for *P. ostreatus* (PO) and 33.00 to 52.33 days for *P. pulmonarius* (PP). In addition to BP*PP interactions, primordia initiation was delayed when *P. pulmonarius* is grown on BP than others. In contrast, *P. ostraetus* responded took few days to primordia initiation. Furthermore, MnS did not have any DP (0.00 days). *Pleurotus ostreatus* grown on BP and MH at 33.33 days while *P. pulmonarius* grown on BP (33.33) took long time to primordial initiation (52.33 days). .The results of this study correspond with those of Garuba *et al*. (2017), who found that *P. pulmonarius* and *P. ostreatus* grown on altered sawdust took 35.33 ± 0.33 and 33.33 ± 0.33 days to initiate primordial initiation, respectively. *P. pulmonarius* cultivated on BP had a shorter cropping cycle due to the number of days it took for primordial initiation. These findings contrast with those of Onyeka *et al*. (2018), who found that pinhead development took roughly 10 ± 0.82 days. These findings demonstrated that substrates were not the only factor influencing DP; species had an impact as well.

*Pleurotus* species on varied substrates showed considerable changes in the number of days to fruiting initiation (IF). Although there were no significant differences between MS, BP, and MH; MS had the lowest IF (8.16 days) and PO had the lowest IF among the species (5.17 days). A significant interaction of substrates and species were observed on IF. Few days of IF were seen on MS and MH at 5.67 and 8.33 days from PO and PP, respectively in the interaction of substrates and species. Long periods of fruiting could be due to unfavourable environmental circumstances or negative strain responses, increasing the likelihood of contamination on the substrates. These findings corroborate those of Onyeka *et al*. (2018), who found that the minimum days to fruiting were 60.00 days and the highest were 100.82 days for various substrates. However, the current findings contradict those of Garuba *et al*. (2017), who found the lowest IF of 25 ± 00 and 23 ± 33 days for PO and PP grown on banana leaves, respectively. It was discovered that the number of days until fruiting varies depending on the substrates and species used, as well as the substrates*species interactions.

Table 6 shows significant differences in the number of days to flushing (DF) among the substrates. Although there were no significant differences between MS, BP, and MH, BP and MH had greater DF at 5.83 and 5.33 days, respectively. On MnS, no DF could be obtained. These findings contradict those of Senghie *et al*. (2021), who reported that *Pleurotus Sajor-caju* cultivated on 100 sago-bark and 100 sawdust yielded a few days of initial harvest (flush) at 50.33 ± 3.35 and more days at 71.86 ± 5.17. However, the current findings are consistent with those of Onyeka *et al*. (2018), who found that Oyster mushrooms cultivated on sawdust + rice bran + $CaCO_3$ took the longest to harvest at 6 ± 0.20 days. As a result, the type of substrate remained the most important determinant of DF.

According to the findings, the type of substrate, species, and interactions between substrates and species all had a significant impact on the quantity of fruiting bodies (FB). The substrate (MH) had the highest FB at 8.50, while the species (PP) had the lowest at 6.92. PO and PP grown on MH at 5.00 and 12.00, respectively, produced the most fruiting bodies among the substrates and species interactions. These findings contradict those of Senghie *et al*. (2021), who found the fewest fruiting bodies (1.67 ± 0.12) on both 25 sawdust and 75 sago bark from *Pleurotus Sajor-caju*. The results, on the other hand, are quite similar to those of Onyeka *et al*. (2018), who obtained the lowest FB of 14 on Cassava peel exclusively for the cultivation of oyster mushrooms. As a result, differences in substrates and species had a big impact on FB.

The stipe length (SL) among the substrates used to cultivate *Pleurotus* species differed significantly (Table 6). On both BP and MH, the greatest SL was 3.17. Only the type of substrate has a significant impact on the SL. The current findings are consistent with those of Islam *et al*. (2017), who found a minimum SL of 4.5±1.3 cm on diverse substrates. These findings, on the other hand, contradict those of Besufekad *et al*. (2019), who measured a minimum SL of 5.1 ± 0.33 cm and maximum SL of 9.88 ± 4.27 cm from diverse substrates. Senghie *et al*. (2021) reported the lowest SL of 6.07 ± 0.32 on 25 sawdust 75 sago frond and the maximum SL of 6.87 ± 1.27 on 50 sawdust 50 sago bark in their study. The availability of carbohydrate and protein contents affects the growth of the stalk (Ajonina and Tata., 2012).

**Table 6. The effects of various substrates on the growth and yield of oyster mushrooms during the period from March to May 2019 cont'd**

| Variable/Effect | Mean values of the variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | NBC (bags) | DC (days) | DP (days) | IF (days) | DF (days) | FB | SL (cm) |
| **Substrates** | | | | | | | |
| *MS* | - | 10.33b | 36.83c | 8.16b | 4.83b | 7.33c | 2.67b |
| *BP* | - | 11.17bc | 42.83d | 8.67b | 5.83b | 4.17b | 3.17b |
| *MnS* | - | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a |
| *MH* | - | 11.67c | 33.17b | 8.67b | 5.83b | 8.50c | 3.17b |
| **Species** | | | | | | | |
| *PO* | - | - | 24.58a | 5.17a | - | 3.08a | - |
| *PP* | - | - | 31.83b | 7.58b | - | 6.92b | - |
| **Substrate*Species** | | | | | | | |
| *MS*PO* | - | - | 31.67b | 5.67b | - | 4.00b | - |
| *BP*PO* | - | - | 33.33b | 6.00b | - | 3.33bc | - |
| *MnS*PO* | - | - | 0.00a | 0.00a | - | 0.00a | - |
| *MH*PO* | - | - | 33.33b | 9.00c | - | 5.00b | - |
| *MS*PP* | - | - | 42.00c | 10.67c | - | 10.67c | - |
| *BP*PP* | - | - | 52.33d | 11.33c | - | 5.00b | - |
| *MnS*PP* | - | - | 0.00a | 0.00a | - | 0.00a | - |
| *MH*PP* | - | - | 33.00b | 8.33b | - | 12.00c | - |

Means with the same column followed by the same letters are not significantly different at $p \leq 0.05$. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nutshells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

On the stipe diameter (SD), there were significant variations between substrates. On MS, a higher SD of 19.42 mm was recorded (Table 7). Because of the *Trichoderma* species' attack, no SD was discovered in the MnS. This research agrees Varghese & Amritkumar's (2020) findings of a maximum stipe diameter of 2 cm. These findings contradict with those of Hoa *et al*. (2015), who found that *P. ostreatus* grown solely on sawdust had the lowest SD of 8.52 mm. The results, on the other hand, contradict those of Girmay *et al*. (2016), who found SD varying from 3.11 to 4.85 cm among the substrates. These findings revealed that the kind of substrate had the greatest impact on SD.

The pileus diameter (PD) obtained from PO and PP cultured on different substrates exhibited significant differences (Table7). MS (60.22 mm) has higher PD than the other

substrates. There is a significant species and substrates' interactions for the PD, thus there were differences in sizes of oyster mushrooms. The interactions between substrates and species resulted in the maximum PD on MH (59.00 mm) and MS (67.83 mm) from PO and PP, respectively. These findings demonstrated that the type of substrates and even relationships between substrates and species influenced the PD of *Pleurotus* species. Muswati *et al*. (2021) measured the greatest pileus diameter at 5.52 cm, which is similar to the current findings. The current findings contradict those of Senghie *et al*. (2021), who showed PD levels ranging from 6.70 to 8.37 mm. The highest PD indicated the best quality. Furthermore, a substrate with a high PD offered a lot of potential for being used in the production of *Pleurotus* species.

There were significant variations in pileus thickness (PT) within the substrates utilised to grow *P. ostreatus* and *P. pulmonarius*, according to the data (Table 7). Macadamia husk (41.56 mm) yielded the highest PT while MnS yielded the lowest (0.00 mm). Maize stalks and MH showed no bigger differences. In comparison to the other substrates, BP showed thin mushroom pileus. One of the characteristics that helps to maximize yield is PT. Varghese & Amritkumar (2020) reported that hay + sawdust produced the smallest Pileus diameter of 0.1 cm when compared to other materials. However, current findings support Ahmed *et al*. (2013), who found the lowest PT of 5.2 ± 0.2 and the maximum PT of 8.2 ± 0.3 cm from various substrates.

