COMPARATIVE GROWTH AND PRODUCTIVITY EVALUATION OF PHOENIX OYSTER MUSHROOM (*pleurotus* sp.) UNDER RESIDUAL AGRO-WASTE SUBSTRATES IN SEMI-ARID LANDS OF KENYA

JUDY W. WACHIRA

REG. NO. A56/MAC/20411/2013

A Research Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agricultural Resource Management of South Eastern Kenya University

2022

DECLARATION

I understand that plagiarism is an offence and I therefore declare that this thesis is my original work and has not been presented to any other institution for any other award.

Signature: ……. Date……..

Judy W. Wachira

Reg. No. A56/MAC/20411/2013

This thesis has been submitted for examination with our approval as university supervisors

Signature: …… Date……..

Dr. Simon Nguluu

Senior Lecturer

Department of Agricultural Sciences

South Eastern Kenya University

Signature: ……... Date …….

Prof. Josphert N. Kimatu

Associate Professor

Department of life Sciences

South Eastern Kenya University

DEDICATION

Especially to my dear husband Dr. Elijah Muange, our three children Esther, David and Victoria, and my loving mum Virginia Wachira together with my loving mother-in law Esther Muange.

ACKNOWLEDGEMENT

I thank the almighty God for His grace that sustained me through the entire research period.

This work would not have been possible without the contributions of the following persons; first are my supervisors Dr. Simon Nguluu and Prof. Josphert Kimatu who tirelessly helped to nurture the proposal concept into a fully developed project, to both of you, I greatly acknowledge your efforts and guidance.

Secondly, the staff at the South Eastern Kenya University (SEKU), who provided all the necessary assistance in making my work successful, I greatly appreciate your kindness.

Gratitude is also owed to the Chief Officer, Department of Agriculture Machakos County, Mrs. Damaris Mativo for the encouragement and support, especially in granting me permission to use the facilities at Machakos Agricultural Training Centre (ATC) to undertake this study. I also thank the staff of Machakos ATC for the much support they accorded me during my study.

I highly acknowledge the National Research Fund (NRF) for providing financial support towards this research.

Finally, I wish to appreciate my loving husband Dr. Elijah Muange, who tirelessly encouraged and gave both moral and technical support. To all my family members and friends that I cannot enumerate, I say big thank you for every small thing that you did that directly or indirectly contributed to this work.

May the Almighty God bless you all.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	**:**	Analysis of Variance
ASALs	**:**	Arid and Semi-Arid Lands
ATC	**:**	Agricultural Training Centre
BE	**:**	Biological efficiency
C/N Ratio	**:**	Carbon Nitrogen Ratio
cm	**:**	Centimeters
CO_2	**:**	Carbon Dioxide
DMRT	**:**	Duncan Multiple Range Test
FAO	**:**	Food and Agricultural Organization of the United Nation
g	**:**	Grams
GOK	**:**	Government of Kenya
Hrs	**:**	Hours
JKUAT	**:**	Jomo Kenyatta University of Agriculture and Technology
Kg	**:**	Kilograms
KIRDI	**:**	Kenya Industrial Research Development Institute
MC	**:**	Moisture Content
mm	**:**	Millimeters
NAFIS	**:**	National Farmers Information Service
NRF	**:**	National Research Fund
RCBD	**:**	Randomized Complete Block Design
SEKU	**:**	South Eastern Kenya University
SPSS	**:**	Statistical Package for the Social Sciences

ABSTRACT

Two major challenges facing arid and semi-arid areas (ASALs) of Kenya today are food insecurity and poverty, occasioned by low agricultural production. Diversification into low input agriculture like mushroom cultivation can help address these challenges. However, commonly used mushroom substrates in Kenya, (rice and wheat straws) are not widely and cheaply available. Several crop residues are found in the ASALs, while *Melia volkensii* is becoming popular agroforest trees in these areas. Literature suggests that these materials can serve as alternative substrates, but their effectiveness have not been adequately evaluated. This study aimed at evaluating the potential of different agro-waste materials as substrates for cultivation of phoenix oyster mushroom (*Pleurotus* spp.) in the ASALs of Kenya. Five different agro-waste materials and their combinations were tested, namely: maize stalks, beans straw, maize cobs, rice straw, and *Melia volkensii* leaves. The study was conducted from March - May 2019, and assessed the effects of these substrates on different mushroom growth parameters. All the substrates were routinely prepared with buffers and supplements and the experiment set in a randomized complete block design (RCBD). Relative humidity was maintained at 80 - 90% and an average room temperature of 23 - 24°C. Mushroom morphological data was collected over a 45-days harvest period and subjected to two-way analysis of variance (ANOVA) using SPSS (version 21) to detect differences between treatments. Correlation between mushroom growth parameters (days to first harvest, stipe length, cap diameter, fruit bodies, weight and biological efficiency) were also determined. Results showed that substrates containing *Melia volkensii* leaves failed to colonize fully, with only the bean straw + *Melia volkensii* leaves combination (BSMV) yielding little, while all the other substrates yielded mushrooms. Days to first harvest varied significantly, with the mean ranging from 35.1 to 48.1 days for the maize cobs + rice straw (MCRS) and BSMV, respectively. The tallest mushroom was obtained from rice straw (6.8 cm) and was followed closely by maize straw + bean straw combination (MSBS) with 6.7 cm, while the shortest was obtained from BSMV with 4.4 cm. The highest average number of marketable fruit bodies per 1kg of wet substrate bag was 9.5 from MSBS, while the lowest was from BSMV (6.2). The overall average yields per 1kg of wet substrate varied from 136.2 g in BSMV to 434.9 g in rice straw, while the average biological efficiency varied from 37.1% to 130.6% for BSMV and rice straw respectively. BSMV substrate gave the worst performance, taking the longest time to first harvest (48.1 days) and gave the lowest yields, indicating that *Melia volkensii* leaves are not suitable mushroom substrates. Further result showed positive correlation between cap diameter, fresh weight and biological efficiency. In conclusion, this study showed that combinations of maize stalks, bean straw and maize cobs are suitable alternatives to rice straw. Among the combinations, MSBS produced the highest (403.7 g) after rice (437.9 g), and therefore recommended as a suitable alternative substrate to where rice straw is not readily available or economical to use.

Key Words: Oyster mushroom, substrates, agro-waste materials, biological efficiency.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Agriculture is the mainstay of Kenyan economy (GOK, 2010). The long-term economic development blueprint for Kenya, the "Vision 2030", has identified agriculture as one of the key sectors to deliver a 10 percent annual economic growth rate (GOK, 2007). To achieve the envisioned growth rate requires improvement of agricultural productivity through diversification to high value crops and transformation of smallholder agricultural sector from subsistence to an innovative, commercially oriented modern sector (GOK, 2007). Food security is paramount to the ever-growing world population of the 21st Century, and scientists all over the world are continuously exploring ways and means to bring more food on the table. Venturing into edible mushrooms cultivation on local substrates is one such effort (Kinge *et al*., 2016).

Cultivated mushrooms have become popular all over the world with about 12 species grown for food and/or medicinal purposes. These species includes the Common mushroom (*Agaricus*), Shiitake (*Lentinus*), Oyster (*Pleurotus*), Straw (*Volvariella*), Lion's Head or Pom Pom (*Hericium*), Ear (*Auricularis*), Ganoderma (*Reishi*), Maitake (*Grifola frondosa*), Winter (*Flammulina*), White jelly (*Tremella*), Nameko (*Pholiota*), and Shaggy Mane mushrooms (*Coprinus*) (Marshall and Nair 2009). Oyster mushrooms (*Pleurotus* spp.) are distributed worldwide, and are being grown commercially on large and small scale basis in many countries (Bernabé-González and Cayetano-Catarino, 2009). About 80% of the mushroom grown in Kenya is oyster variety whilst 20% is button variety (Odendo *et al*., 2011). Since the cultivated mushroom does not require access to land, it is a viable and attractive activity for both rural farmers and peri-urban dwellers. Small-scale growing of mushrooms requires minimal capital investment and mushroom substrate can be prepared from various agricultural waste materials (Marshall and Nair 2009). The oyster mushrooms are renowned for good marketability and are relatively easy to grow.

Mushroom are macro fungus with distinctive fruiting bodies that are large enough to be seen with naked eyes, and they fall into four broad categories. The categories includes; edible mushrooms (e.g. *Agaricus bisporus*), mushrooms that are considered to have medicinal applications (e.g. *Ganoderma lucidum*), those proven or suspected to be poisonous, (e.g. *Amanita phalloides*) and those in a miscellaneous category, whose properties remain less well defined. These may tentatively be grouped together as "other mushrooms" (Chang and Miles, 2004). There are two types of edible mushrooms that are commonly being commercialized in Kenya, the button (*Agaricus bisporus*) and oyster (*Pleurotus* species). Of the two, oyster mushrooms are renowned for good marketability and are relatively easy to grow. They only require agricultural waste materials as the growing media (substrate), thus providing a more economically and environmentally sound disposal system for the agro-wastes (Kimenju *et al*., 2009). Mushrooms start as very small spores (reproductive structures like very tiny seeds in fungi). The spores grow in the substrate to produce a network of fine white filaments called mycelium (portion of the mushroom that grows underground). From the mycelium the mushroom fruit is produced and this is the part that is harvested.

Mushroom of *Pleurotus* spp. occupy the second position among cultivated edible mushrooms worldwide due to their nutrition and medicinal value (Khan *et al*., 2008). Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Belewu *et al*., 2005). They are also a good source of vitamins (B-complex and C), essential amino acids, and carbohydrates, but are low in fat and fibre and contain no starch. In general mushrooms on dry weight bases, composes of 10%- 40% proteins, 2%-8% fat, 3%-28% carbohydrates, 3%-32% fibers, 8%-10% mineral while when fresh they have a very high-water content of around 90-95% (Bogale, 2017). Mushrooms also have minerals present including phosphorus, potassium, iron, calcium, zinc and copper. They have high availability of lysine and tryptophan and other amino acids usually absent in cereals making them ideal food for patients suffering from hypertension, diabetes and weight-watchers (Pathania *et al*,, 2017). Oyster mushroom has also been reported to lower the cholesterol levels in the body (Poppe, 2000) and thus can serve as an alternative source of protein for

the vegetarians. Mushrooms have components of water-soluble polysaccharides obtained from the fruiting bodies which have ability to inhibit the growth of tumors. A major fraction of the acidic polysaccharide designated as H51 is reported to have strong antitumor activity, and structurally this component consists of a skeleton of b (1, 3)-linked glucose residues, probably having branches of galactose and mannose residues and also containing acidic sugars (Chang and Miles, 2004). Being organically grown, mushrooms are thus most recommended for cancer and HIV-positive victims.

Agricultural wastes disposal is of great concern in today's world as its mismanagement can pose a great risk in environmental pollution. Mushroom cultivation is another ecofriendly and cost-effective method of agricultural waste management. The fungus has a property of breaking down lignin cellulosic components that are always difficult to breakdown into simpler compound thus used to transform the less useful agricultural waste into valuable products that can later be utilized as manure on agricultural farms (Kamthan and Tiwari, 2017).

While wild edible mushrooms are popular, most people are not familiar with cultivated mushrooms (Chioza and Ohga 2014). This could be attributed to limited availability and lack of awareness on the economic, nutritional and medicinal benefits of cultivated mushrooms. However, the demand for mushroom has been increasing due to population growth, market expansions, changing of consumer behaviour and developments in the manufacturing to industries, storage, transportation and retailing. World mushroom production has gradually increased reaching 33.4 million tons in 2007 from 26 million tons in the year 2000 (Prince *et al*., 2016). As reported by Gwanama *et al*. (2011), Africa produces very small quantities of cultivated mushrooms, accounting for less than 1% of the world's total tonnage, with most of this production being done in South Africa. African countries have high potential for mushroom production due to availability of abundant materials from agricultural wastes. These agro-waste materials could be used as substrates for mushroom production. Kenya produced 500 tonnes of mushrooms per year as at 2014, of which 476 tons were button mushroom, against an annual demand of 1200 tonnes

(NAFIS, 2015). The mushrooms are produced by small-scale farmers and mainly in Western, Nyanza and Coastal areas. Only a handful of cultivators are present in the arid and semi-arid (ASALs) regions of Kenya, and the main substrate materials used are rice and wheat straws that are not locally available hence resulting to high cost of production.

1.2 Statement of the problem

Smallholder farmers in Kenya lack cheaper alternative substrates for oyster mushroom growing (Kimenju *et al.*, 2009); yet successful production of high-quality mushroom highly depends on the type, availability, and cost of substrates (Chitamba *et al*., 2012). Mushroom cultivation is important due to its high nutritional and medicinal value, employment creation and income generation, (Chang and Miles, 2004). Despite this, its demand in Kenya is largely unsatisfied. Little research has been carried out in Kenya to evaluate the suitability of locally available substrates in various agro-ecological environments. Some of the studies done on suitability of available mushroom substrates includes; Kimenju *et al,* 2009. They evaluated some substrates including; water hyacinth (*Eichhornia crassipes*), maize cobs (*Zea mays*), coconut fibre (*Cocos nucifera*), finger millet straw (*Seteria microcheata*), banana fibre (*Musa* sp.), sawdust (*Eucalyptus* sp.), rice straw (*Oryza sativa*) bean straw (*Phaseolus vulgaris*) and wheat straw (*Tritichum aestivum*) for suitability in mushroom production. In their study, the results showed that, bean straw, rice, finger millet and wheat straws had the highest biological efficiency (BE), respectively while the Sawdust had the least BE.

The main substrates used by most farmers for mushroom farming in the country are rice (*Oryza sativa*) and wheat (*Triticum aestirum*) straws. However, rice and wheat are grown only in few regions in Kenya, implying that their straws are not always cheaply available across the country. In the semi-arid regions of Kenya such as Machakos and Kitui, there are many other organic substrates that have high potential for use in mushroom production (Onyango *et al*., 2011). In those regions, maize (*Zea mays* L) and beans (*Phaseolus vulgaris*) are the staple foods and are widely cultivated by most households. Hence, maize straw (stalks), maize cobs, and bean straw are readily available. *Melia volkensii,* locally

known as "*Mukau"* in Kamba dialect, is becoming a popular agroforest tree in the ASAL (Orwa *et al*., 2009). These trees are occasionally pruned making available herbage that can be utilized as substrates for oyster mushroom cultivation. However, its effectiveness in mushroom production in the ASALs of Kenya has not been adequately evaluated.