The number of flushes (F) observed from the various substrates differed significantly. Table 7 shows that the F ranged from 0.00 to 2.50, MS (2.50) having the highest F. These results are like those of Onyeka *et al*. (2018) and Getachew *et al*. (2019), who found a maximum of four flushes in all substrates. These findings corroborate with Varghese & Amritkumar (2020), who found a maximum of three flushes on varied substrates. More flushes might result in a higher yield while also lengthening the cropping period. The frequency of flushes is determined not only by the type of substrate, but also by the ambient circumstances.

The period between flushes (PBF) varied significantly among distinct *Pleurotus* species and substrates. On MH, the highest PBF was observed (5.00 days). The largest PBF was noted among the interactions between substrates and species on MS (5.67 days) and MH (6.67 days) from PO and PP, respectively. As a result, the cropping season was extended. Short cropping period was observed o BP*PO and

BP*PP interactions at 1.00 days. The substrate MnS received a score of 0.00 days because no PBF was found; however, the substrate BP received a score of 1.00 days indicating a faster cropping time. As a result, the kind of substrates as well as the interaction of substrates with the species had a significant impact on PBF. This study contradicts Islam *et al*. (2017), who found that the shortest time between the first and fourth flushing was 33.5 ± 2.3 days. The BP substrates have the capability of completing a cropping session in a short amount of time. In another study, cropping cycle took between 26 and 46 days (Varghese & Amritkumar, 2020). As a result, it is essential to choose a substrate that shortens the cropping cycle while still promoting the optimum fruiting quality.

Fresh weight (FW) was considerably affected by differences in substrates, species, and interactions between substrates and species, according to the findings (Table 7). On MS, the FW was higher at 75.73 g. As a result, PO and PP grown on MS had great FW (94.40 and 57.07 g, respectively) than those grown on BP (74.87 and 21.67 g, respectively). The MS*PO interactions was positive than others. Furthermore, no fruiting body formed on MnS, therefore, no FW was observed. The current findings contradict those of Oseni *et al*. (2012), who found that the FW from pine sawdust treated with varied quantities of wheat bran supplements ranged from 0.0 to 683.9 g. In comparison, Varghese & Amritkumar (2020) obtained 50.67 g on sawdust, 52.48 g on Hay + Sawdust + Banana Leaves, and 50.87 g on sawdust from flush 1, 2, and 3, respectively. The substrates MS and MH, as well as BP, showed tremendous promise in terms of being acceptable substrates for the culturing of *Pleurotus* species. Attributes including SD and PD, as well as PT have an impact on the amount of FW produced by *Pleurotus* species.

On diverse substrates, the results revealed significant variations in biological efficiency (BE) between the *Pleurotus* species (PO and PP). MS from *P. ostreatus* and *P. pulmonarius* exhibited significantly higher BE at 18.88 % and 11.41 %, respectively. There were significant interactions for BE, superior BE was observed on MS*PO and MS*PP. The variations differ with the substrate and species used. Due to a strong *Trichoderma* species infection, no BE was recorded from MnS. As a result of BE acquired on MS and MH, *Pleurotus* species had a stronger ability to convert substrates into edible Mushrooms which could be attributed to the availability of critical nutrients

in the substrates. These findings contrast with those of Sharmila *et al*. (2015), who found BE levels ranging from 48 to 76 percent on diverse substrates. Varghese & Amritkumar (2020), on the other hand, reported superior BE of 5.07, 5.45 and 5.09 percent on diverse substrates from flush 1, 2 and 3, respectively. Biological efficiency was greatly altered by the use of various substrates and species, and the interaction between substrates and species. Furthermore, the higher FW resulted in increased BE.

**Table 7. The effects of various substrates on the growth and yield of oyster mushrooms during the period from March to May 2019 cont'd**

| | Mean values of the variables | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable/Effect | SD (mm) | PD (mm) | PT (mm) | F | PBF (days) | FW (g) | BE (%) |
| **Substrates** | | | | | | | |
| *MS* | 19.42c | 60.22c | 39.63c | 2.50d | 4.67a | 75.73d | 15.15d |
| *BP* | 15.34bc | 47.32b | 30.62b | 1.00b | 1.00a | 48.27b | 9.65b |
| *MnS* | 0.00a | 0.00a | 0.00a | 0.00a | 0.00b | 0.00a | 0.00a |
| *MH* | 15.89b | 60.12c | 41.56c | 2.00c | 5.00b | 61.30c | 12.26c |
| **Species** | | | | | | | |
| *PO* | - | - | - | - | - | 61.07a | 12.21b |
| *PP* | - | - | - | - | - | 31.58b | 6.32a |
| **Substrate*Species** | | | | | | | |
| *MS *PO* | - | 52.60ab | - | - | 5.67bc | 94.40c | 18.88c |
| *BP*PO* | - | 52.63ab | - | - | 1.00a | 74.87b | 14.97b |
| *MnS*PO* | - | 0.00a | - | - | 0.00a | 0.00a | 0.00a |
| *MH* PO* | - | 59.00b | - | - | 3.33b | 75.00b | 15.00b |
| *MS *PP* | - | 67.83ab | - | - | 3.67b | 57.07c | 11.41c |
| *BP*PP* | - | 42.00b | - | - | 1.00a | 21.67b | 4.33b |
| *MnS*PP* | - | 0.00a | - | - | 0.00a | 0.00a | 0.00a |
| *MH*PP* | - | 61.23c | - | - | 6.67c | 47.60c | 9.52c |

Means with the same column followed by the same letters are not significantly different at p≤0.05. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nut-shells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

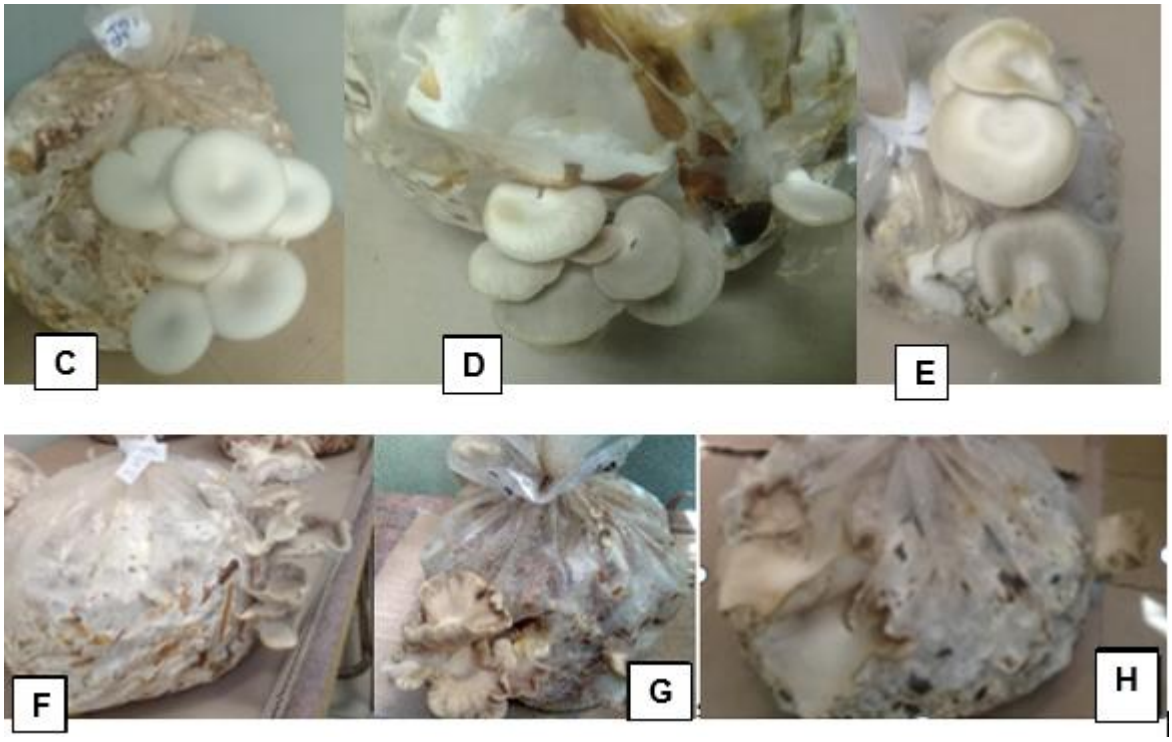


Figure 3. *P. ostreatus* grown on letter C - MS, D - BP and E - MH; and *P. pulmonarius* grown on letter F - MS, G - BP and H - MH.

Table 8 showed significant variations between the substrates on the NBC, DC, and DF; however, there were no significant differences between the species or the interaction between the substrates. On DP, considerable changes were found on the substrate, species, and even substrates with species used on Oyster mushroom growing. however, there was a considerable variation on IF between the species and their interactions with the substrates and each other.

**Table 8. Results of analysis of variance on growth and yield of oyster mushrooms during the period from June to August 2019**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of bags contaminated (NBC)** | | | | | |
| *Substrates* | 3 | 0.9444 | 11.3333 | 0.0003 | Sig |
| *Species* | 1 | 0.0000 | 0.0000 | 1.0000 | No-Sig |
| *Substrates*Species* | 3 | 0.1111 | 1.3333 | 0.2985 | No-Sig |
| *Error* | 16 | 0.0833 | | | |
| **Number of days from inoculation colonization (DC)** | | | | | |
| *Substrates* | 3 | 96.111 | 6.1023 | 0.0057 | Sig |
| *Species* | 1 | 13.500 | 0.8571 | 0.3683 | No-Sig |
| *Substrates*Species* | 3 | 15.833 | 1.0053 | 0.4160 | No-Sig |
| *Error* | 16 | 0.0833 | | | |
| **Number of days to primordia initiation (DP)** | | | | | |
| *Substrates* | 3 | 1057.944 | 1154.12 | 0.0000 | Sig |
| *Species* | 1 | 541.500 | 590.73 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 60.611 | 66.12 | 0.0000 | Sig |
| *Error* | 16 | 0.917 | | | |
| **Number of days to fruiting (IF)** | | | | | |
| *Substrates* | 3 | 61.0000 | 31.8261 | 0.0000 | Sig |
| *Species* | 1 | 2.6667 | 1.3913 | 0.2554 | No-Sig |
| *Substrates*Species* | 3 | 6.3333 | 3.3043 | 0.0472 | Sig |
| *Error* | 16 | 1.9167 | | | |
| **Number of days to flushing (DF)** | | | | | |
| *Substrates* | 3 | 48.8194 | 32.5463 | 0.0000 | Sig |
| *Species* | 1 | 1.0417 | 0.6944 | 0.4169 | No-Sig |
| *Substrates*Species* | 3 | 1.0417 | 0.6944 | 0.5688 | No-Sig |
| *Error* | 16 | 1.5000 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 9 reveals a significant variation on FB between substrates and species, even though there were no significant differences in the interactions between substrates and species. On PT, there were significant variations in the substrates, species, and interactions between species and substrates. There were significant differences among the substrates on PD and SD, but there were significant differences among the substrates and on the interaction between species and substrates on SL.

**Table 9. Results of analysis of variance for the growth and yield of oyster mushrooms during the period from June to August 2019 cont'd.**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of fruiting bodies (FB)** | | | | | |
| *Substrates* | 3 | 24.9306 | 7.6464 | 0.0022 | Sig |
| *Species* | 1 | 22.0417 | 6.7604 | 0.0193 | Sig |
| *Substrates*Species* | 3 | 5.8194 | 1.7849 | 0.1906 | No-Sig |
| *Error* | 16 | 3.2604 | | | |
| **Stipe length (SL)** | | | | | |
| *Substrates* | 3 | 15.8344 | 101.6114 | 0.0000 | Sig |
| *Species* | 1 | 0.4267 | 2.7380 | 0.1174 | No-Sig |
| *Substrates*Species* | 3 | 0.7433 | 4.7701 | 0.0146 | Sig |
| *Error* | 16 | 0.1558 | | | |
| **Stipe diameter (SD)** | | | | | |
| *Substrates* | 3 | 245.598 | 55.0986 | 0.0000 | Sig |
| *Species* | 1 | 17.957 | 4.0286 | 0.0619 | No-Sig |
| *Substrates*Species* | 3 | 9.656 | 2.1663 | 0.1319 | No-Sig |
| *Error* | 16 | 4.457 | | | |
| **Pileus diameter (PD)** | | | | | |
| *Substrates* | 3 | 4829.20 | 100.8806 | 0.0000 | Sig |
| *Species* | 1 | 5.98 | 0.1249 | 0.7284 | No-Sig |
| *Substrates*Species* | 3 | 141.68 | 2.9596 | 0.0638 | No-Sig |
| *Error* | 16 | 47.87 | | | |
| **Pileus thickness (PT)** | | | | | |
| *Substrates* | 3 | 562.024 | 123.273 | 0.0000 | Sig |
| *Species* | 1 | 1747.115 | 383.206 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 258.824 | 56.770 | 0.0000 | Sig |
| *Error* | 16 | 4.559 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 10 displays the ANOVA findings for F and PBF which revealed significant differences solely between the substrates. On both substrates and the interaction between substrates and species, the characteristics FW and BE changed dramatically.

**Table 10. Results of analysis of variance for the growth and yield of oyster mushrooms during the period from June to August 2019 cont'd**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Number of flushes (F)** | | | | | |
| *Substrates* | 3 | 5.4861 | 21.9444 | 0.0000 | Sig |
| *Species* | 1 | 0.0417 | 0.1667 | 0.6885 | No-Sig |
| *Substrates*Species* | 3 | 0.1528 | 0.6111 | 0.6175 | No-Sig |
| *Error* | 16 | 0.2500 | | | |
| **The period between flushing (PBF)** | | | | | |
| *Substrates* | 3 | 31.7049 | 10.8659 | 0.0004 | Sig |
| *Species* | 1 | 0.4161 | 0.1426 | 0.7107 | No-Sig |
| *Substrates*Species* | 3 | 1.7172 | 0.5885 | 0.6313 | No-Sig |
| *Error* | 16 | 2.9178 | | | |
| **Fresh weight (FW)** | | | | | |
| *Substrates* | *3* | *9065.71* | *26.9858* | *0.0000* | *Sig* |
| *Species* | 1 | 1225.65 | 3.6484 | 0.0742 | No-Sig |
| *Substrates*Species* | 3 | 2739.46 | 8.1545 | 0.0016 | Sig |
| *Error* | 16 | 335.94 | | | |
| **Biological efficiency (BE)** | | | | | |
| *Substrates* | 3 | 362.647 | 26.9667 | 0.0000 | Sig |
| *Species* | 1 | 49.049 | 3.6473 | 0.0743 | No-Sig |
| *Substrates*Species* | 3 | 109.604 | 8.1503 | 0.0016 | Sig |
| *Error* | 16 | 13.448 | | | |

Sig = significant difference, No-Sig = No-significant difference

The number of bags contaminated (NBC) was significantly different only among the substrates from both *Pleurotus* species, according to the findings (Table 11). On MH (0.83 days), the highest NBC was found and on BP the lowest (0.00 days). On MnS, a severe attack by *Trichoderma* species (green mould) was documented, resulting in unsatisfactory performance. These findings contradict those of Dlamini *et al*. (2012), who looked at maximal NBC owing to bacterial attack on substrates. These findings, on the other hand, agree with Akhter *et al*. (2017), who identified *Trichoderma* species as one of the most common pollutants during spawning. Contaminations on the substrates may have occurred as a result of an inefficient substrate pre-treatment procedure, unfavourable environmental conditions, or a poor strain.

Significant changes were found on DC between various substrates such as MS, BP, MnS, and MH. The time from inoculation to colonization was short on MS when compared to other substrates. Furthermore, poor strain performance could explain why the

bags containing MnS were not attacked by green mould before or after colonization and did not produce fruiting bodies. These findings are similar to those of Neupane *et al*. (2018), who found the highest spawn flowing on sawdust in the mushroom cultivation in 42.00 days. Varghese and Amritkumar (2020) acquired the largest number of days to colonization using hay. These findings are similar to those of Garuba *et al*. (2017), who found the lowest spawn running of 17.00 ± 0.00 and 15.33 ± 0.67 days respectively from banana leaves in the cultivation of *P. pulmonarius* and *P. ostreatus*. The nature and type of substrates were shown to have the greatest impact on the number of days to colonization (DC).