1.3 Objectives of the study

1.3.1 Broad objective

The study aimed at contributing towards improved production of oyster mushrooms by identifying alternative growth substrates that are locally available in the arid and semi-arid lands of Kenya.

1.3.2 Specific objectives

i. To determine the effects of five substrates; bean straw, maize stalks, rice straw, maize cobs and *Melia volkensii* leaves on growth and productivity of oyster mushrooms.
ii. To assess interaction effects of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on oyster mushroom growth and productivity.
iii. To assess the correlations of different mushroom growth parameters as influenced by varied substrates and their combinations.

1.4 Null Hypotheses (H_0)

i. Maize stalks, maize cob, bean straw, rice straw and *Melia volkensii* leaves as mushroom substrates do not significantly influence the growth and productivity of oyster mushroom.
ii. There are no significant interaction effects of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* as mushroom substrates on oyster mushroom growth and productivity.
iii. There is no significant correlation in levels of different mushroom growth parameters as influenced by varied substrates and their combinations.

1.5 Justification of the study

Food insecurity and poverty are major development challenges in the semi-arid lands (ASALs) of Kenya. These areas cover 80% of Kenya's landmass (Mganga *et al*., 2010) and are characterized by low rainfall and high temperatures (Kahi *et al*., 2006). These areas like the rest of the country are currently facing a decline in per-capita land and climate change also threatening agricultural production and farm incomes. A potential solution to this challenge is diversification into high value enterprises like mushroom cultivation that will require less land, water and minimal pesticides. Mushroom cultivation is important due to its high nutritional and medicinal value, employment creation and income generation. Despite this, its demand in Kenya outstrips current production and this presents an alternative farming opportunity, especially for farmers in dry regions. The commonly used agro-waste products in mushroom growing (usually referred to as substrates) are rice and wheat straws, but these are not locally available in the semi-arid areas of Kenya. No study has evaluated effects of different substrates in the semi-arid regions of Kenya. Furthermore, the available studies conducted elsewhere in the country have not looked at use of different combination of substrates in mushroom production. There is need for studies to identify suitable substrates that are locally available in the semi-arid areas as cheaper alternatives for oyster mushroom production as conceptualized in figure 1.1.

1.6 Significance of the study

The mushroom substrates evaluated in this study are cheaper and available on most farms in the drier parts of lower Eastern Kenya. This study aimed at providing information on cheaper alternative substrates for small-scale farmers in these regions. It also provides information to academicians interested in further research. Similarly, policy makers may use the information to enhance farmers' income and food security.

1.7 Limitations of the study

There are several mushroom species that can be grown in the semi-arid areas of Kenya. However, due to time and resource constraints, this study was limited to only one species, *Pleurotus* spp. This species is the most cultivated since it is easier to cultivate, favorable to

eat, and grows economically on different kinds of organic waste raw material (Sitaula *et al*,.2018).

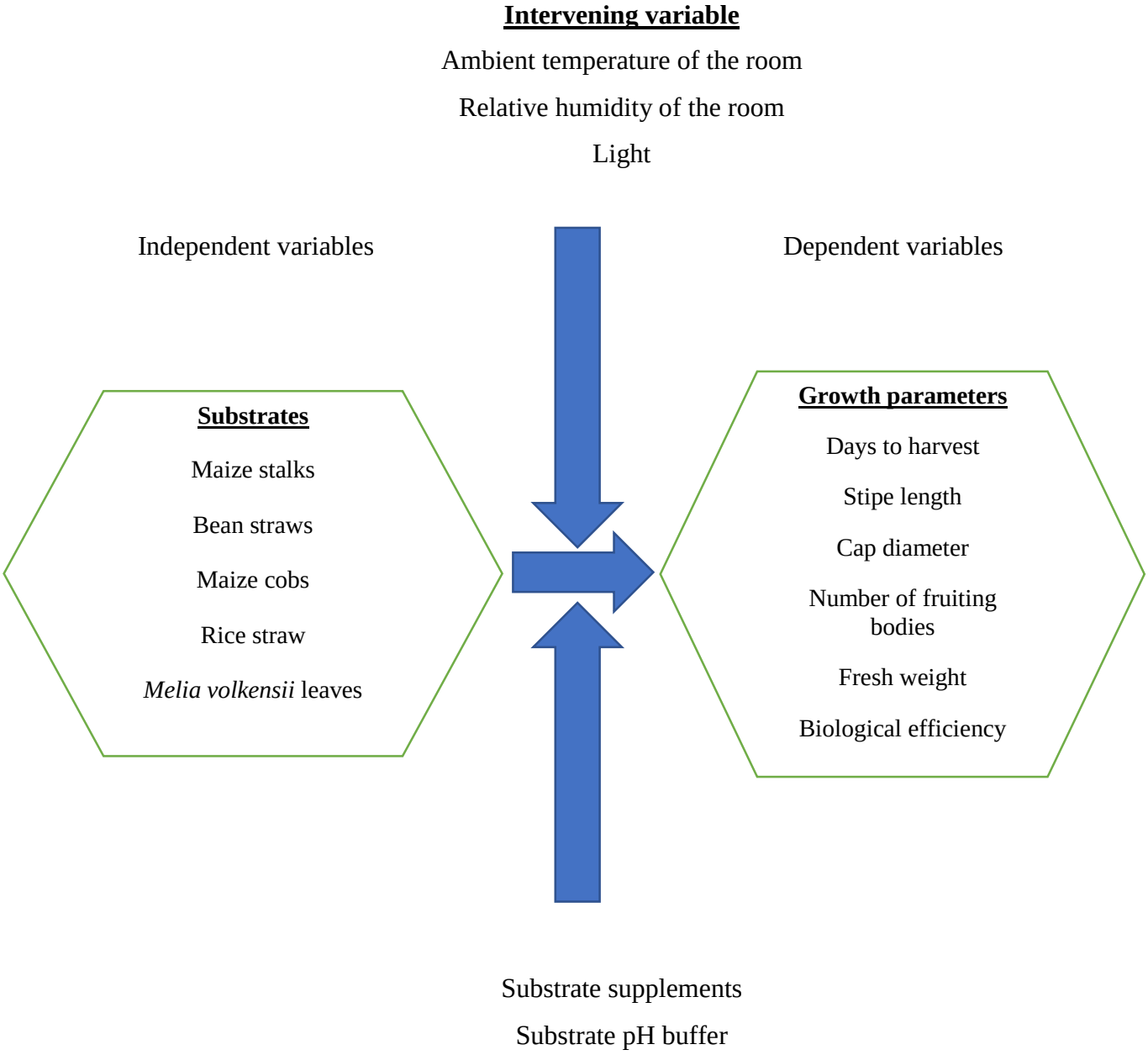


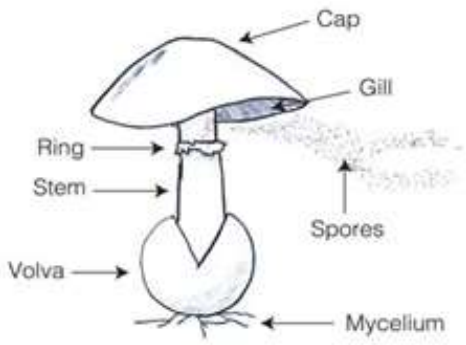
Figure 1.1 Conceptual Framework as adapted from (Mwami, 2017).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botanical aspects of Mushrooms

Mushrooms have been defined as fruiting bodies of macrofungus, similar to apples on a tree (Onuoha, 2007). Mushrooms can either be epigeous (lifted above ground in germination) or hypogenous (occurring below the surface of the ground) and visible enough with the naked eyes and can also be picked by hand.



1A: Different parts of Mushroom.

1B: Oyster mushroom.

1C: *Agaricus bisporus* showing pileus, gills, stipe, and annulus

1D: *Amanita phalloides* (extremely poisonous), showing pileus, stipe, annulus, and volva.

Figure 2.3: Plate 1A; Shows key mushroom parts, plate 1B, 1Cand 1D shows different mushroom species.

Source: Kang (2004) and Chang and Miles (2004).

There exists, an estimate of 1.5 million different species of fungi that comes in countless varieties and forms (Chang and Miles, 2004). Some are umbrella shaped with pileus (cap) and stipe (stem), e.g. *Lentinula edodes* and some species have an annulus (ring) e.g., *Agaricus bisporus* or a volva (cup), e.g., *Volvariella volvacea* or both, e.g., *Amanita phalloides*. Additionally, some mushrooms are in the form of pliable cups, and others are round like golf balls. Some are in the shape of small clubs; some resemble coral; others are yellow or orange jellylike globs; and some even resemble the human ear.

The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cordlike strands that branch out through the soil, compost, wood log or other lignocellulosic material on which the fungus is growing. After a period of growth, and under favorable conditions, the established (matured) mycelium produces the fruiting structure, which we call the mushroom (Chang and Miles, 2004). Various edible mushroom strains are cultivated worldwide. Some of them include; button (*Agaricus* spp.), Oyster (*Pleurotous* spp.), Shiitake (*Lentinula edodes*), *Volvallella volvacea* and the Chinese mushroom (*Ganoderma*), (Kamthan and Tiwari, 2017). Figure 2.1 shows the parts and some different types of mushrooms.

2.2 Mushroom biology

Mushrooms are fungi and they reproduce sexually and some asexually in a variety of ways (Ali, 2013). They lack chlorophyll and consequently cannot carry on photosynthesis. They are not able to manufacture their own food from simple inorganic materials, such as water, carbon dioxide, and nitrates, using energy from the sun, like the green plants.

Therefore, nutritionally the fungi are described as saprophytic as they obtain nutrients from nonliving organic materials, (Bogale, 2017). Mushrooms have been categorized into four major groups including the edible mushroom, e.g., *Agaricus bisporus,* medicinal mushrooms, e.g., *Ganoderma lucidum,* poisonous mushrooms, e.g., *Amanita phalloides and* those in a miscellaneous category, which includes a large number of mushrooms whose properties remain less well defined (Bogale, 2017). Mushrooms play an irreplaceable role

in the decomposition of organic materials in nature (Maszlavér, 2008), thus deriving their food from the complex organic materials found in dead or living tissues of plants and animals. Those obtaining their nutrients from dead organic materials like agricultural crop residues, wood of dead trees and animal dung, are referred to as saprophytic fungi while those deriving their food substances from living plants and animals and causing harm to the hosts are called parasitic fungi, (Rinker *et al.*, 2004).

2.3 Mushroom Nutrition (Substrates)

Mushrooms may be grouped into saprophytes, parasites and mycorrhizae depending on their trophic patterns with the most commonly grown being saprophytic, (Odero, 2009). Saprophytic mushrooms are often quite specific in their nutritional and ecological requirements. Some grow on fresh or almost fresh wood residues (e.g., *Lentinula, Pleurotus, Flammulina, Auricularia, Pholiota, Tremella, Agrocybe, Ganoderma*), others on only slightly composted lignocellulosic materials (e.g., *Volvariella, Stropharia, Coprinus*), some grow on well-composted materials or on animal dung (e.g., *Agaricus*) while others grow on soil and humus (e.g., *Lepiota, Leptista, Morchella, Gyromitra*). Moreover, some saprophytic mushrooms grow only on dead grass and straw, while some grow only on dead wood of specific tree species and shrubs. Some mushrooms prefer cool moist climatic conditions, whereas others grow only under warm climatic conditions. However, production techniques can be adjusted and adopted to local conditions to ensure continuous mushroom production throughout the year, (Lal, 2006).

Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose or lignin. *Pleurotus* species of mushrooms have high efficiency in degradation of a wide range of lignocellulosic residues, including wheat straw, cotton wastes, coffee pulp, corn cobs among others, (Poppe, 2000). Their mycelium produces enzymes (cellulases, hemicellulases and extracellular enzymes), which degrade lignocellulosic residues and use them as nutrients for their growth and fructification (Philippoussis, 2009). Lignins have a more complicated structure thus more difficult to break down than cellulose or

hemicellulose. The main extracellular enzymes participating in lignin degradation are lignin peroxidase, manganese peroxidase, and laccase, with manganese peroxidase proving to be the most common lignin-modifying peroxidase produced by almost all wood-degrading basidiomycetes (Philippoussis, 2009).

2.4 Cultivated mushrooms

The ever-growing need of cheap nutritious food and the lack of protein in developing countries led to the development of the mushroom cultivation industry (Alananbeh *et al*., 2014). There exist wild edible mushrooms that are seasonal, and are only available during the rainy seasons. The cultivated mushrooms are not popular with most people due to lack of awareness on their benefits (Chioza and Ohga 2014). Among the edible mushrooms, Oyster mushrooms (*Pleurotus* spp.) are widely cultivated all over the world (Uddin *et al*., 2011). The genus *Pleurotus* comprises about 40 different species commonly referred to as "Oyster mushroom". Among several species of this genus are; *Pleurotus ostreatus* (*P. ostreatus*), *Pleurotus sajor-caju* (*P. sajor-caju*), *Pleurotus.erygii* (*P. erygii*) and *Pleurotus pulmonarious* among others. *Pleurotus ostreatus* and *Pleurotus pulmonarious* are more popularly all over the world due to their taste, flavour, high nutritional values and medicinal properties. Due to the presence of numerous nutritional compositions and various active ingredients in the *Pleurotus* spp, they have been reported to have antidiabetic, antibacterial, anticholestrolic, antiarthritic, antioxidant, anticancer, eye health and antiviral activities (Deepalakshmi and Mirunalini, 2014). These species can also be cultivated and exploited for a profitable agribusiness (Mohamed *et al*., 2014).

2.5 Consumption of Mushrooms

Consumption of fresh mushrooms has been found to increase also due to their contribution of anti–β- glucan antibodies in the serum of humans, and provision of better defense against pathogens (Wasser, 2014). *Pleurotus* species is also the most preferred because it's easier to cultivate using the low-cost cultivation methods. It is also characterized by its rapid growth on agro-wastes such as olive cake, tomato tuff, pine needles, wheat straw,

and banana leaves, cotton waste, maize stover, palm oil and other wastes (Alananbeh *et al*., 2014).