The number of days to primordia initiation (DP) on *P. ostreatus* and *P. pulmonarius* cultured on diverse substrates varied significantly. Although there were no significant changes on MS, BP and MH; BP had the highest DP maximum DP was found on BP and MH at 26.67 days. Between *Pleurotus* species, PO and PP there were more differences. *Pleurotus pulmonarius* had the least DP, indicating the potential to cause early flushing. There is no significant interactions of substrate and species. however, the highest DP was recorded on the substrates BP (33.33 days) and MH (20.67 days), which were utilized to grow *P. ostreatus* and *P. pulmonarius*, respectively. These findings revealed that DP was affected by species, substrates, and substrate*species' interaction. Garuba *et al*. (2017) identified the lowest number of days to pinhead appearance on banana leaves from *P. ostreatus* and *P. pulmonarius* at 22.33 ± 0.02 and 20.33 ± 0.67 days, respectively. These findings support Onyeka *et al*. (2018), who found that sawdust + corn waste + $CaCO_3$ took a minimum of 60.00 days to fully colonize. Getachew *et al*. (2019) found that primordial initiation happened between 2.50 and 4.00 days among the substrates in their investigation.

Table 11 shows that the number of days to fruiting initiation (IF) varied considerably across all substrates employed in *Pleurotus* species cultivation as well as the interaction of substrates with species. The IF of the substrate MH (7.17 days) was the highest than other substrates. There is no significant interaction for IF on *P. ostreatus* and *P. pulmonarius* grown on MS, BP and MH. *Pleurotus ostreatus* cultivated on MH (9.00 days) and *P. pulmonarius* cultivated on MS and MH at 6.00 days had the highest number of days to fruit commencement. These findings are like those of Sharmila *et al*. (2015), who recorded fruiting initiation times ranging from 18 to 34 days depending on

substrate type. Garuba *et al*. (2017) found that *P. pulmonarius* and *P. ostreatus* grown on altered sawdust had the greatest IF of 39.33 ± 0.33 and 37.33 ± 0.33 days respectively. It was discovered that factors like DC and DP have an impact on IF.

The results revealed considerable differences in flushing (DF) seen from different substrates utilised in growing of oyster mushrooms over numerous days. The obtained DF was in the range of 0.00 to 6.50 days. The DF on MnS was 0.00 days, thus, there were no days dedicated to flushing. Muswati *et al*. (2021) reported a minimum of 26.0 days until first harvesting which differs from the current findings. However, BP takes few days to flushing resulting in shortening cropping time. These findings contrast with Senghie *et al*. (2021), who had the lowest DF of 50.33 ± 3.35 and 71.86 ± 5.17 days on 100 sawdust and 100 sago bark from *Pleurotus Sajor-caju*, respectively. Different DFs are seen to differ from substrate variation.

Significant variations in the number of fruiting bodies (FB) were detected only across the various substrates and species. Maize stalk had the highest number of FB at 4.33, while MnS had the lowest at 0.00. *Pleurotus pulmonarius* produced significantly more fruiting bodies than PO. These findings are very similar to those of Muswati *et al*. (2021), who found the greatest FB of around 29.8 on 100% cotton waste from *P. ostreatus*. These results contradict those of Girmay *et al*. (2016), who achieved a maximum FB of 32.00 from cotton. Getachew *et al*. (2019) obtained a superior number of fruiting bodies of 41.44 and the least number of fruiting bodies of 27.44. Variations in substrates were shown to have a significant effect on the quantity of fruiting bodies.

*Pleurotus ostreatus* and *P. pulmonarius* cultivated on diverse substrates showed significant differences on stipe length (SL). When compared to other substrates, MS had the longest stipe length (3.52 cm). There is a significant interaction for SL on *P. ostreatus* grown on MS, BP and MH when compared to *P. pulmonarius*. Long stipe was obtained by *P. ostreatus* and *P. pulmonarius* at MS (3.77 cm) and BP (3.67 cm) respectively. These findings contradict those of Muswati *et al*. (2021), who reported a superior SL of 6.07± 0.32 mm on 25% sawdust + 75% sago frond from *P. ostreatus*. Girmay *et al*. (2016) discovered that cotton seed had the lowest SL of 2.81 cm and the paper waste utilized to cultivate *P. Sajor-caju* had the highest SL of 3.81 cm.

**Table 11. The effects of different substrates on growth and yield of oyster mushrooms during the period from June to August 2019.**

| Variable/Effect | Mean values of the variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | NBC (bags) | DC (days) | DP (days) | IF (days) | DF (days) | FB | SL (cm) |
| **Substrates** | | | | | | | |
| *MS* | 0.00a | 3.17a | 26.33b | 5.50b | 5.33bc | 4.33b | 3.52b |
| *BP* | 0.00a | 11.00b | 26.67b | 6.00b | 4.67b | 3.83b | 3.08b |
| *MnS* | 0.17a | 11.17b | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a |
| *MH* | 0.83b | 11.33b | 26.67b | 7.17b | 6.50c | 4.00b | 3.07b |
| **Species** | | | | | | | |
| *PO* | - | - | 24.67b | - | - | 2.08a | - |
| *PP* | - | - | 15.17a | - | - | 4.00b | - |
| **Substrate*Species** | | | | | | | |
| *MS*PO* | - | - | 32.67b | 5.00b | - | - | 3.77d |
| *BP*PO* | - | - | 33.33b | 6.00b | - | - | 2.50b |
| *MnS*PO* | - | - | 0.00a | 0.00a | - | - | 0.00a |
| *MH* PO* | - | - | 32.67b | 9.00c | - | - | 2.87c |
| *MS*PP* | - | - | 20.00b | 6.00b | - | - | 3.27b |
| *BP*PP* | - | - | 20.00b | 6.00b | - | - | 3.67b |
| *MnS*PP* | - | - | 0.00a | 0.00a | - | - | 0.00a |
| *MH*PP* | - | - | 20.67b | 5.33b | - | - | 3.27b |

Means with the same column followed by the same letters are not significantly different at p≤0.05. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nut-shells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

Table 12 revealed that there wre significant differences on SD among the various substrates utilized to grow oyster mushrooms. The SD obtained ranged from 0.00 to 13.54 mm with BP having the highest SD. Due to its low performance, no SD was found on MnS. Muswati *et al*. (2021) obtained a minimum of 3.5 cm SD and minimum of about 3.5 cm SD on 50 percent cotton waste + 50 percent wheat straw to grow *P. ostreatus*. These findings contradict those of Girmay *et al*. (2016) who found stipe diameters ranging from 3.11 to 4.85 cm in *P. ostreatus* growing on diverse substrates. These findings revealed that substrate variation influenced SD.

The results revealed a statistically significant variation in PD between the various substrates. Maize stalks gave the highest PD (59.41 mm) while MnS caused the least

(0.00 mm). These results are like those of Garuba *et al*. (2017) who obtained PD on *P. pulmonarius* ranging from 4.50 ± 0.00 to 6.33 ± 0.33 cm. This study contradicts Onyeka *et al*. (2018), who found a minimum PD of 10 cm and a maximum PD of 17 cm from diverse substrates. Furthermore, it has been stated that a greater pileus diameter is preferable, the substrate is acceptable (Onyango *et al*., 2011). As a result, MS and BP have the potential to be used as a viable substrate for the culture of *P. ostreatus* and *P. pulmonarius* if MH is unavailable.