2.6 Oyster mushrooms cultivation process

2.6.1. Spawn Preparation

Cultivation of oyster mushroom begins with spawn preparation. Study has shown that spawn medium is inoculated with a pure culture of fungal mycelium and is generally composed of a solid organic matrix such as grains (sorghum, rye, millet, or wheat) or a liquid broth (Friel and McLoughlin, 2000). After the grains in jars are inoculated with the fungi, they are then incubated in the laboratory and during that time, the grains are regularly shaken to prevent aggregation and spawn is usually ready within 10 to 14 days, (Bechara, 2007). Another literature by Ragupathi *et al*., (2016), showed that the grains for spawn preparation were half cooked and mixed with calcium carbonate @ 20g per kg of grains (dry weight), thoroughly mixed and filled in polypropylene bags provided with PVC rings as neck. The bags were tightly plugged with non-absorbent cotton and sterilized at 1.42-kg/cm2 pressure and 126 ºC temperature for 1.5-2.0 hours in an autoclave. When the bags were cooled, they were aseptically inoculated with fresh cultures of oyster mushroom fungus, and kept in a clean room for 15-20 days before use. Nazir *et al*. (2012) also stated that spawn was prepared from sorghum seeds in clean and autoclaved jam bottles. Supplemented with 2% calcium carbonate to maintain pH at (6.5-7.2), the bottles covered with lid and then kept in incubation chamber at 25+2^{o}C before use.

2.6.2. Growth conditions for oyster mushrooms

Growth of oyster mushrooms is greatly affected by a number of factors including nutrition factors, chemical factors and environmental factors (Chang and Miles, 2004). Some of the environmental factors includes; temperature, relative humidity, light, aeration, gravity, carbon dioxide and acidity of substrate among others. Nutritional factors include Carbon: Nitrogen (C/N) ratio, carbohydrates and vitamins requirements. Chemical factors include phenol oxidase and tyrosinase.

2.6.2.1 Nutritional Factors

Cellulose, hemicelluloses and lignin are the main nutritional sources for the growth of oyster mushroom (Hoa *et al*., 2015). The Carbon: Nitrogen (C/N) ratio of the substrate is an important factor for the optimal growth of any mushroom. According to Chang and Miles, (2004) any consideration of nutrition in mushroom growth must include a discussion of nitrogen requirements. Nitrogen is required at different levels at different stages of growth, minimum concentration of nitrogen necessary for fruiting body formation may be slightly greater than the concentration supporting mycelial growth. A high concentration of nitrogen encourages mycelial growth and decreases sporulation which is as a result of accumulation of toxic metabolic products or exhaustion of some essential metabolite due to the excessive mycelial growth. The carbon-to-nitrogen ratio (C: N ratio) is important in fruiting body formation and a C:N ratio of 20:1 is suitable, (Chang and Miles,2004).

Different cellulosic plant materials contain different amounts of nitrogen: sugarcane bagasse 1.20%, corn cobs have 1.16 % (Hoa *et al*., 2015), while maize stalks is 0.65%, and bean straw 0.64% (Lynch et al., 2016). Different studies give different levels of nitrogen in different materials. Ruiz-Vega *et al*. (2010) gave nitrogen levels in different materials as follows; Common beans, 2.32%, Crotalaria 1.94%, Mungo beans, 2.76% and Dolichos beans, 3.21%. Wheat straw contains about 0.62% nitrogen and rice straw has 0.8% total nitrogen (Kamthan and Tiwari, 2017). *Lentinula edodes* and *Pleurotus* spp. are fungi that can grow on wood. In addition to being distinguished by its high lignin content, wood can also be distinguished from other plant materials by its very low nitrogen content. Woody tissues contain 0.03 to 1.0% nitrogen as compared to 0.85 to 1.71% in herbaceous residues (Chang, 2009). The C/N ratio in most woody tissues is in the order of 350 to 500:1. Wood-inhabiting mushrooms are unique in that they can grow in such substrates. This suggests that these mushrooms can metabolise large amounts of carbohydrates, including lignin, in the presence of a very small amount of nitrogen. The optimum C/N ratio for *Agaricus bisporus* is about 17:1 for mycelial running, while *V. volvacea* is capable of growing on plant material with low nitrogen content (Chang, 2009). Oyster mushroom requires much carbon and less nitrogen than button mushroom (*Agaricus bisporus*) for its growth.

Nevertheless, most of the commonly used substrates need supplementation of a nitrogen source to reach optimal C/N ratio for oyster mushroom (Chang, 1999). However, there is no consensus on materials to be used for supplementation or the rates at which these should be applied.

2.6.2.2 Mushroom Substrates Supplements

A number of studies have evaluated substrate supplementation. For instance, Ruegger *et al*., (2001) carried out a study on cultivation of different edible mushroom species (*Oudemansiella canarii*, *Agrocybe perfecta* and *Pleurotus ostreatus*) in Brazil using sugar-cane bagasse and eucalyptus sawdust, both supplemented with wheat bran at two different rates (20% and 30%.) of the dry weight. According to their results, sugar-cane bagasse recorded higher productivity than eucalyptus sawdust. Moreover, the substrates supplemented with 30% wheat bran had higher mushroom productivity than the one supplemented with 20% wheat bran. In another study, Odero, (2009) used maize germ, rice bran and wheat bran to supplement wheat straw, finger millet straw, rice straw and bean straw at 3% (dry weight basis) while saw dust was supplemented with wheat bran at a rate of 5% (dry weight basis). The results showed that the best mycelial development and sporophore yields were observed on the sawdust substrate.

In his study on the effects of various substrates on comparative growth and yield performance of oyster mushroom, Kinge *et al*., (2016), used rice bran and corn flour as supplements in maize stover and on Eucalyptus sawdust substrates both in the ratio of 4:2. The results indicated that Eucalyptus sawdust on corn flour gave the best performance compared to the other substrates used for the cultivation of *Pleurotus ostreatus* in terms of growth and yield parameters measured.

2.6.2.3 Relative humidity and temperature

Oyster mushrooms require different environmental conditions at each growing stage. Mushroom productivity is greatly affected by environmental factors including temperature and relative humidity. Most studies have shown that relative humidity and temperatures

are paramount in mushroom productivity. According to Chang and Miles (2004), rising of room temperature increased relative humidity and optimum mycelial growth was found to occur over the range of 5°C to 33°C; whereas fruiting occurred only from 13°C to 24°C. The optimal temperature for mycelial growth was 27°C and for fruiting body formation 18°C to 21°C. Optimum relative humidity of 80-95% and room temperature of 24-28°C should be maintained in the cropping room while proper ventilation for gaseous exchange should also be observed in the chamber. Relative humidity is maintained by spraying water twice a day on the walls and floor of the room. Ragupathi *et al*., (2016) and Sitaula *et al*., (2018) maintained the temperatures at 20-30°C and a relative humidity of 80-90% at various growing stages of mushroom by hanging the wet jute bags around the wall of the house and keeping the floor wet. Water was sprayed about 4-5 times a day on the jute bag and floor. Upon the completion of incubation, pinning induction is done by changing the environmental conditions in order to discontinue the vegetative growth of mycelia and convert to a reproductive growth mode, which initiates fruit body formation. Pinning induction includes cold shock, watering and lighting. Once the pins come out, the pinning induction should stop and environmental conditions that are favorable to fruiting are maintained. Fruit body formation requires high relative humidity (80- 95%) and a temperature 10^{o}C lower than that of optimal mycelial growth. According to Kamthan and Tiwari, (2017), the optimum conditions for temperatures during cultivation of oyster mushrooms ranges between, 15-35°C, and humidity between 86-90%.

2.6.2.4 Aeration

Most fungi require adequate aeration for vegetative growth, and the requirements for fruiting are even more stringent. Generally, fruiting bodies of higher fungi typically form best under conditions of good aeration and failure of fungi to fruit is frequently attributed to the accumulation of carbon dioxide from respiration, (Chang and Miles, 2004). According to Kamthan and Tiwari, (2017), the optimum Carbon dioxide (CO_2) requirement levels for oyster mushrooms growth range between 15-20%. Improper management of the aeration in the mushroom house can also lead to malformation of fruiting bodies and poor primordial formation due to a high concentration of CO_2 (Chang and Miles, 2004).

2.6.2.5 Light

Many fungi are apparently uninfluenced in reproduction by light in the visible range; i.e., they do equally well in darkness, continuous light, or alternating darkness and light. However, there are some fungi that do not fruit without light, among the Ascomycetes are the *Pyronema and Coprinus cinereus* among the Basidiomycetes, light should be regulated, with some strains needing light intensity of 50-500 lux for primordial formation (Kang, 2004) while according to Chang and Miles, 2004, light intensity of about 1600–3200 lux should be maintained in the cropping room while proper ventilation for gaseous exchange should also be observed in the chamber.

2.6.2.6 Hydrogen Ion Concentration (pH)

Kamthan and Tiwari, (2017), reported that the optimum pH during cultivation of oyster mushrooms ranges between 6.0 and 6.5. Different studies have shown that different species differ in their optimal pH values for fruiting (Odero, 2009). During the course of an experiment the pH value of the medium may change because the fungus has produced metabolites, e.g., organic acids that affect the hydrogen ion concentration, (Chang and Miles, 2004).

2.6.3 Substrates for oyster mushroom cultivation

Mushroom substrate may be simply defined as a kind of lignocellulosic material which supports the growth, development, and fruiting of mushroom mycelium (Chang and Miles, 2004). Substrates for edible mushrooms vary depending on the species. While species like *Agaricus*, which includes button mushroom, require composted substrates, oyster mushroom can be grown on uncomposted organic materials (Tisdale, 2004).

Over the last two decades, research on suitable substrates for mushroom growth has been of interest to the scientific community (Gregori *et al*., 2007; Kumari, *et al*., 2008; Datta, 2014). However, there seems to be no consensus on the best substrate for mushroom cultivation. Several literatures have revealed that it is possible to use various agro- waste materials in mushroom cultivation. A study in India by Pathania *et al*., (2017), used apple

pomace, wheat straw and a combination of the two substrates. Apple pomace gave the highest performance, followed by the combination of wheat straw + apple pomace while wheat straw gave the least yield in his study.

In their study, Nazir *et al.*, (2012) used corn cob, wheat straw, rice straw and sugarcane bagasse as substrates on evaluation of yield parameters and nutritional aspects of different strains of Oyster mushrooms. He observed that mushroom mycelial growth was fastest in corn cob in comparison to the other substrates used.

Dlamini *et al*., (2012) used banana leaves, sugarcane tops, maize stalks and maize stover + maize cobs combination in the ratio of (1:1) to evaluate growth and yield response of oyster mushroom on different substrates. They observed that maize stover + maize cob combination yielded the highest among the four substrates. An experiment by Sitaula *et al*., (2018), was conducted at Rampur, Chitwan, Nepal to find out the growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) using four different substrate i.e. paddy straw (100%), maize cob + paddy straw (1:1), sugarcane bagasse + paddy straw (1:1) and sawdust + paddy straw (1:1). Several parameters were taken for the observation during the experiment including the fresh weight the biological efficiency of mushrooms of various substrates. Among the substrates used, the biological efficiency (BE) was found to be highest in case of the paddy straw (96.30 %) followed by maize cob + paddy straw (1:1), sugarcane bagasse + paddy straw (1:1) and sawdust + paddy straw (1:1) respectively.

In Kenya, Wachira, (2003), compared sugarcane waste (bagasse), maize cobs, saw dust and papers, as substrates for oyster mushroom cultivation and concluded that bagasse was the best among the four. Similarly, Kimenju *et al*., (2009) evaluated the suitability of ten different materials as substrates for oyster mushroom production in Kenya and reported bean straw as the best substrate in terms of yields, followed by rice, finger millet and wheat straws, banana leaves, maize cob, bagasse, coconut fiber, water hyacinth and saw dust, in that order. Another study conducted at Kenya Industrial Research Development Institute (KIRDI), Kenya, by Musieba *et al*. (2012), evaluated the growth and yield performance of

the Kenyan Indigenous Golden Oyster Mushroom (*Pleurotus citrinopileatus* Singer) on seven substrates. The substrates used were; bean straw, sawdust of African mahogany, rice straw, maize cob, wheat straw, sugarcane bagasse and banana leaves. The study showed that bean straw was the best in terms of yields and biological efficiency.

The conclusions of each study in terms of the yields and performance of mushroom depend on the type of materials used, and handling of the substrates. The use of recommended substrate materials by farmers depends to a large extent on their availability and affordability. In the Kenyan case, wheat and rice straw are the commonly used substrates for mushroom production, yet they are not readily available in most semi-arid areas as the production of these crops is uncommon. Studies have also shown that locally available substrates similar to those found in the Eastern semi-arid regions of Kenya can be suitable for Oyster Mushroom growing (Bogale, 2017). Hence, there is the need to evaluate agro-waste materials from commonly cultivated crops in local conditions in South Eastern Kenya to establish their suitability for oyster mushroom production.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Area of Study

The experiment was conducted at Machakos Agricultural Training Centre (ATC), in Machakos County between March and May, 2019, whereby an existing room within the farm facility was renovated and used to set the experiment. The center is located near Machakos people's park, about 4.5 Km from Machakos town. The Centre has the following latitude and longitude coordinates; 1.544742^0 S and 37.240981^0E respectively. An average room temperature of 23±1°C was maintained during the period of cultivation.