Significant differences on pileus thickness (PT) were found among substrates in the current study. MS (59.41 mm) had higher PT while MnS (0.00 mm) had a poor performance due to the lack of PT. There were variations among the species with PO (22.50 mm) having higher PT than PP (5.43 mm). Greater interactions were observed for PT on *P. ostreatus* grown on MS, BP and MH. *Pleurotus ostreatus* respond positively to all substrates than *P. pulmonarius*. *Pleurotus ostreatus* and *Pleurotus pulmonarius* planted on MS and BP, respectively, had the highest PT among the interactions between substrates and species. Varghese & Amritkumar (2020) reported the largest pileus thickness of 0.75 cm from oyster mushrooms growing on hat + banana leaves, but these findings contradict them. These findings are different from those of Mondal *et al*. (2010), who discovered the lowest PT of 0.35 cm on sawdust used in oyster mushroom production during the first flush. It was discovered that the usage of diverse substrates has a significant impact on pileus thickness.

Table 12 showed variations on the number of flushes (F) observed between different substrates utilized in oyster mushroom growing. The minimum F was recorded on MnS (0.00) and the maximum on MS (2.17). Moreover, during the flushing period, there was *Trichoderma* infections. As a result, the current findings contradict those of Dlamini *et al*. (2012), who found that maize stover produced the least amount of F when compared to other substrates. However, On MnS no flushes were observed. The lower the number on F, the shorter the period of production and the greater likelihood of producing fewer fruiting bodies. Current research contradicts Tesfaw *et al*. (2015) who reported four flushes inside the substrates. As a result, the variation in F differed depending on the substrate.

According to current findings there were differences in the time between flushes (PBF) among the substrates. The largest number of days MS took to flushing was 4.83 days

while there were no days observed on MnS (0.00 days) because there was no fruiting body formed. This study contradicts Islam *et al*. (2017), who claimed the shortest flushing time of around 33.5 ± 2.3 days. These findings support Onyeka *et al*. (2018) who recorded short flushing period of 22 ± 0.00 days on sawdust + banana leaves and sawdust + cassava peel + $CaCO_3$ from oyster mushroom had a lengthy flushing period of 36 ± 0.50 day. Varghese & Amritkumar (2020) found that oyster mushrooms grown on hay extended the cropping cycle by 46 days, which argue current experiment. Substrates like BP had the ability to complete the cropping cycle in a short time.

Fresh weight (FW) differences between substrates and even substrates of species were significant (Table 12). The FW reported on BP (82.46 g) was higher than others. The significant interactions were observed for FW. The interactions between substrates and species grown on MS (120.19 g) and (92.63 g) showed significantly higher FW from *P. ostreatus* and *P. pulmonarius*, respectively. The type of substrates and species have influence on the interactions. Due to strong *Trichoderma* species attack and poor performance of the *Pleurotus* strain, no fruiting body grew on MnS. These present findings contradict those of Muswati *et al*. (2021), who reported fresh weights ranging from 0.64 to 1.292 kg. Varghese & Amritkumar (2020) found maximum FW of 50.67 and 50.87 g on *P. ostreatus* grown on sawdust during the first and third flushes, respectively. MS showed considerable promise as a good substrate followed by BP and MH, respectively. The differences could be caused by differences in substrates and species, as well as traits like SD, PD, and PT.

On the BE of *P. ostreatus*, there were significant differences among substrates. The best BE was found on BP (16.49 %) compared to the other substrates. There were significant interactions for BE on oyster musrooms. The interaction between the substrates and species had the highest BE on MS (24.04 %) and BP (18.53 %) from *P. ostreatus* and *P. pulmonarius*, respectively. These results are consistent with those of Senghie *et al*. (2021) who reported an inferior BE of 11.50 ± 1.64 percent from *P. sajor-caju* grown on 75 % sawdust + 25 % sago bark. Muswati *et al*. (2021) reported BE ranging from 42.5 to 86.2 percent depending on substrate. The high concentration of cellulose in lignocellulose materials was reported to be the cause of the greatest BE (Rizki & Tamai, 2011). Girmay *et al*. (2016) found a high BE of 74,17 percent in cottonseed and a low BE of 9.73 percent in *P. ostreatus* cultivated on sawdust in their

investigation. The findings revealed that the kind of substrates may have an impact on oyster mushrooms' ability to convert lignocellulose resources into value-added food.

**Table12. The effects of different substrates on the growth and yield of oyster mushrooms during the period from June – August 2019 cont'd**

| Effects | Mean values of the variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | SD (mm) | PD (mm) | PT (mm) | F | PBF (days) | FW (g) | BE % |
| **Substrates** | | | | | | | |
| *MS* | 12.86b | 59.41b | 22.19c | 2.17c | 4.83b | 81.85c | 16.37c |
| *BP* | 13.54b | 57.74b | 17.80b | 1.17b | 1.67a | 82.46c | 16.49c |
| *MnS* | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a |
| *MH* | 11.71b | 52.01b | 15.86b | 1.83c | 4.42b | 48.10b | 9.62b |
| **Species** | | | | | | | |
| *PO* | - | - | 22.50b | - | - | - | - |
| *PP* | - | - | 5.43a | - | - | - | - |
| **Substrate*Species** | | | | | | | |
| *MS*PO* | - | - | 38.21 | - | - | 120.19c | 24.04c |
| *BP*PO* | - | - | 26.83b | - | - | 72.28b | 14.46b |
| *MnS*PO* | - | - | 0.00a | - | - | 0.00a | 0.00a |
| *MH* PO* | - | - | 24.94b | | | 48.52b | 9.70b |
| *MS*PP* | - | - | 6.17b | - | - | 43.50b | 8.70b |
| *BP*PP* | - | - | 8.77b | - | - | 92.63c | 18.53c |
| *MnS*PP* | - | - | 0.00a | - | - | 0.00a | 0.00a |
| *MH*PP* | - | - | 6.78b | - | - | 47.69b | 9.54b |

Means with the same column followed by the same letters are not significantly different at p≤0.05. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nut-shells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

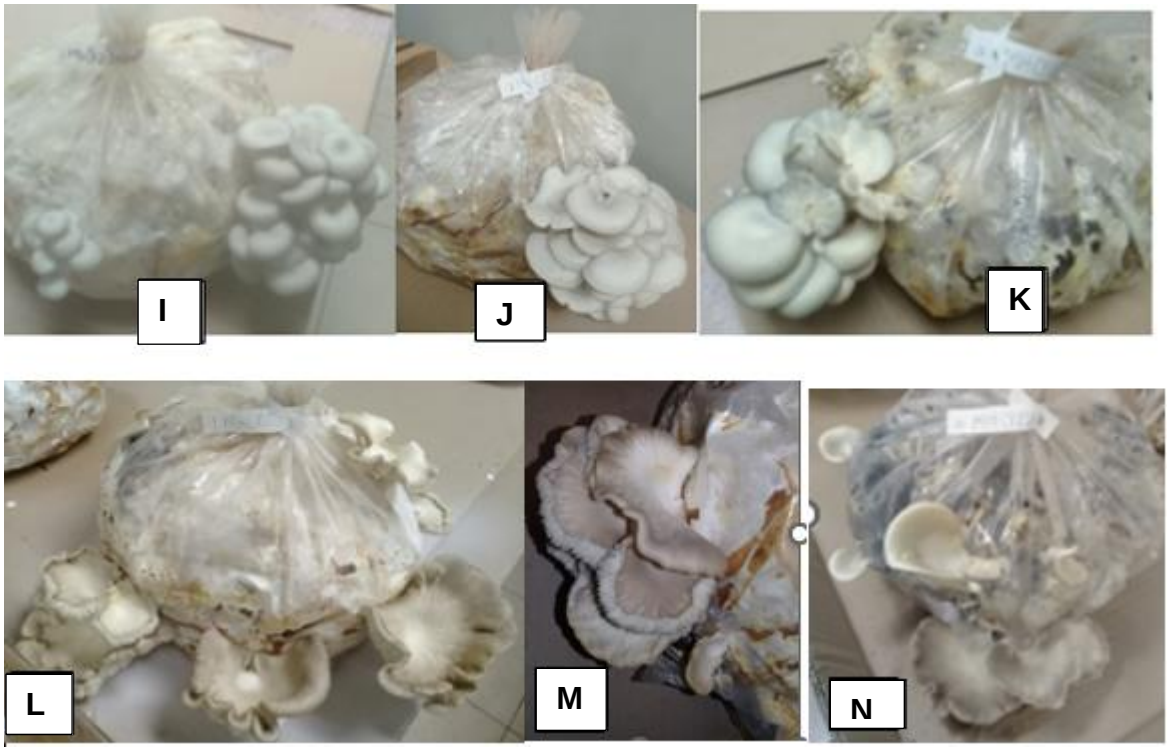


Figure 4. *Pleurotus ostreatus* grown on letter I - MS, J - BP and K - MH and *Pleurotus pulmonarius* grown on letter L - MS, M - BP and N - MH.