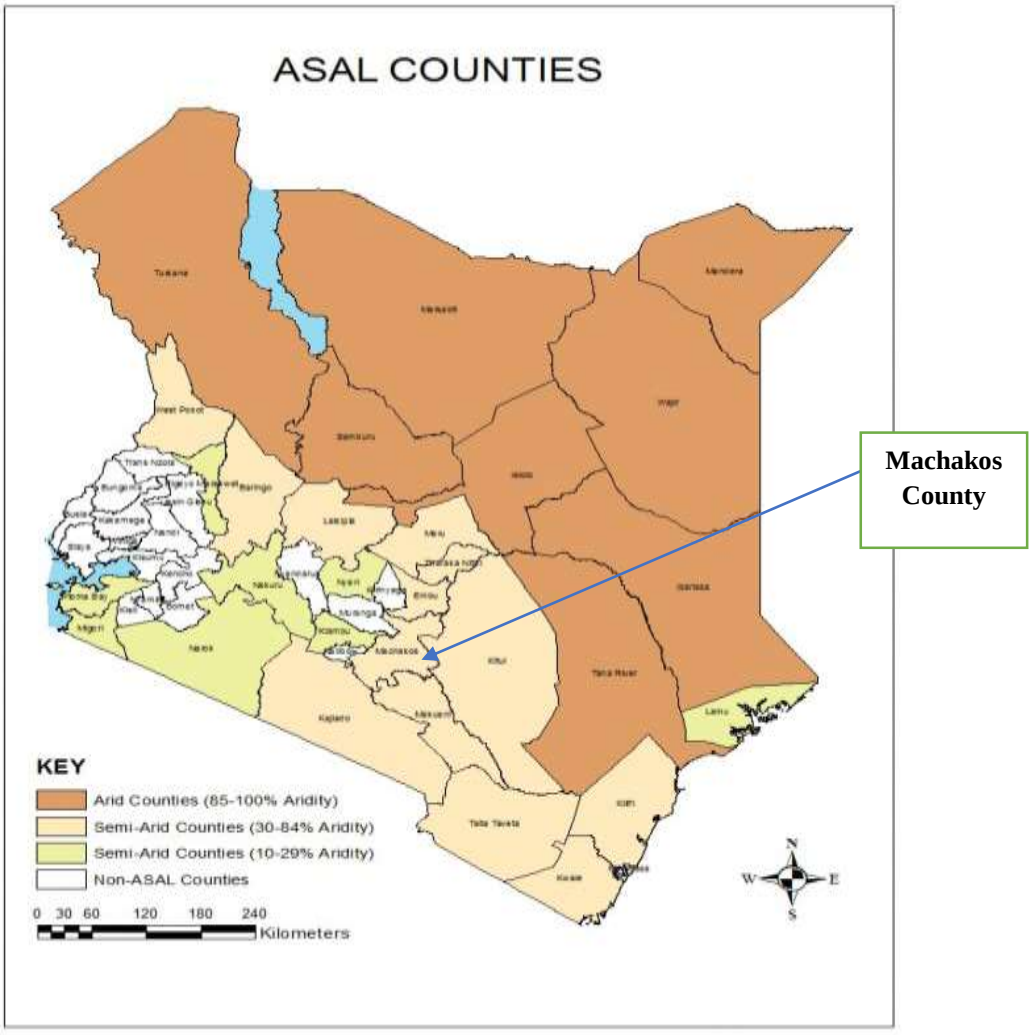


Figure 3.1 Map of the study area

3.2 Treatments and experimental design

Five dry substrates materials were used in this experiment. These were maize stalks, bean straw, maize cobs, rice straw and *Melia volkensii* leaves. Each of these substrates was used at a rate of 1kg wet weight, and combination of each two substrates each weighing 500gms wet weight was used making a total of 15 treatments. Hence the experiment consisted of the following treatments;

Table 3.1: Treatments: Substrates and their combinations

Treatment	Substrate	Composition
T1	Substrate 1	Maize stalks
T2	Substrate 2	Bean straw
T3	Substrate 3	Maize cobs
T4	Substrate 4	Rice straw
T5	Substrate 5	*Melia volkensii* leaves
T6	Substrate 6	Maize stalks + Bean straw (MSBS)
T7	Substrate 7	Maize stalks + Maize cob (MSMC)
T8	Substrate 8	Maize stalks + Rice straw (MSRS)
T9	Substrate 9	Maize stalks + *Melia volkensii* leaves (MSMV
T10	Substrate 10	Bean straw + Maize cobs (BSMC)
T11	Substrate 11	Bean straw + Rice straw (BSRS)
T12	Substrate 12	Bean straw + *Melia volkensii* leaves (BSMV)
T13	Substrate 13	Maize cobs + Rice straw (MCRS)
T14	Substrate 14	Maize cobs + *Melia volkensii* leaves (MCMV)
T15	Substrate 15	Rice straw + *Melia volkensii* leaves (RSMV)

The treatments were replicated three times and arranged in a randomized complete block design (RCBD) and six culture bags per treatment. Paddy straw served as the control as it is the most commonly used substrate for the growth of the mushroom.

Plate 3.4: The Mushroom growth chambers showing the RCBD at the ATC. The sprays were to sterilize the air around the treatments.

3.3 Spawn and substrates

Mushroom spawn (Phoenix oyster mushroom, *Pleurotus* spp.) were purchased from Jomo Kenyatta University of Agriculture and Technology (JKUAT). Dry maize cobs from Duma 43 variety, dry maize stalks (Duma 43 variety) and dry bean straw (KAT B1 variety) were obtained from farms within Machakos County, *Melia volkensii* leaves were collected from farms in Kitui County and sun dried, while dry rice straw from Basmati varieties was obtained from paddy rice farmers in Mwea irrigation scheme.

3.4 Substrate preparation

Substrate preparation followed whereby it involved buffer and supplements addition, then sterilization. Sterilization of substrates is a common practice in mushroom production. In a number of studies, the procedure is done in a laboratory, but in this study, physical sterilization of substrate was followed; drawing from the findings by Caral *et al*., (2013) that this method gives better results than chemical sterilization. The experiment adopted a procedure recommended by the ministry of Agriculture and the one similar to a study by Mamiro *et al*., (2014). All the materials were ground separately into small pieces of 5-10mm using a shredding machine which had a sieve of 10mm diameter. They were then weighed into 10 kilograms each, put into sacks and soaked into water for 24hrs to attain

adequate moisture content. The materials were then hanged to drain excess water from the substrate until only 2 or 3 drops came out when the fist squeeze test was applied in order to ensure moisture retention of 65% to 75%.

The optimal pH range for production of *Pleurotus* spp. is 5.4-6.0 (Chang and Miles, 2004). The commonly used buffers are; Calcium carbonate at a rate of 0.2% - 1% of the substrate wet weight (Uddin *et al*., 2011; Ruegger *et al*., 2001) and gypsum at 1% - 2% of the substrate wet weight (Chang, 2009). Calcium Carbonate at 1% of the wet weight of substrate is the most commonly used in experimental and commercial mushroom production in Kenya. Therefore, the same was used in this experiment where 1 % calcium carbonate (lime/buffer) was also added into the wet substrate materials, with the aim of raising the pH. Supplement of wheat bran was also added up to 25% of substrate dry weight making a mixture of substrate material, buffer and supplement, usually referred to as the substrate mixture. The straw, supplement, buffer and water, referred to as the substrate mixture, were then well blended on a polythene sheet. The blended mixture was then put in the smaller (9" x 15") mushroom polythene bags ¾ ways and pressed firmly. The bags were then closed but a plastic neck and sterile cotton wool were introduced to make a breather.

Decontamination through steaming for 2 hours then followed where clean drums were filled with water to about 4 inches height, then iron screen placed inside so it was out 1 inch higher than water. The bags were then placed in pasteurization system until full and the drum was tightly covered. The lid had a small hole (5mm in diameter) made for reducing pressure build up. After decontamination, care was taken to prevent contaminants from entering the substrate. To achieve sterile conditions in the production room, 99.5% iso-propyl- alcohol was sprayed on the clean working surfaces. The substrate was cooled to 28^{o}C and then spawn was added under sterile conditions. Different studies have used different rates of substrate amounts; 200gms per packet (Ruegger *et al*., 2001), 250gms per packet (Kimenju, *et al*., 2009), 500gms per packet (Uddin *et al*., 2011; Datta, *et al*., 2014) but in this study, substrates and substrate mixture of 1kg was used.

A B

Plate 3.2**:** Shredded substrates of rice straw used to increase surface area (plate A) and sterilization chambers at ATC Machakos (plate B).

3.4.1 Spawning

Spawning is the inoculation of the substrate with mushroom mycelia. Different spawning rates have been used in different studies. Reugger *et al*. (2001) used 3gms of spawn in 200gms of substrate (1.5% spawn rate) while Kimenju *et al*. (2009) used a spawn rate of 3-5%. A training manual on mushroom cultivation technology by Chang (2009) recommends a spawn rate of 2%-4%. Therefore, in this study, a few grains of the spawn were added to each bag at a spawn rate of 5% of substrate wet weight used.

3.4.2 Incubation

The experiment was set in a randomized complete block design (RCBD), with three replicates per treatment. This was done by having similar experimental units grouped into 3 blocks, or replicates whereby the spawned mushroom bags were tagged properly and arranged in the three shelves in the chamber; the top shelves, middle and the lower shelves. The treatments were randomly distributed within the three blocks (Appendix 2: The layout of the experiment). This was done in order to account for any variations in the experiment due to lighting effects within the room since the room had only one window on one side thus light was not uniformly distributed within the room. The window within the chamber was initially covered with opaque curtains to ensure darkness within the room and the temperatures ranged within 20^{o}C-24^{o}C to let the mushroom mycelia grow through the substrate. This is referred to as incubation, colonization or spawn run. After all the bags

were fully colonized, some lighting was allowed into the room by withdrawing the curtains. The mushroom bags were opened by making 3 holes of 5cm diameter on each bag. During incubation, a relative humidity of between 75-90% in the growing room was maintained by spraying the growing room and bags 3 times a day with a mist of water until the water could starts dropping from the walls and bags. Since mushroom fungi is aerobic, fresh air especially during reproductive stage was ensured by opening the window since high CO_2 concentration makes the mushroom fruiting bodies look unappealing, i.e. swollen stipes (stems). The room temperatures were monitored using a thermometer while the room humidity was monitored using a hygrometer.

3.4.3. Harvesting

The process of harvesting involved the removal of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The mature mushrooms were held on their stipe below the pileus and close to the substrate level and were gradually pulled out. Harvesting was done continuously over a period of 45 days, with harvesting intervals of three to five times for each bag of substrate. All fruiting bodies of a particular substrate bag were harvested at the same time since each bag had to be watered after harvest. Watering was done by spraying a mist of water on the room and on the substrate bags, three times daily. This was done to enable the substrate to have moisture that enables fruiting to occur again for harvest.

3.5 Morphological data collection

Data was collected from five bags in each replicate and the parameters measured were; fresh weight of mushrooms, stipe height, number of fruiting bodies, diameter of pileus, and days from spawning to harvesting, harvesting intervals and Biological efficiency (BE). The yields of the mushroom on the different substrates which was determined by the fresh weight of mushrooms, number and size of the fruit bodies produced during the first 45 days (5 flushes), time from spawning to first harvest was counted and recorded, the data was collected from different replicates and the mean of each set of data calculated. The fruiting

bodies of the fungus were harvested daily or on the second day, depending on observed maturation.

3.5.1 Number of fruit bodies

Harvesting of the well-developed fruiting bodies was done, the process which involved the removal of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The number of mature fruit bodies per bag were counted for each treatment separately and recorded. Tiny dry pinheaded fruiting bodies were discarded and not included in the counting. The mean number of fruiting bodies was then calculated.

3.5.2 Stipe length

In order to measure the stipe length, the mature caps were held on their stipe below the pileus and close to the substrate level and gradually pulled out. Three samples of fruiting bodies were randomly selected from each harvested bunch per bag; large, medium and small sized caps. Stipe length of the three fruiting bodies were measured in centimeters using a ruler, from the base of the stipe where it was attached to the substrate to the point where the gills on the pileus start on the stipe and measurements recorded.

3.5.3 Diameter of pileus

In order to get the diameter of pileus, harvesting of the mature fruiting bodies was done on each treatment. Like in the measuring of the stipe length, using visual judgement, three samples of fruiting bodies were randomly selected from each harvested bunch; large, medium sized and small sized caps. The diameter of pileus was measured in centimetres using a string to get the circumference of the pileus, then the string measurement taken using a ruler, and then the diameter calculated using the formula:

$$\text{Diameter} = \text{Circumference}/\pi, \text{ Where } \pi = 3.14 \quad (1)$$

This formula was used to compute the diameters of three fruit bodies, from which the mean pileus diameter was calculated since oyster mushrooms pileus, are not perfectly uniform in size.

3.5.4 Mushroom weight

Harvesting of mature fruiting bodies was done and the fruiting bodies per treatment weighed immediately after harvest using an electronic balance. Each weight per bag and per treatment was recorded. Watering of the bags was done after every harvest to enable the substrate to have adequate moisture that enables fruiting to occur again.

Plate 3.3: Weighing of oyster mushroom from various substrates

3.5.5 The harvesting intervals

This involved counting the number of days from spawning to the first days of harvesting, which took place three to four days after the emergence of pinheads and mature mushrooms harvested. Subsequently, the harvesting intervals from the first flush, to the last per bag per replicate and per treatment were recorded by recording the dates for each harvest.

3.5.6 Biological efficiency (BE)

Mushrooms are involved in degradation of lignocellulosic residues and the productivity of the conversion is expressed by biological efficiency (Philippoussis, 2009). BE is expressed

as a ratio (percentage) of fresh fruiting body weight (g) per dry weight of substrates (g) (Hoa et al., 2015). The BE was worked out for each substrate using the expression:

BE = (Mushroom fresh weight / Substrate dry weight) x 100

All the above measurements were done on 5 selected bags per replicate per treatment, after harvest and then the means calculated.

3.6 Data Analysis

The collected data was subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Tests (DMRT) used to compare the mean significant differences ($p \leq 0.05$) among treatments by using computer software Statistical Package for Social Science (SPSS) version 21. The pairwise treatment comparisons were done and presented in a tabular form whereby means were followed by superscripted letters allowing the reader to infer at a glance whether the treatments means were significantly different ($P \leq 0.05$) or not. Means followed by at least one common letter were not significantly different, while means with no common letter were significantly different at ($P \leq 0.05$) significance level (Piepho, 2018).

CHAPTER FOUR

4.0 RESULTS

4.1.1 Effects of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* on days from mushroom spawning to harvesting

Incubation took about 4 weeks for most substrates, with the end of incubation being marked by mycelia fully colonizing the substrate turning it completely white, after which pinning followed. There were no bags showing signs of other fungal contamination during the experiment. Among the five substrates, *Melia volkensii* had insignificant mushroom spawn run of about 5% and after 7 days the life cycle ceased to continue, thus no mushroom primordia induction nor mushroom fruiting bodies were obtained, and therefore no mushroom data was obtained from *Melia volkensii* leaves substrate.

A **B**

Plate 4.1.1 Plate A: Spore germination in *Melia volkensii*, Plate B: 100% colonization observed in some other substrates

Table 4.1.1 below shows the results on duration (days) taken by mushroom from spawning to harvesting in different substrates for four harvests. Days from spawning to first, second and fourth harvest were significantly different ($P \leq 0.05$) across all the four treatments. However, no significant differences ($P \leq 0.05$) were observed in days from spawning to third harvest. During the first harvest, mushroom took the shortest time in rice straw (40.5 days), followed by maize stalks (41.7 days), bean straw took 43.9 days while maize cobs took 44.1 days. The duration taken in rice straw was not significantly different ($P \leq 0.05$) from

that taken in maize stalks, but it differed significantly from that taken in maize cobs and bean straw. However, there was insignificant difference ($P \leq 0.05$) in the days taken from spawning to first harvest between maize cobs and bean straw. During the second harvest, there was also significant difference ($P \leq 0.05$) in the duration mushroom took from spawning to harvest in rice straw (49.9) and bean straw (56.3) substrates. However, the duration did not differ significantly ($P \leq 0.05$) in bean straw (56.3), maize cobs (54.7 days) and maize stalks (53.5 days). During the fourth harvest the duration differed significantly ($P \leq 0.05$) between rice straw (61.9 days) and in bean straw (72.8 days), but not in maize cobs and maize stalks. The overall mean of number of days to first harvest for the mushrooms in all the four substrates was 42.6 days.

Table 4.1.1 Effects of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* on days from mushroom spawning to harvesting

	Mushroom number of days from spawning to harvesting			
Treatments	1st harvest	2nd harvest	3rd harvest	4th harvest
Rice straw	40.5^{b}	49.9^{b}	60.1^{a}	61.9^{b}
Maize cobs	44.1^{a}	54.7^{ab}	60.8^{a}	70.1^{ab}
Maize stalks	41.7^{ab}	53.5^{ab}	63.5^{a}	66.7^{ab}
Bean straw	43.9^{a}	56.3^{a}	66.2^{a}	72.8^{a}
Overall mean	**42.6**	**53.6**	**62.44**	**67.4**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.1.2 Effects of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* leaves on harvesting intervals

The results on mushroom harvest intervals are show in table 4.1.2 below. The mean length of time taken by mushrooms between the first and the second harvest varied between 9.4 days in rice straw, 10.6 days in maize cobs, 11.8 days in maize stalk and 12.4 days in bean straw. The duration from the second to third flush was 10.2 days in rice straw, 6.1 days in maize cobs and 10 days in maize stalks, while it was 9.9 days in bean straw. The mushroom harvest intervals between third and fourth harvest was shortest in rice straw (1.8 days) and in maize stalks (3.2 days), while it was 9.3 days for the maize cobs and 6.6 days for the bean straws. The average number of days between the first harvest and the second harvest for all the four substrates was 11 days while it was 8.8 and 4.9 days between 2nd harvest and third and between third and four harvest respectively.

Table 4.1.2 Effects of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* leaves on harvesting intervals

Treatments	Days between mushroom harvest intervals		
	1st -2nd Harvest	2nd -3rd Harvest	3rd -4th Harvest
Rice straw	9.4	10.2	1.8
Maize cobs	10.6	6.1	9.3
Maize stalks	11.8	10	3.2
Bean straw	12.4	9.9	6.6
Overall mean	**11**	**8.8**	**4.9**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.1.3 Effect of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* leaves on mushroom stipe length (cm), cap diameter (cm) and number of fruiting bodies

The results for effects of different substrates on mushroom stipe length, cap diameter and fruiting bodies are shown in table 4.1.3 below. The tallest mushroom was obtained from maize cobs (5.8cm) while the shortest was obtained from bean straw (4.9cm) and they were both significantly different ($P \leq 0.05$) from each other. The mean stipe length mean for rice (5.7cm) and for maize cobs (5.8cm) were not significantly different ($P \leq 0.05$), while the overall mean stipe length for the four substrates was 5.4cm.

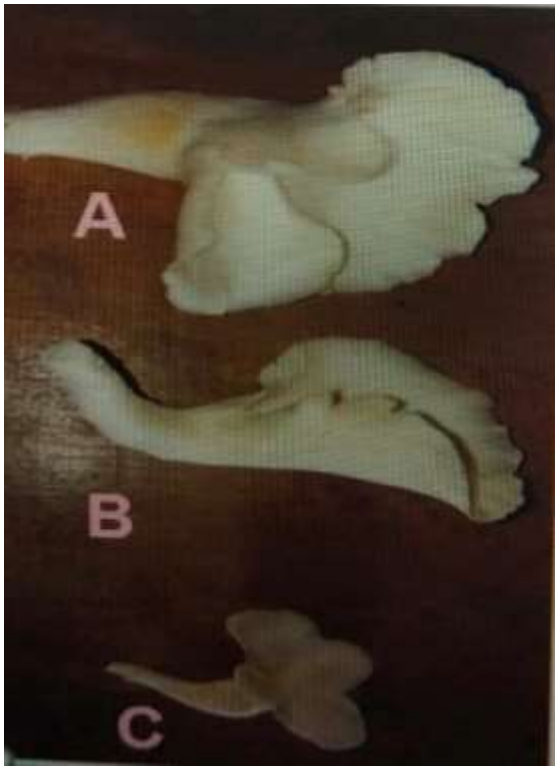


Plate 4.1.2: A shows the large sized fruiting body, plate B Medium sized cap, and plate C, Small sized cap.

The mean mushroom cap diameter in rice straw (8.1cm) varied significantly ($P \leq 0.05$) with the cap diameter in maize stalks (7.3cm) and but it was not significantly different ($P \leq 0.05$) from all the other two substrates (Table 4.1.3). The mean mushroom cap diameter varied

between 7.3cm to 8.4cm in maize stalk and bean straw, respectively and were significantly ($P \leq 0.05$) different from each other. The overall average cap diameter was 8.0cm, table 4.3. The mean number of fruiting bodies ranged from 6.6 in maize stalks to 9.3 in rice straw, and they were both significantly different ($P \leq 0.05$) from each other. The mean number of fruiting bodies in rice straw (9.3) and in bean straw (8.5) were not significantly different ($P \leq 0.05$) from each other while they both differed significantly ($P \leq 0.05$) from the mean number of fruiting bodies in maize stalks. However, the mean fruiting bodies in maize cobs (7.7) was not significantly different ($P \leq 0.05$) from the other three substrates, table 4.1.3.

Table 4.1.3 Effect of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* leaves on mushroom stipe length (cm), cap diameter (cm) and number of fruiting bodies

Treatment	Mean stipe length(cm)	Mean cap diameter (cm)	Mean number of fruiting bodies
Rice straw	5.7^{a}	8.1^{a}	9.3^{a}
Maize cobs	5.8^{a}	8.3^{a}	7.7^{ab}
Maize stalks	5.3^{ab}	7.3^{b}	6.6^{b}
Bean straw	4.9^{b}	8.4^{a}	8.5^{a}
Overall mean	**5.4**	**8.0**	**8.1**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.1.4 Effect of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* on oyster mushroom weight (Ranked - largest) and biological efficiency

The results showed that, different substrates had significant difference ($P \leq 0.05$) on yields of mushrooms. The mean fresh weight of mushroom was highest in rice straw (434.9 grams) and lowest in maize stalk (223.1 grams) and the weight difference was significantly different ($P \leq 0.05$) from each other. The mushroom mean weight in rice differed significantly ($P \leq 0.05$) from all the other three substrates, while there was no significant difference ($P \leq 0.05$) in mushroom weight between maize cobs (336.1 grams) and bean

straw (284.1 grams), and between bean straw and maize stalks. The overall mean fresh weight of the mushrooms per 1kg bag of wet substrate was 319.6 grams, (table 4.1.4). The mushroom average biological efficiency in rice straw (130.6%) was significantly different ($P \leq 0.05$) from the other substrates. Similarly, there was a significant difference ($P \leq 0.05$) between the biological efficiency in maize cobs (106.0%) and in the other substrates, while there was no, significant difference ($P \leq 0.5$) between the biological efficiency in bean straw (80.9%) and in maize stalks (71.0). The overall mean biological efficiency among the four substrates was 97.2% (table 4.1.4).

Table 4.1.4 Effect of rice straw, maize cobs, maize stalks, bean straw and *Melia volkensii* on Oyster mushrooms weight (Ranked - largest) and biological efficiency (%)

Treatment	Mushroom weight in grams per bag and biological efficiency (%)	
	Total Weight (g)	Biological efficiency (%)
Rice straw	434.9[a]	130.6[a]
Maize cobs	336.1[b]	106.0[b]
Bean straw	284.1[bc]	80.9[c]
Maize stalks	223.1[c]	71.0[c]
Overall	319.6	97.2

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.1 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves substrates on days from spawning to harvesting of oyster mushrooms, *Pleurotus* spp.

All the substrates that had *Melia volkensii* leaves combinations failed to colonize full with only bean straw combination having mycelial colonization of up to 70% of the bag. The other *Melia volkensii* substrate combinations went up to 30% for maize stalks and maize cobs combination and up to 40% for the rice straw combination. There was no primordial

induction experienced in the *Melia volkensii* leaves substrates combinations apart from the beans straw that yielded little. Therefore, no data on mushroom growth parameters was obtained from the *Melia volkensii* leaves substrates combinations with maize stalk, maize cobs, and with rice straw. The interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii*, and their combinations on days from spawning to harvesting is shown in table 4.2.1 below. There was significant interaction ($P \leq 0.05$) effect in maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii*, and their combinations on days from spawning to first, second, third and fourth harvests. The results (Table 4.2.1) showed differences in the duration the mushroom took from spawning to harvesting across all substrates during the harvesting. During the first harvest, the duration was shortest in maize cob and rice straw (MCRS) combination (35.1 days) followed by maize stalks + maize cob (MSMC) combination (36.4days) and maize stalks + bean straw (MSBS) combination (37.9 days). Days to first harvest in MCRS were significantly different ($P \leq 0.05$) from MSBS but not from MSMC. The longest duration was experienced in bean straw + *Melia volkensii* (BSMV) combination with 48.1 days and was significantly different ($P \leq 0.05$) from all the other substrates. The average number of days it took for the mushrooms to first harvest was 41.9 days. During the second harvest, the duration was shortest in maize stalk + maize cob combination (48.2 days) followed by maize stalk + beans straw combination (49 days) and maize cob + rice straw combination (50.3 days) but was not significantly different ($P \leq 0.05$) from each other, but was significantly different ($P \leq 0.05$) from BSRS, MSRS, BSMC and BSMV. Mushrooms took longest duration from spawning to second harvest in MSRS with 57.5days, BSMC combination (57.3 days) and in BSMV (56.5 days), but was not significantly different ($P \leq 0.05$) from each other. The average number of days it took for the mushrooms to second harvest was 53.2 days. Similarly, the duration was shortest in MSMC combination (61.7 days) during the third harvest and this differed significantly ($P \leq 0.05$) by the longest duration, 69.8 days in BSMV. The average number of days it took for the mushrooms to third harvest was 64.4 days. During the fourth harvest, the duration was shortest in BSMV combination (67.0 days), and was only significantly different ($P \leq 0.05$) from the longest duration, (73.7 days) in BSMC. The average number of days it took for the mushrooms to fourth harvest was

69.2 days. Therefore, the experiment showed that mixing substrates had significant effects on maturity of oyster mushrooms.

Table 4.2.1 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves substrate on days from spawning to harvesting and harvest intervals.

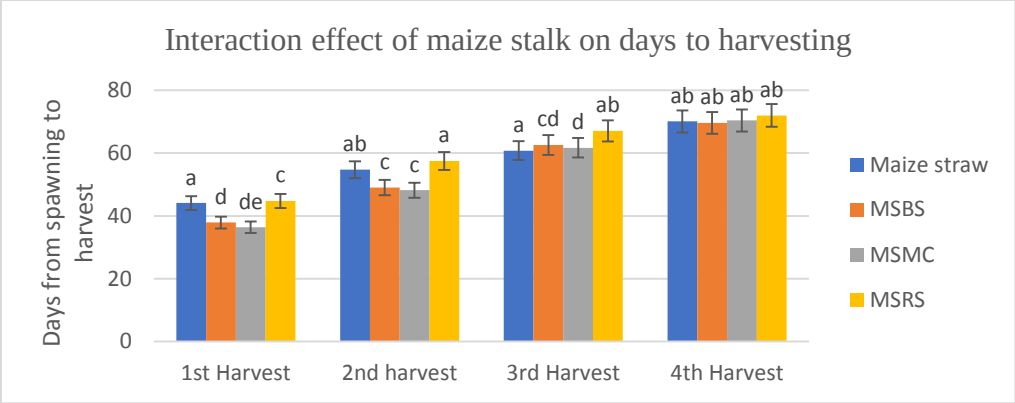
	Mushroom days from spawning to harvesting			
Treatments	1st harvest	2nd harvest	3rd harvest	4th harvest
Maize cobs + rice straw	35.1^{e}	50.3^{bc}	66.4^{abc}	69.0^{ab}
Maize stalks + maize cobs	36.4^{de}	48.2^{c}	61.7^{d}	70.4^{ab}
Maize stalks + bean straw	37.9^{d}	49.0^{c}	62.6^{cd}	69.6^{ab}
Bean straw + rice straw	42.8^{c}	53.1^{b}	65.1^{bcd}	70.9^{ab}
Maize stalks + rice straw	44.8^{bc}	57.5^{a}	67.1^{ab}	72.0^{ab}
Bean straw + maize cobs	45.7^{b}	57.3^{a}	68.3^{ab}	73.7^{a}
Bean straw + *Melia volkensii*	48.1^{a}	56.5^{a}	69.8^{a}	67.0^{b}
Total	**41.9**	**53.2**	**64.4**	**69.2**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.2 Interaction effect of maize stalks on days from spawning to harvesting

Interaction effect of maize stalks on days from spawning to first, second, third and fourth harvest is shown in figure 4.2.1 below. Combining maize stalks with bean straw and maize cobs reduced the duration to maturity significantly ($P \leq 0.05$) while the maturation period increased significantly ($P \leq 0.05$) when the maize stalk was combined with rice straw (figure 4.2.1). There were also significant difference ($P \leq 0.05$) in number of days from spawning to second and third harvest while no significant difference ($P \leq 0.05$) in days from spawning to fourth harvest was experienced (figure 4.2.1).