## 4.3 STUDY 3: THE EFFECT OF VARIOUS SUBSTRATES ON THE NUTRITIONAL COMPOSITIONS OF OYSTER MUSHROOMS

Table 13 shows that the substrates and species employed in the study had significant changes in M, however the interaction between substrates and species had no significant difference at $P \leq 0.05$. Furthermore, species, substrates, and even substrates with species all had a significant impact on CP. Only across substrates did the characteristic Fat vary considerably.

**Table 13. The results of the analysis of variance for the nutritional compositions of oyster mushrooms.**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Moisture (M)** | | | | | |
| *Substrates* | 3 | 10116.65 | 385.225 | 0.0000 | Sig |
| *Species* | 1 | 237.51 | 9.044 | 0.0084 | Sig |
| *Substrates*Species* | 3 | 65.54 | 2.496 | 0.0969 | No-Sig |
| *Error* | 16 | 26.26 | | | |
| **Crude protein (CP)** | | | | | |
| *Substrates* | 3 | 1468.41 | 151.462 | 0.0000 | Sig |
| *Species* | 1 | 80.67 | 8.321 | 0.0108 | Sig |
| *Substrates*Species* | 3 | 46.10 | 4.755 | 0.0148 | Sig |
| *Error* | 16 | 9.69 | | | |
| **Fat** | | | | | |
| *Substrates* | 3 | 5.1299 | 23.9357 | 0.0000 | Sig |
| *Species* | 1 | 0.1734 | 0.8091 | 0.3817 | No-Sig |
| *Substrates*Species* | 3 | 0.3352 | 1.5639 | 0.2370 | No-Sig |
| *Error* | 16 | 0.2143 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 14 indicate significant differences between substrates and species on AC, but no significant changes between substrates and species interactions at $P \leq 0.05$. Furthermore, CF, C, and ME revealed significant disparities in substrates and species, as well as interactions between them.

**Table 14. The results of analysis of variance for the nutritional compositions of oyster mushrooms cont'd**

| Variable/Effect | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Ash content (AC)** | | | | | |
| *Substrates* | 3 | 38.1771 | 62.1186 | 0.0000 | Sig |
| *Species* | 1 | 5.5104 | 8.9661 | 0.0086 | Sig |
| *Substrates*Species* | 3 | 0.6771 | 1.1017 | 0.3773 | No-Sig |
| *Error* | 16 | 0.6146 | | | |
| **Crude fibre (CF)** | | | | | |
| *Substrates* | 3 | 919.816 | 312.106 | 0.0000 | Sig |
| *Species* | 1 | 353.050 | 119.795 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 39.528 | 13.412 | 0.0001 | Sig |
| *Error* | 16 | 2.947 | | | |
| **Carbohydrate (C)** | | | | | |
| *Substrates* | 3 | 2649.57 | 142.828 | 0.0000 | Sig |
| *Species* | 1 | 1004.40 | 54.144 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 146.27 | 7.885 | 0.0019 | Sig |
| *Error* | 16 | 18.55 | | | |
| **Metabolizable energy content (ME)** | | | | | |
| *Substrates* | 3 | 99389.2 | 3245.99 | 0.0000 | Sig |
| *Species* | 1 | 5768.9 | 188.41 | 0.0000 | Sig |
| *Substrate*Species* | 3 | 671.5 | 21.93 | 0.0000 | Sig |
| *Error* | 16 | 30.6 | | | |

Sig = significant difference, No-Sig = No-significant difference

Table 15 shows the effect of substrates in Ca and Mg mineral content. However, substrates, species, and interactions between substrates and species had effect on Fe content. Diverse substrates have a significant impact on the number of minerals found.

**Table 15. The results of the analysis of variance for the nutritional compositions of oyster mushrooms.**

| Variables | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Calcium (Ca)** | | | | | |
| *Substrates* | 3 | 0.0031 | 3.8739 | 0.0294 | Sig |
| *Species* | 1 | 0.0005 | 0.6269 | 0.4401 | No-Sig |
| *Substrates*Species* | 3 | 0.0007 | 0.8480 | 0.4877 | No-Sig |
| *Error* | 16 | 0.0008 | | | |
| | | | | | |
| **Magnesium (Mg)** | | | | | |
| *Substrates* | 3 | 0.0412 | 430.072 | 0.0000 | Sig |
| *Species* | 1 | 0.0002 | 2.130 | 0.1638 | No-Sig |
| *Substrates*Species* | 3 | 0.0000 | 0.739 | 0.5440 | No-Sig |
| *Error* | 16 | 0.0000 | | | |
| | | | | | |
| **Iron (Fe)** | | | | | |
| *Substrates* | 3 | 12084.3 | 48.3774 | 0.0000 | Sig |
| *Species* | 1 | 3266.1 | 13.0755 | 0.0023 | Sig |
| *Substrates*Species* | 3 | 918.0 | 3.6749 | 0.0346 | Sig |
| *Error* | 16 | 249.8 | | | |

Sig = significant difference, No-Sig = No-significant difference

Copper (Cu) was affected by substrates as shown in table 16, while species and interactions between substrates and species had no effect. Furthermore, both Zn and K mineral concentration differed significantly among substrates, species, and substrate-species interactions.

**Table 16. The results of analysis of variance for the nutritional compositions of oyster mushrooms cont'd.**

| Variables | Df | MS | F | P | Remarks |
|---|---|---|---|---|---|
| **Copper (Cu)** | | | | | |
| *Substrates* | 3 | 280.963 | 35.4656 | 0.0000 | Sig |
| *Species* | 1 | 12.427 | 1.5687 | 0.2284 | No-Sig |
| *Substrate*Species* | 3 | 18.885 | 2.3838 | 0.1075 | No-Sig |
| *Error* | 16 | 7.922 | | | |
| | | | | | |
| **Zinc (Zn)** | | | | | |
| *Substrates* | 3 | 10891.13 | 221.940 | 0.0000 | Sig |
| *Species* | 1 | 2088.00 | 42.549 | 0.0000 | Sig |
| *Substrates*Species* | 3 | 278.22 | 5.670 | 0.0077 | Sig |
| *Error* | 16 | 49.07 | | | |
| | | | | | |
| **Potassium (K)** | | | | | |
| *Substrates* | 3 | 16.0187 | 235.008 | 0.0000 | Sig |
| *Species* | 1 | 0.4082 | 5.989 | 0.0263 | Sig |
| *Substrates*Species* | 3 | 0.3308 | 4.854 | 0.0138 | Sig |
| *Error* | 16 | 0.0682 | | | |

Sig = significant difference, No-Sig = No-significant difference

*P. ostreatus* and *P. pulmonarius* grown on various substrates exhibited variations in the results. *Pleurotus ostreatus* and *P. pulmonarius* had the maximum moisture content (M) when cultivated on BP (84.25 %) and MS (82.82 %), respectively (Table 17). Furthermore, these findings revealed that *P. ostreatus* retained a lot of moisture when compared to *P. pulmonarius*. According to Tirkey *et al*. (2017), the lower moisture content is caused by ageing, harvesting time and packaging as well as the poor quality of the substrate and low water retention capacity. *Pleurotus ostreatus* had the highest M among the species, at 64.67 %. The results are similar to Dinssa *et al*. (2020) who found that *P. ostreatus* had the greatest moisture content at 87.00 %. These findings are similar to those of Onyeka *et al*. (2018), who produced the lowest M of 72.23 ± 2.2 % using sawdust + banana leaves as a growing medium. Due to a severe *Trichoderma* infection that resulted in no growth of fruiting bodies the amount of moisture within the substrate, MnS could not be assessed. The moisture content of mushrooms is influenced by their age, growth circumstances, mushroom strain and postharvest settings (Kurtzman, 2005). Furthermore, moisture content may be one of the determinants of mushroom quality. As a result, a suitable substrate may be required for cultivation.