Figure 4.2.1 Interaction effect of maize stalks on days from spawning to harvesting

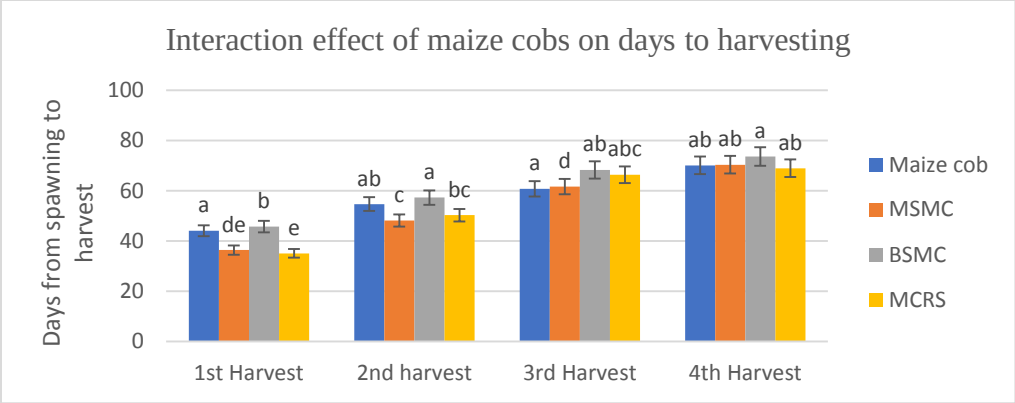


Means within the same harvest with the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.3 Interaction effect of maize cobs on days from spawning to harvesting

The interaction effect of maize cobs with maize stalks, bean straw and rice straw in days from spawning to first, second, third and fourth harvest is shown in figure 4.2.2 below. There was significant reduction ($P \leq 0.05$) of days from spawning to first harvest of mushrooms when maize cobs were mixed with maize stalks and with rice straw, while the duration increased significantly ($P \leq 0.05$) when the same substrate was mixed with bean straws (figure 4.2.2). Similarly, during the second harvest, it was also observed that mixing cobs with maize and rice straw reduced the duration significantly ($P \leq 0.05$) while the increase in days when the maize cobs were mixed with bean straw was not significantly different ($P \leq 0.05$). During the third and fourth harvest, there was no significant difference ($P \leq 0.05$) in duration when the cobs were mixed with the other three substrates, apart from MSMC which had a significant increase in duration during the third harvest.

Figure 4.2.2 Interaction effect of maize cobs on days from spawning to harvesting

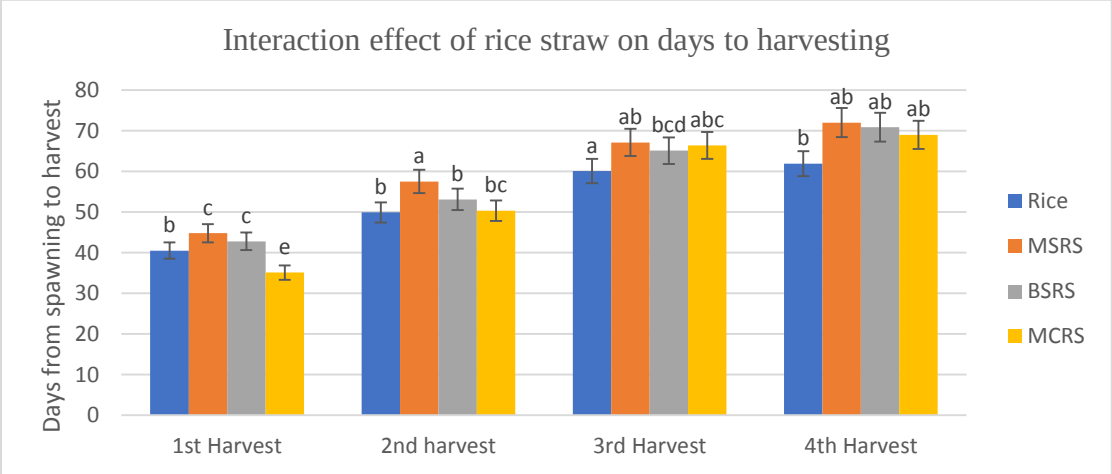


Means within the same harvest with the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.4 Interaction effect of rice straw on days from spawning to harvesting

There was significant increase ($P \leq 0.05$) in days from spawning to first harvest when rice straw was mixed with maize stalks and with bean straw, while the duration decreased insignificantly when the same was mixed with maize cobs. The duration increase was only significant in MSRS and in BSRS during the second and third harvests respectively while there was no significant increase in days to fourth harvest when the rice straw was mixed with the other three substrates (figure 4.2.3).

Figure 4.2.3 Interaction effect of rice straw on days from spawning to harvesting

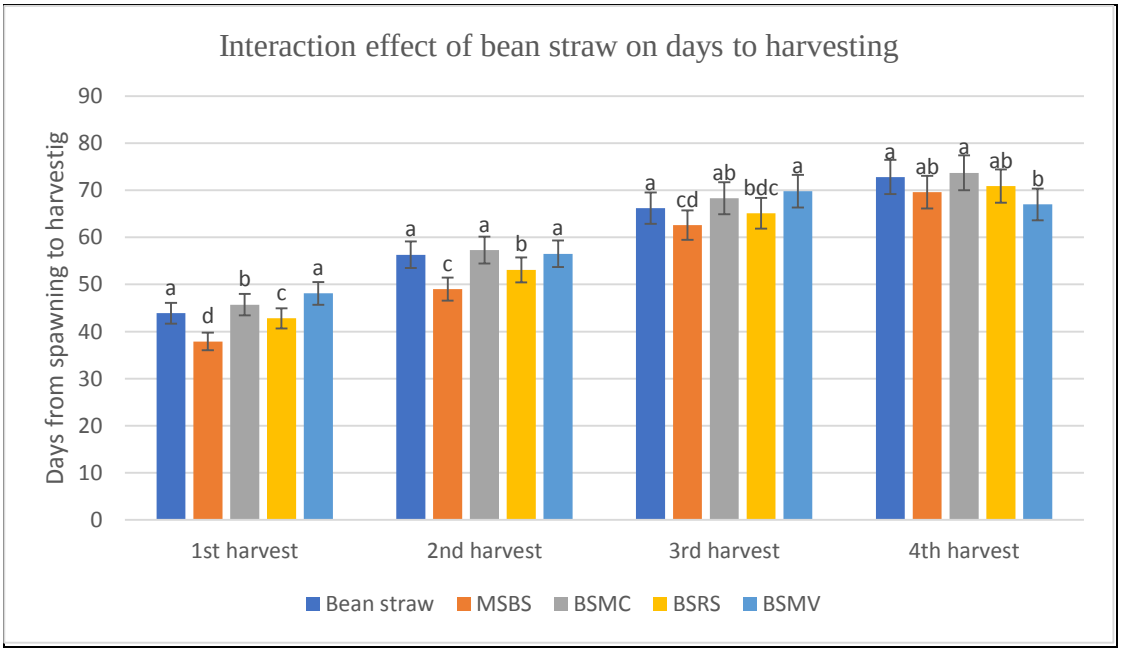


Means within the same harvest with the same letters are not significantly different at p ≤ 0.05 according to Duncan's multiple range test (DMRT)

4.2.5 Interaction effect of bean straw on days from spawning to harvesting

Interaction effect of bean straw on days from spawning to harvesting is shown in figure 4.2.4 below. The number of days from spawning to first harvest reduced significantly (P≤0.05) when bean straw was mixed with maize stalk and rice straw while the duration increased significantly in BSMC but not in BSMV. During the second harvest and third harvest, the duration decrease was significant (P≤0.05) in MSBS and BSRS (second harvest) and in MSBS and BSRS during the third harvest. While there was insignificant change in duration when the substrate was mixed with the other three substrates during the same harvests. During the fourth harvest, the duration change was only significant when bean straw was mixed with *Melia volkensii* leaves.

Figure 4.2.4 Interaction effect of bean straw on days from spawning to harvesting



Means within the same harvest with the same letters are not significantly different at p ≤ 0.05 according to Duncan's multiple range test (DMRT)

4.2.6 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on harvest intervals.

The duration from first to second harvest was shortest in bean straw + *Melia volkensii* combination (8.4 days) while it was longest in MCRS (15.2 days). However, during the second to third harvest, the harvest interval was shortest in BSMC (11 days) and longest in MCRS, (16.1 days) while it was shortest in MSBS (7days) and longest (8.7 days) in MSMC during the third to fourth harvest. It was observed that the harvest intervals between the first to the forth harvest varied from 11.3 days and 11.2 days for first to second harvest and second to third harvest respectively. During the third to fourth harvest, the harvest interval was 4.8 days, (table 4.2.2).

Table 4.2.2 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* on harvest intervals.

	Days between mushroom harvest intervals		
Treatments	1^{st} -2^{nd} harvest	2^{nd} -3^{rd} harvest	3^{rd} -4^{th} harvest
Maize cobs + rice straw	15.2	16.1	2.6
Maize stalks + maize cobs	11.8	13.5	8.7
Maize stalks + bean straw	11.1	13.6	7
Bean straw + rice straw	10.3	12	5.8
Maize stalks + rice straw	12.7	9.6	4.9
Beans straw + maize cobs	11.6	11	5.4
Bean straw + *Melia volkensii*	8.4	13.3	2.8
Total	**11.3**	**11.2**	**4.8**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.7 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on stipe length (cm), Cap diameter (cm), number of fruiting bodies.

The results (table 4.2.3) below shows significant interaction effect ($P \leq 0.05$) in maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on average stipe length, cap diameter and number of fruiting bodies in oyster mushrooms, *Pleurotus* spp. There was significant interaction effect ($P \leq 0.05$) of mushroom substrates on stipe length as shown in table 4.2.3. The tallest mushroom was obtained from bean straw + maize cob combination (6.1cm) while the shortest mushroom was obtained from bean straw + *Melia volkensii* substrates (3.8cm) and they were both significantly different ($P \leq 0.05$) from each other (Table 4.2.3). The average stipe length of the mushroom was 5.3 cm. There was also a significant interaction effect in maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on the average mushroom cap diameter (table 4.2.3). The largest cap diameter was obtained from bean straw + maize cob (9.2cm) while the shortest was obtained from bean straw + *Melia volkensii* (7.6cm) and were both significantly different ($P \leq 0.05$). There was significant interaction effect in maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii*, and their combinations on the average mushroom number of fruiting bodies, with MSBS producing the most while BSMV produced the least and they were both significantly different ($P \leq 0.05$). The experiment showed that mixing substrates had significant effects on the production of oyster mushrooms.

Table 4.2.3 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* leaves on stipe length (cm), cap diameter (cm), number of fruiting bodies

	Mean stipe length (cm), cap diameter (cm) and number of fruiting bodies		
Treatment	Mean stipe length (cm)	Cap diameter	Number of fruiting bodied
Maize stalks + maize cobs	5.9^{ab}	8.5^{b}	9.3^{a}
Maize stalks + bean straw	5.5^{bc}	8.3^{b}	9.5^{a}
Bean straw + rice straw	5.3^{c}	8.3^{b}	7.4^{bc}
Maize stalks + rice straw	5.3^{c}	8.5^{b}	6.7^{cd}
Maize cobs + rice straw	5.2^{c}	8.3^{b}	8.0^{b}
Bean straw + *Melia volkensii*	3.8^{d}	7.6^{c}	6.2^{d}
Bean straw + maize cobs	6.1^{a}	9.2^{a}	6.9^{bcd}
Overall	**5.3**	8.3	**7.8**

Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.8 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* on mushroom weight and biological efficiency

There was significant interaction effect in maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* on the average mushroom weight and biological efficiency. The experiment showed that mixing substrates had significant effects on the yield of oyster mushrooms. The results showed that mushroom mean weight was greatest for MSBS combination with 403.7g, followed by MSMC combination with 374.2g and were both significantly different ($P \leq 0.05$), while BSMC ranked third with mean weight of 303.1 grams and was not significantly different ($P \leq 0.05$) from MSMC. The least weight was obtained from BSMV with 136.2g and was significantly different ($P \leq 0.05$) from all the others. There was also a significant interaction effect in maize stalks, maize cobs, beans

straw, rice straw and *Melia volkensii* on the productivity (BE), as shown in table 4.8 below. The average biological efficiency was highest in MSMC (118.4%), followed by MSBS (112.8%), and they were not significantly different ($P \leq 0.05$) from each other. The BE ranged from 37.1% to 118.4% for Bean Straw+ *Melia Volkensii* and for MSMC combination respectively and they were both significantly different ($P \leq 0.05$) from each other. The overall mean biological efficiency was 91.1%. The experiment showed that mixing substrates had significant effects on the weight and biological efficiency of oyster mushrooms.