There were significant variations at P≤0.05 on CP among the substrates in the cultivation of *P. ostreatus* and *P. pulmonarius*. Greater CP was observed on MH (34.41 %) among the various substrates and on PP (23.41 %) among the species. There were significant interactions for CP on oyster mushrooms. The variation of Crude protein content is mostly influenced by the type of substrates and species. Interactions results showed the highest CP of 34.20 and 34.21 % on MH, and the lowest CP of 14.32 and 26.04 % on MS from *P. ostreatus* and *P. pulmonarius*, respectively. These findings supported Sifat *et al*. (2020) who had reported the protein content of 31.63±0.96 % from *P. ostreatus*. It is indicated that the differences in protein were influenced by nitrogen content variations of substrates (Hoa *et al*., 2015). In contrast, these results disagree with Onyeka *et al*. (2018) who had recorded the highest protein content of 3.98 ± 0.05 % from sawdust + banana leaves and the lowest protein of 2.11 ± 0.11 % on Sawdust + Corn waste + $CaCO_3$. The results found in this study demonstrated the best protein contents observed on MH and BP compared to MS. This could be due to the differences in the type, nature, and sources of substrates as well as the amount of nitrogen within the substrates. This showed that MH can be considered as the best substrate followed by BP due to its potential to stimulate high protein that is essential for human consumption.

Fat levels varied significantly across the substrates employed in the cultivation of *P. ostreatus* and *P. pulmonarius*, according to the findings on Table 17. The highest fat content was found on MS (1.95 %) while the lowest was found on MH (1.55 %). There was no fruiting body on MnS, thus, there was no fat content determined. Macadamia husks had the highest fat content when compared to other substrates. Due to a severe attack by *Trichoderma* species, there was no fat content observed on MnS. These findings contradict those of Sifat *et al*. (2020) who found the highest fat content of 4.13 ± 0.25 % on *P. ostreatus*. The recent findings agree with Onyeka *et al*. (2018), who achieved the maximum quantity of fat 1.83 ± 0.02 % on sawdust + banana leaves utilised to cultivate *P. ostreatus*. These results are consistent with those of Garuba *et al*. (2017), who obtained the highest fat content of 2.18 % on cassava peels and the lowest fat content of 1.31 % on modified sawdust. Fat differs from the substrates. Oyster mushrooms are typically low in calories, they have a low-fat content.

*Pleurotus ostreatus* and *P. pulmonarius* planted on MS, BP, and MH showed significant differences in ash content (AC). The highest AC (3.67 %) was found on PO while the lowest was found on PP (2.71 %). The highest AC was recorded for MH*PO and MS*PP interactions at 4.83 and 5.33 %, respectively. Table 15 shows that the results differ from those of Onyeka *et al*. (2018) who found the greatest ash was 1.27 ± 0.56 % and the lowest ash was 0.25 ± 0.04 % among *Pleurotus* species. Variations in AC could be due to the quantity of water that substrates can hold throughout the manufacturing process. These results differ with Sifat *et al*. (2020) who found AC of 7.86±0.6 %. This demonstrated that species and substrate type had an impact on AC.

On crude fibre (CF), the MH (25.75 %) was much higher than on other substrates, but PP (22.35 %) was higher than PO. There were no significant interactions for CF on oyster grown on MS, BP and MH. Maximum CF was respectively reported on MH at 20.95 and 30.55 % in the cultivation of *P. ostreatus* and *P. pulmonarius* based on substrates*species interactions. This could be related to the decomposition of lignin and cellulose by mushrooms during the manufacturing process. These findings are consistent with those of Sifat *et al*. (2020) who calculated a fibre content of 24.02 ± 0.10 % on *P. ostreatus*. These findings contradict those of Dinssa *et al*. (2020) who showed that the amount of fibre content varied from 9.03 to 15.94 %. The discrepancies found on CF could be attributable to the species chosen and their nature. These findings contradict those of Garuba *et al*. (2017) who found fibre content in *P. ostreatus* and *P. pulmonarius* growing on diverse substrates ranging from 5.31 to 5.88 %. The variations in crude fibre could be related to differences in *Pleurotus* sp. compositions and substrates (Hoa *et al*., 2015).

In the culture of *P. ostreatus* and *P. pulmonarius*, the findings revealed significant variations in carbohydrate estimations (C) within the substrates MS, BP, and MH at $P \leq 0.05$. The substrate, MnS, was found to be devoid of C. There were differences in the substrates; MS had a higher C than the other substrates. There were significant interactions for C on oyster, the greatest amount of C was observed on MS. PO, on the other hand, received the highest C between the *Pleurotus* species. From *P. ostreatus* and *P. pulmonarius*, the quantity of C discovered on the substrates*species interactions varied from 38.77 to 59.56 percent and 26.17 to 35.69 percent, respectively. These differences could be attributed to changes in substrates and species used.

Aspects such as CP, CF, and fat, as well as AC, play a major role in the carbs content, in addition to C changes. Dinssa *et al*. (2020) reported the highest amount of C of 37.29 percent and these data support a recent experiment. These findings are consistent with those of Hoa *et al*. (2015), who found the maximum carbohydrate content of 51.93 percent and the lowest carbohydrate content of 55.92 percent in *P. ostreatus* and *P. cystidiosus*, respectively. These findings contradict Garuba *et al*. (2017) who found the lowest C of roughly 22.25 % in modified sawdust and the highest C of 26.41 % in banana leaves. Tirkey *et al*. (2017) reported wheat straw with a higher carbohydrate content of 4.46 %. This suggested that the production of oyster mushrooms was influenced differently by the usage of diverse substrates of varied natures and the types of *P*. species.

There were significant variations in ME found in the culture of *P. ostreatus* and *P. pulmonarius* on diverse substrates at $P \leq 0.05$. Banana pseudostem had a higher ME (269.67 kcal/g) amongst the substrates and among the species, PO (208.02 kcal/g) had the highest ME. There were significant varioations for ME on *pleurotus* species. *Pleurotus ostreatus* and *P. pulmonarius* had the greatest ME between substrates*species interactions on BP at 289.55 and 249.78 kcal/g, respectively. Furthermore, *P. ostreatus* and *P. pulmonarius* grown on MH had much lower ME, with 270.29 and 235.45 kcal/g, respectively. This implies that ME changes are influenced by species, substrates, and interactions between substrates and species. According Dinssa *et al*. (2020), *P. ostreatus* had the best ME of 253.97±8.25 (kcal/g) among the mushrooms. Furthermore, ME's composition is heavily influenced by factors such as protein, fat, and carbohydrate.

**Table 17. The effects of various substrates on the nutritional compositions of oyster mushrooms.**

| Variable/Effect | Mean values of the variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | M % | CP % | FAT % | AC % | CF % | C % | ME (Kcal/g) |
| **Substrates** | | | | | | | |
| *MS* | 82.42b | 20.18b | 1.95b | 5.33c | 24.92bc | 47.62d | 253.84c |
| *BP* | 84.25b | 31.94c | 1.94b | 2.33b | 23.39b | 40.40c | 269.67d |
| *MnS* | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a |
| *MH* | 79.42b | 34.41c | 1.55b | 5.08c | 25.75c | 32.47b | 246.55b |
| **Species** | | | | | | | |
| *PO* | 64.67b | 19.75a | - | 2.71a | 14.68a | 36.59b | 208.02b |
| *PP* | 58.38a | 23.41b | - | 3.67b | 22.35b | 23.65a | 177.01a |
| **Substrate*Species** | | | | | | | |
| *MS*PO* | - | 14.32b | - | - | 19.68b | 59.56d | 272.23b |
| *BP*PO* | - | 30.46c | - | - | 18.09b | 48.05c | 289.55c |
| *MnS*PO* | - | 0.00a | - | - | 0.00a | 0.00a | 0.00a |
| *MH* PO* | - | 34.20c | - | - | 20.95b | 38.77b | 270.29b |
| *MS*PP* | - | 26.04b | - | - | 30.16b | 35.67c | 235.45c |
| *BP*PP* | - | 33.40c | - | - | 28.70b | 32.76c | 249.78d |
| *MnS*PP* | - | 0.00a | - | - | 0.00a | 0.00a | 0.00a |
| *MH*PP* | - | 34.21c | - | - | 30.55b | 26.17b | 222.81b |

Means with the same column followed by the same letters are not significantly different at p≤0.05. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nut-shells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

According to Singh & Singh (2021), oyster mushrooms' mineral composition is highly important due to their content of microelements, particularly numerous microelements. The maximum Ca was detected on MS and MH as shown on Table 18. On MnS there was no unformed fruiting structures, therefore, no amount of Ca was detected. This result agrees with Silva *et al*. (2002), who reported Ca levels varying from 0.0034 ± 0.0002 to 0.0684 ± 0.0011 % amongst several substrates. This current study support Masevhe *et al*. (2016) who reported a tiny amount of 0.067 percent on wood chips. This research agrees with Youssef *et al*. (2021) who found the greatest Ca level of 0.004 ± 0.001 % on discarded oyster substrate mixed with wheat straw.

The magnesium (Mg) content of the substrates employed in oyster mushroom cultivation varied greatly. In contrast to other substrates, MS obtained the superior amount

of Mg (0.18 %). These findings agree with Masevhe *et al*. (2016) who found that the amount of Mg in various substrates ranged from 0.024 to 0.282 %. These findings contrast with Youssef *et al*. (2021) who found Mg levels varying from 0.01 ± 0.000 to 0.04 ± 0.0006% from *P. ostreatus* grown on a variety of substrates. The amount of Mg varies depending on the substrate. As a result, the substrate continues to be the most important element in oyster mushroom performance.

The highest iron (Fe) concentration was found on MH (102.71 mg/kg). The amount of Fe from oyster mushrooms (PO and PP) differed greatly. However, PO have a higher Fe content. There were significant interactions for Fe on both substrates and species. Among the substrates*species' interaction, the largest amounts of Fe were found on MH at 107.95 and 80.33 mg/kg from PO and PP, respectively. It is noted that interactions between substrates and species influence Fe content. This work contradicts Masevhe *et al*. (2016) who observed the highest concentration of Fe (845.00 mg/kg) from *P. ostreatus* cultivated on wheat straw. These findings contradict the findings of Zahid *et al*. (2020) who found a minimum quantity of Fe (300 ± 5 mg/kg) in cotton waste.

Elattar *et al*. (2019) reported similar results on the approximation of Cu concentrations from oyster mushrooms cultivated on a variety of agrowaste materials. On MH, the oyster mushroom produced the highest amount of copper (Cu) (16.37 mg/kg). It should be mentioned that the amount of Cu in the substrates differed greatly. This research agrees with Elattar *et al*. (2019) who got a lot of Cu (16.95 mg/kg) from mushrooms grown on water hyacinth. The findings are consistent with those of Masevhe *et al*. (2016) who obtained the highest concentration of Cu (24.33 mg/kg) in wheat straw and the lowest concentration of Cu (3.00 mg/kg) in wood chips. On the other hand, oyster mushrooms cultivated on cotton waste contained the least quantity of Cu at 47.2 ± 2 mg/kg (Zahid *et al*., 2020).

In the cultivation of *P. ostreatus* and *P. pulmonarius*, a significant amount of Zn was detected on BP. The type of substrate had a considerable impact on the amount of Zn. Furthermore, MH contained a considerably high level of Zn (91.14 mg/kg) whereas MnS had negligible Zn content due to unformed fruiting bodies. The zinc concentration of PO (72.85 mg/kg) was higher than that of PP (54.19 mg/kg). There were significant interactions for Zn; least Zn was found on MS from PO and on BP from PP. On MH,

the substrate species interactions yielded the most Zn with 101.95 and 80.33 mg/kg from PO and PP, respectively. These findings agree with Elattar *et al*. (2019), who observed Zn levels ranging from 38.84 to 61.58 mg/kg. This study differs from Masevhe *et al*. (2016), who found that thatch grass had the highest Zn content of 36.33 mg/kg while wood chips had the lowest Zn level of 8.67 mg/kg. The type of substrates, instead, had a significant effect on the amount of Zn present.

According to Singh & Singh (2021), K is the mineral with the highest content accounting for 45 percent of total ash content. The amount of potassium (K) in Oyster mushrooms (PO and PP) cultivated on diverse substrates varied significantly. When compared to other species, MS had a high concentration of K (3.66 percent). There was no fruiting body grown on MnS, thus, the amount of K was not measured. The species PP had a higher K content when compared to PO. There were significant interactions for K on oysters and substrates. Oyster grown on BP gave least K content when compared to other substrates. The largest quantity of K was recorded at 3.55 and 3.77 percent from PO and PP grown on MS, according to the results of the interaction between substrates and species. Youssef *et al*. (2021) discovered the largest level of K at 0.37 ± 0.05 percent on discarded oyster substrate mixed with wheat straw dosed with 3 $kg^{-1}$ Lithovit-Urea50 after spawning which contradicts current result. These findings support Silva *et al*. (2002) who found the amount of K in diverse substrates ranged from 0.5790±0.0020 to 2.3709 ± 0.0020%.

**Table 18. The effects of various substrates on the nutritional compositions of oyster mushrooms cont'd.**

| Effect | Mean values of the variables | | | | | |
|---|---|---|---|---|---|---|
| | Ca % | Mg % | Fe mg/kg | Cu mg/kg | Zn mg/kg | K % |
| **Substrates** | | | | | | |
| *MS* | 0.05b | 0.18g | 82.22b | 9.88b | 80.13b | 3.66d |
| *BP* | 0.04b | 0.14b | 74.93b | 11.21b | 82.80b | 1.87b |
| *MnS* | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a |
| *MH* | 0.05b | 0.16c | 102.71c | 16.37c | 91.14c | 3.17c |
| **Species** | | | | | | |
| *PO* | - | - | 76.63b | - | 72.85b | 2.04a |
| *PP* | - | - | 53.30a | - | 54.19a | 2.30b |
| **Substrate*Species** | | | | | | |
| *MS*PO* | - | - | 95.25b | - | 90.29b | 3.55c |
| *BP*PO* | - | - | 103.32b | - | 99.15b | 1.40b |
| *MnS*PO* | - | - | 0.00a | - | 0.00a | 0.00a |
| *MH* PO* | - | - | 107.95b | - | 101.95b | 3.22d |
| *MS*PP* | - | - | 69.18b | - | 69.97c | 3.77d |
| *BP*PP* | - | - | 46.54c | - | 66.46b | 2.33b |
| *MnS*PP* | - | - | 0.00a | - | 0.00a | 0.00a |
| *MH*PP* | - | - | 97.47d | - | 80.33d | 3.11c |

Means with the same column followed by the same letters are not significantly different at p≤0.05. MS = maize stalks; BP = banana pseudo-stem; MnS = macadamia nut-shells, MH = macadamia husks. PO = *P. ostreatus*, PP = *P. pulmonarius*.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATIONS

## 5.1 CONCLUSION

Due to its acceptable pH of 6.84, EC, total nitrogen, and C/N ratio, it is concluded that MS can be advantageous when utilized in agriculture. When compared to other substrates, MS gave the best results in terms of yield and BE on both *P. ostreatus* and *P. pulmonarius*. The substrate BP showed tremendous promise in terms of yield and BE. During colonizing and flushing, the MnS was mostly susceptible to *Trichoderma* species infection, and performed poorly. When compared to other substrates, *P. ostreatus* and *P. pulmonarius* cultivated on MH produced the highest value of CP, Fat, and CF. The highest concentrations of minerals Ca, Mg, Fe, Cu, and K were found on MH compared to other substrates, indicating that MH has the potential to be employed in mixed substrates for the cultivation of Oyster mushroom.

## 5.2 RECOMMENDATIONS

Maize stalk is recommended as the best substrate for the production of oyster mushrooms. Banana pseudo stem is specifically recommended for *P. plumonarius* production because of the significantly higher yield obtained. The significant impact of macadamia husks on oyster mushrooms nutrient enrichment needs to be further explored to obtain insight into the best strategy of combining it with other substrate for optimum results.

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