Table 4.2.4 Interaction effect of maize stalks, maize cobs, beans straw, rice straw and *Melia volkensii* on mushroom fresh weight and biological efficiency

Treatment	Total Fresh mushroom weight/bag (grams)	Biological Efficiency (%)
Maize stalks + maize cobs	374.2^{b}	118.4^{a}
Maize stalks + bean straw	403.7^{a}	112.8^{a}
Maize cobs + rice straw	295.7^{bc}	91.0^{b}
Beans straw + maize cobs	303.1^{b}	90.8^{b}
Maize stalks + rice straw	273.4^{bc}	84.4^{bc}
Beans straw + rice straw	260.2^{c}	76.1^{c}
Bean straw + *Melia volkensii*	136.2^{d}	37.1^{d}
Overall	**303.3**	**91.1**

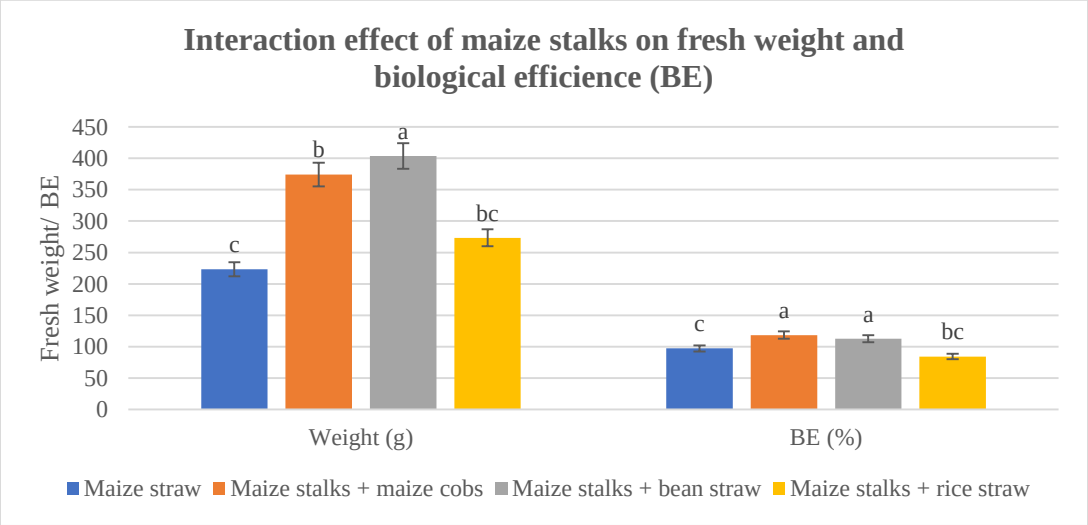
Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.9 Interaction effect of maize stalk on mushroom fresh weight and biological efficiency

There was significant interaction effect ($P \leq 0.05$) in mushroom fresh weight when maize cobs and bean straw was added to the maize stalks while no significant interaction effect ($P \leq 0.05$) was observed when rice straw was added to maize stalks (figure 4.2.5). There was

significant interaction effect (P≤0.05) on biological efficiency when maize cobs were added to maize stalk but not when bean straw or rice straw were added.

Figure 4.2.5 Interaction effect of maize stalk on mushroom fresh weight and biological efficiency

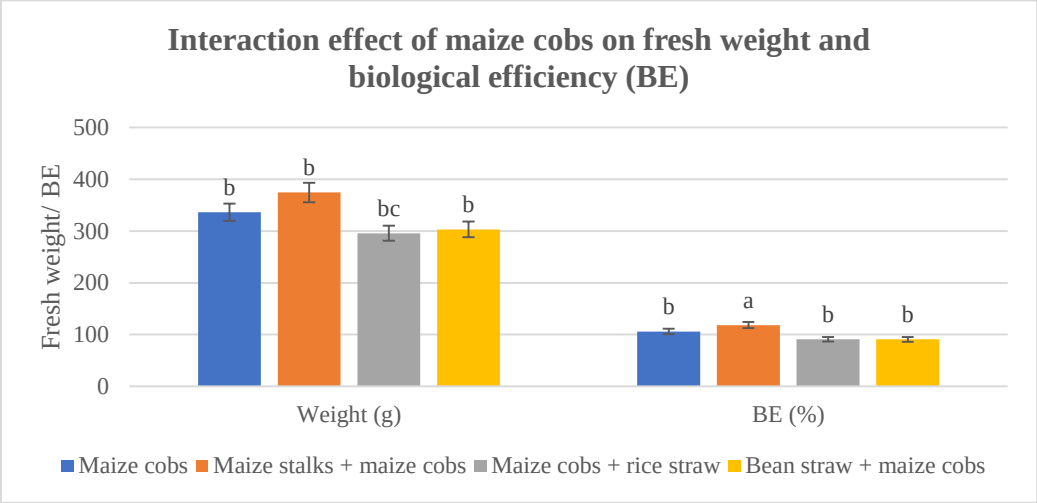


Means within the bars followed by the same letters are not significantly different at p ≤ 0.05 according to Duncan's multiple range test (DMRT)

4.2.10 Interaction effect of maize cobs on mushroom fresh weight and biological efficiency

There was no significant interaction effect of maize cobs in fresh weight when maize cobs, rice straw and bean straw were added to maize cobs. The biological efficiency of mushrooms in maize cob substrates increased significantly (P≤0.05) when maize stalks were added to maize cobs. However, the BE decreased insignificantly (P≤0.05) when rice and bean straws were added to the maize cobs.

Figure 4.2.6 Interaction effect of maize cobs on mushroom fresh weight and biological efficiency

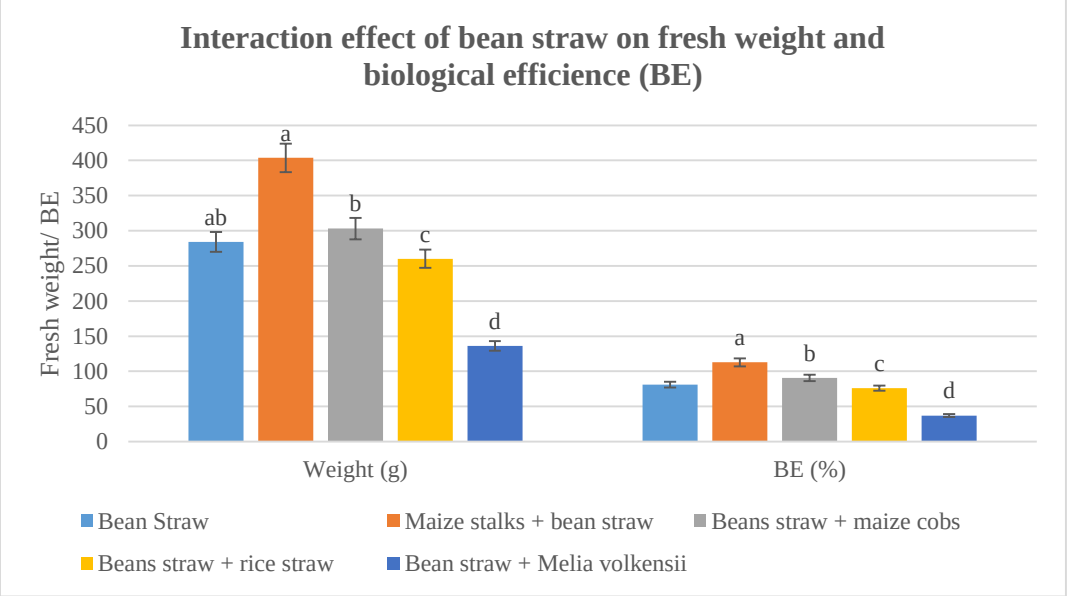


Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.11 Interaction effect of bean straw on mushroom fresh weight and biological efficiency

There was insignificant interaction effect of bean straw on mushroom weight when maize cobs and stalks were added, while the mushroom weight decreased significantly when rice straw and *Melia volkensii* were added to the bean straws (figure 4.2.7). There was significant interaction effect ($P \leq 0.05$) on biological efficiency when maize stalks, maize cobs, and *Melia volkensii* substrates were added to the bean straw but not when rice was added.

Figure 4.2.7 Interaction effect of bean straw on mushroom fresh weight and biological efficiency

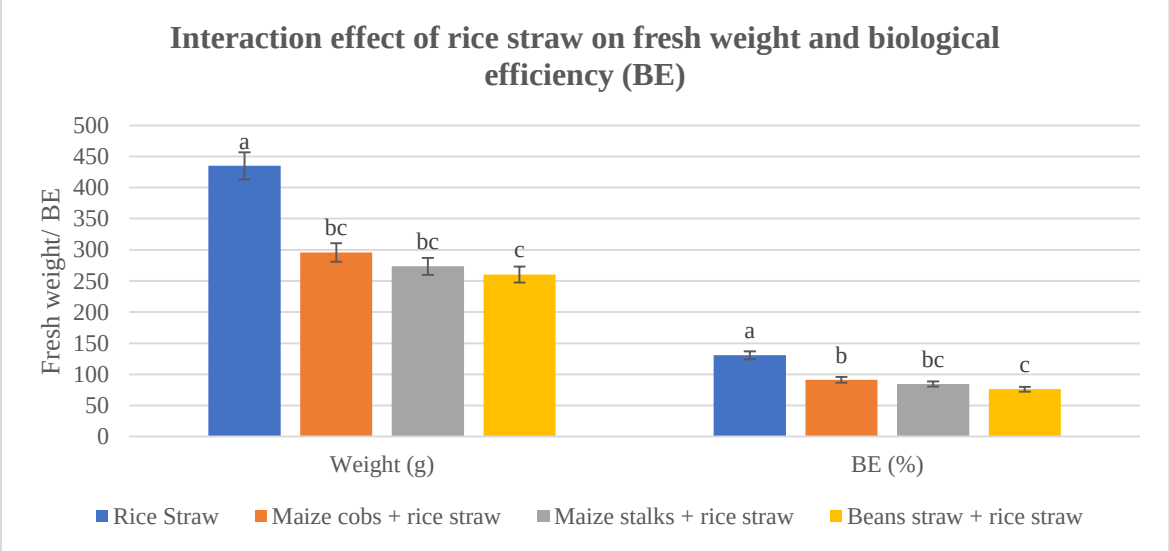


Means within the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test (DMRT)

4.2.12 Interaction effect of rice straw on mushroom fresh weight

The weight of mushrooms reduced significantly ($P \leq 0.05$) when maize cobs, maize stalks and bean straws were added to the rice straw (figure 4.2.8) below. There was significant decrease ($P \leq 0.05$) in biological efficiency when maize cobs, maize stalks and bean straw substrates were added into the rice straw substrates.

Figure 4.2.8 Interaction effect of rice straw on mushroom fresh weight and biological efficiency



Means within the same harvest with the same letters are not significantly different at p ≤ 0.05 according to Duncan's multiple range test (DMRT)

4.3.1 Correlation of different mushroom growth parameters, substrate dry weight and substrate moisture content

The result showed that the mushroom cap diameter had a significant positive correlation (P≤0.01) to the stipe length, the wider was the diameter, the longer the stipe length, (Table 4.3.1). The cap diameter also had a significant negative correlation (P≤0.05) with the fruiting bodies whereby the number decreased with increased diameter. There was also a significant positive correlation (P≤0.05) between the stipe length and mushroom weight and biological efficiency, mushrooms with longer stipes weighed heavier than those with shorter stipe length and gave a higher biological efficiency. There was also a significant positive correlation (P≤0.05) between the mushrooms cap diameter, the weight and the biological efficiency, whereby the wider the diameters the heavier the mushrooms and the higher the biological efficiency. The number of fruit bodies also had a significant positive correlation (P≤0.05) with the fresh weight of the mushrooms since the more the number the heavier the mushrooms were and the higher the biological efficiency. The substrates

that took a shorter time to the first harvest produced more fruit bodies and also gave the highest fresh weight (table 4.3.1)

Table 4.3.1 Pairwise correlations between different mushroom growth parameters

	Average stipe length	Average cap diameter (cm)	Number of fruit bodies	Total fresh weight	Dry substrate weight (g)	BE (%)	Days to first harvest	Substrate MC
Average stipe length	1							
Average cap diameter (cm)	0.398** (0.000)	1						
Number of Fruit body	0.060 (0.448)	-0.216** (0.006)	1					
Total fresh mushroom weight	0.440** (0.000)	0.290** (0.000)	0.487** (0.000)	1				
Dry Substrate Weight (g)	-0.379** (0.000)	-0.024 (0.760)	0.013 (0.867)	-0.157* (0.045)	1			
Biological Efficiency (%)	0.466** (0.000)	0.284** (0.000)	0.472** (0.000)	0.992** (0.000)	-0.266** (0.001)	1		
Days to first harvest	-0.120 (0.127)	-0.019 (0.813)	-0.416** (0.000)	-0.431** (0.000)	0.217** (0.005)	-0.438** (0.000)	1	
Substrate moisture content	0.379** (0.000)	0.024 (0.760)	-0.013 (0.867)	0.157* (0.045)	-1.000** (0.000)	0.266** (0.001)	-0.217** (0.005)	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

N= 164

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Effects of five substrates; bean straw, maize stalks, rice straw, maize cobs and *Melia volkensii* leaves on growth and productivity of oyster mushrooms.

Melia volkensii substrate showed insignificant mycelial density (5% spawn run) and no mushroom produced. There is no research work that had been done before on *Melia volkensii* in mushroom production. Some literature by Orwa *et al.*, 2009 indicates that *Melia* leaves extracts could be used as flea and fly repellents. They are also said to have antifeed activities against *Schistocerca gregaria* while they are also growth inhibitory against mosquitoes. Another study by Kamau *et al.* (2015) on antimicrobial compounds from root, stem bark and seeds of *Melia volkensii* showed that some compounds extracted from those plant parts exhibited high activity against *Aspergillus niger*. In his study, Kamau *et al.,* (2015) carried out both phytochemical and antimicrobial investigations on *Melia volkensii* focusing on antimicrobial activity and revealed for the first time the existence of toosendanin and scopoletin in this plant together with kulactone, which had antifungal, antibacterial and antiplasmodial activities. This antimicrobial effect could therefore probably explain why *Melia volkensii* did not perform well in the present study.

The other four substrates; maize stalks, maize cobs, bean straw and rice straw attained full colonization and yielded mushrooms. The hypothesis that the maize, bean and rice agro-waste materials and *Melia volkensii* leaves do not significantly influence the morphology and productivity of oyster mushroom and that there is no effect of different substrate on oyster mushrooms productivity was therefore not sustained. This is because the results showed that most of the yield parameters were significantly different among the different substrates. This finding is in conformity with (Kinge *et al*., 2016) who reported the same while studying use of different agro-waste materials as substrates for *Pleurotus* spp. production. He compared the effects of different agro-wastes materials on the growth and yield of oyster mushrooms. Among the agro wastes used, Kinge et al., (2016) also used maize cobs and the substrate showed significant differences in total colonization period and fruiting period, yield and biological efficiency of the oyster mushrooms. The days to

fruiting for mushrooms in this study using rice straw (40.5) differed with the finding by Sitaula et al., (2018), where the oyster mushrooms (*Pleurotus ostreatus*) took 21.75 days to fruiting in rice straw. However, in this study, mushroom stipe length (5.7 cm) and cap diameter (8.1 cm) in rice straw agreed with the same study by Sitaula et al., (2018) who obtained stipe length of 5.7 cm and cap diameter of 8 cm in their study with rice straw. The total yields of mushrooms in rice straw; 434 g fresh weight and biological efficiency (130.6%), in this study differed slightly with the same study by Sitaula et al,. (2018) who obtained total mushroom fresh weight of 408.3 grams and biological efficiency of 96%. These differences could be been attributed to the differences in environmental conditions of the study area, substrate supplements used and differences in the fungal strains.

5.2 Interaction effects of maize stalks, maize cobs, beans, rice straw and *Melia volkensii* leaves on oyster mushroom growth and productivity.

Melia volkensii substrates combinations performed poorly. The combination of bean straw and *Melia volkensii* leaves took the longest time (48.1 days) to first harvest. It's time to first harvest was almost two weeks after the first harvest of the fastest substrate (rice straw) which took 35.1 days. This could have been attributed to the anti-fungal effects in this agro-forest tree, (Kamau *et al.*, 2015). Nevertheless, most of the results on mushroom growth for the different substrate combinations showed significant interaction effect. This conforms with Kimenju *et al*, 2009 who compared the effects of different agro-wastes materials including; rice straw, bean straw and maize cobs on the growth and yield of oyster mushrooms, *Pleurotus* spp. and most of his results were also significantly different in total colonization period, characteristics of fruiting bodies, yield and biological efficiency of the oyster mushrooms.

In this study, the fruit initiation period for the fastest substrate, maize cob + rice straw combination was 35 days. This differed with the findings of Ahmed, (1998) , who found out that *Pleurotus ostreatus* took 23-27 days for initiation of fruiting bodies in maize cob + rice straw combination. The findings also differed with Sitaula *et al*, (2018) in their study with *Pleurotus ostreatus* where they found that the mushroom took 22 and 23 days to first

harvest on rice straw and on maize cob + rice combination respectively. The mushroom fresh weight in rice straw in this study was 434.9 grams while Sitaula et al. (2018) obtained 408.3 grams from rice straw in their study. He also obtained a biological efficiency of 96.3% in rice straw while in this study the BE of rice was 130.6%. The stipe length in this study that was obtained from rice straw + maize cobs combination (5.2 cm) differed from that in the study by Sitaula et al., (5.8 cm) and Kinge et al., (2016), 6.3 cm. This research has demonstrated that locally available organic substrates are potentially suitable for use in the production of oyster mushrooms. It means that the substrates contain lignin and cellulose, utilized by the mushroom mycelium as a source of nutrition. The diverse range of substrates indicates that mushrooms can grow on many available organic wastes.

5.3 Level of correlations of different mushroom growth parameters as influenced by varied substrates and their combinations.

From this study, it was observed that days to first harvest greatly depends on the substrate and the highly productive substrates like rice straws came into production earlier than the less suitable substrates, bean straw + *Melia volkensii* combination. The tendency of the poorer substrates, bean straw + *Melia volkensii* combination to fruit later could be attributed to antimicrobial stress that mycelium was subjected to. These results differed with (Oei, 2003) whose finding showed that the highly productive substrates came into production later while the poorer substrates came into production earlier. He associated the poorer substrate pinning earlier to nutritional stress.

The significant and negative correlation between cap diameter and number of fruiting bodies also suggests that the number of fruiting bodies per cluster dependents on the cap diameters of mushroom. The wider the diameter, the fewer they were. This finding conforms with the finding by Kimenju et al., (2009) who obtained the same correlations. This correlation could be due to lower competition for space, nutrients and the available Moisture. The number of fruit bodies harvested and time taken to first harvest varied greatly, indicating that the two variables were substrate dependent. The earlier producing substrates produced more fruit bodies than the late producing ones. This report conforms

to other reports by researchers like Nageswaran *et al.* (2003), who found the same correlation between the two parameters. The significant and positive correlation between the mushroom fresh weight and biological efficiency suggests that the yields of mushrooms depends on biological efficiency in different substrates.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Throughout the investigation, the growth substrates significantly ($P \leq 0.05$) affected the productivity of the oyster mushrooms. Among the pure substrates used, (maize stalks, bean straw, maize cobs and rice straws), the highest yields obtained were from rice straw, followed by maize cobs while there was no mushroom production from *Melia volkensii*. From this study, it can be concluded that rice straw is the best substrate for the growth and development of oyster mushroom (*pleurotus ostreatus*) while *Melia volkensii* is not a suitable substrate. The results also showed that, different substrates combination had significant interaction effects ($P \leq 0.05$) on yields. The lowest yields obtained were from bean straw + *Melia volkensii* while the highest obtained was from maize stalks + bean straw combination. This study showed that combining some substrates improved the productivity of mushrooms. Maize stalks and beans straws are relatively abundant in rural communities in the study area where resource poor farmers reside, and they can therefore be economically used in oyster mushroom cultivation. For the correlation study, there was significant correlation of the mushroom growth parameters observed and the strong association was due to the fact that; the more the weight of mushroom, the more the sales. From this study, the performance of the substrates in terms of yields could be arranged in order of decreasing suitability as follows; rice straw, maize stalks + bean straw, maize stalks + maize cobs, maize cobs, bean straw + maize cobs, maize cobs + rice straw, bean straw, maize stalks +rice straw, bean straw +rice straw, maize stalks and bean straw+ *Melia volkensii*. From the present study, it can be concluded that some of the locally available materials in the semi-arid areas; maize stalks, bean straw and maize cobs are suitable substrates for oyster mushroom production.

The study showed that choice for the right substrate for mushroom cultivation is very important to the growers since it determines growth and yields.

6.2 Recommendations

The following recommendations were deduced from the research:

1. Use of readily and available mushroom substrates; maize cobs, bean straw and maize stalks for oyster mushroom production in the ASALs should be initiated and promoted. Rice straw can be used as a supplement.
2. For better results, combination of the above materials; maize stalks + bean straw, maize stalks + maize cobs and bean straw + maize cobs combinations to be used as substrates for oyster mushroom production.
3. The substrates that take a shorter time to the first harvest (rice straw and maize stalk + bean straws combination) are more recommended since they produced more fruit bodies giving more fresh weight of the mushrooms and therefore more economical to the farmers.

6.3 Suggestions for further research

Further research can be done on:- nutritional contents of mushrooms from different substrates. Furthermore, the antimicrobial properties of *Melia volkensii* needs further investigations to break them down for use as mushroom substrates in the ASALs.

REFERENCES

Ahmed, S. (1998). Development of mushroom varieties suitable for rural level in Bangladesh. *Report presented in BARC Annual Review Programme,* 72-73.

Alananbeh, K. M., Bouqellah, N. A., & Al Kaff, N. S. (2014). Cultivation of oyster mushroom (*Pleurotus ostreatus)* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi journal of biological sciences, 21*(6), 616–625.

Ali M.D., (2013). Reproduction of Fungi. *Technical Report,* 1-12.

Ashraf, J., Ali, M. A., Ahmad, W., Ayyub, C. M., & Shafi, J. (2013). Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) production. *Food Science and Technology, 1*(3), 44-51.

Bechara M.A., (2007). Alternative Mushroom Production Systems Using Non-composted Grain-Based Substrates. *Thesis*. Department of Agricultural and Biological Engineering. The Pennsylvania State University.

Belewu, M. A. & Belewu, K.Y. (2005) Cultivation of mushroom (*Volvariella volvacea*) on banana leaves. *African Journal of Biotechnology, 4 (12),1401-1403.,*

Bernabé-González, T., & Cayetano-Catarino, M. (2009). Cultivation of *Pleurotus pulmonarius* on substrates treated by immersion in alkaline water in Guerrero, Mexico. *Micologia Aplicada International, 21*(1), 19-23.

Biswas, P., Ahmad, A., Ahmed, K. U., Hossain, A., Hamja, A., & Haque, M. (2016). Yield of Oyster Mushroom (*Pleurotus Ostreatus*) Influenced by Different Sawdust Substrate. *International Journal of Scientific and Research Publications* 6: 1-6.

Bogale A. (2017). Cultivation of Edible Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*) Using Millet [*Eleusine coracana*(L)] Straw and Broad Bean [*Vicia faba*(L) Stalk as Growth Substrates. *MSC. thesis*. Haramaya University, Haramay. College of natural and Computational Sciences

Caral Dinesh, R., Vinay, P., Manasa, P., Vinothkumar, D., & Ramesh Babu, N. G. (2013). Comparative study of oyster mushroom (*Pleurotus ostreatus*) cultivation by physical and chemical method of sterilization using two different substrates. *International Journal of Scientific and Engineering Research* 4(9):898-902

Chang S.T., & Miles P.G., (1991). Recent trend in world production of edible mushrooms. *The Mushroom Journal*; 503: 15 – 30.

Chang, S. T. (1999). World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing, in China. *International Journal of Medicinal Mushrooms, 1*(4).

Chang, S. T. (2009). Training manual on mushroom cultivation technology. *United nations-Asian and pacific centre for agricultural engineering and machinery (UN-APCAEM), Beijing, China*.

Chang, S. T., & Miles, P. G. (2004). Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. *CRC press*.

Chioza, A., & Ohga, S. (2014). Cultivated mushrooms in Malawi: A look at the present situation. *Advances in Microbiology*, *04*(01), 6-11.

Chitamba, J., Dube, F., Chiota, W., & Handiseni, M. (2012). Evaluation of substrate productivity and market quality of oyster mushroom (*Pleurotus ostreatus*) grown on different substrates. *International Journal of Agricultural Research*, *7*(2), 100-106.

Datta, S., Choudhuri, S. S., & Mitra, A. K. (2014). Increased biomedical potential of *Pleurotus ostreatus* through the usage of effective substrate. *International Journal of Pharma and Bio Sciences*, *5*(4).

Deepalakshmi, K., & Sankaran, M. (2014). *Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties. *Journal of Biochemical Technology*, *5*(2), 718-726.

Dlamini, B. E., Earnshaw, D. M., & Masarirambi, M. T. (2012). Growth and Yield Response of Oyster Mushroom ("*Pleurotus ostreatus")* Grown on Different Locally Available Substrates. *Current Research Journal of Biological Sciences*, *4*(5), 623-629.

Friel, M. T., & McLoughlin, A. J. (2000). Production of a liquid inoculum/spawn of Agaricus bisporus. *Biotechnology Letters*, *22*(5), 351-354.

GOK. (2010). Agricultural sector development strategy 2010-2020: Government Printer.

Gregori, A., Mirjan, V. and Pohleven, J., (2007). Cultivation techniques and medicinal Properties of *Pleurotus* spp. Food Technol. Biotechnol. 45 (3) 238–249.

Gwanama C., Mwale V.M. and Nsibande B, "*Basic Procedures for Small Scale Production of Oyster Mush- rooms*," University of Namibia, 2011.

Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, *43*(4), 423-434.

Kahi, A. K., Wasike, C. B and Rewe, T. O. (2006). *Beef production in the arid and semi-arid lands of Kenya: constraints and prospects for research and development. outlook on agriculture*, 35(3), 217-225.

Kamau, R. W., Juma, B. F., & Baraza, L. D. (2016). Antimicrobial compounds from root, stem bark and seeds of Melia volkensii. *Natural Product Research*, *30*(17), 1984-1987.

Kamthan R. & Tiwari I (2017) Agricultural waste- potential substrates for mushroom cultivation. *Eur J Exp Biol 7(5:31):1-4.*

Kang, S.W., (2004). What is oyster mushroom? In MushWorld (2004), Mushroom Growers' Handbook 1: *Oyster Mushroom Cultivation pp 48-51.*

Khan, M. A., Amin, S. M. R., Uddin, M. N., Tania, M. & Alam, N. (2008). *Comparative Study of the Nutritional Composition of Oyster Mushrooms Cultivated in Bangladesh. Bangladesh J*. Mushroom. 2(1): 9-14.

Kimenju, P.M., Odero, G.O.M., Mutitu, E.M., Wachira, P.M., Narla, R.D. and Muiru, W., (2009). Suitability of locally available substrates for oyster mushroom (*Pleurotus osteaus*) cultivation in Kenya. *Asian Journal of plant sciences 8(7)*: 510-514.

Kinge, T. R., Djidjou, T. A. M., Nji, T. M., Ache, N. A., & Mih, A. M. (2016). Effect of local substrates on the growth and yield of Pleurotus ostreatus K. in the North West Region, Cameroon. *Curr. Res. Environ. Appl. Mycol*, *6*(1), 11-19.

Kumari, D. and Achal, V., (2008). Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (*Oyster mushroom*). Life Science Journal; 5(3): 73 – 76.

Lal R., 2005. World crop residues production and implications of its use as a biofuel. Environ Int 31 (4): 575-584 lignocellulose bioefineries. *J Ind Microbiol Biotechnol* 35: 367-375.

Lynch, M. J., Mulvaney, M. J., Hodges, S. C., Thompson, T. L., & Thomason, W. E. (2016). Decomposition, nitrogen and carbon mineralization from food and cover crop residues in the central plateau of Haiti. *Springerplus*, *5*(1), 1-9.

Mamiro, D.P., Mamiro, P.S. and Mwatawala, M. W., (2014). Oyster mushroom (*Pleurotus* spp.) Cultivation technique using reusable substrate containers and comparison of mineral content with leafy vegetables. *Journal of Applied Biosciences 80*:7060 – 7070.

Marshall E. and Nair N.G., (2009) Make money by growing mushrooms. FAO diversification booklet 7 (1:3): 44-51.

Maszlavér, P. (2008). *Cultivation Possibilities for Production of Reishi Ganoderma Lucidum* (curt.: fr.) Farst in Hungary. Unpublished Thesis, University of Budapest.

Mganga, K.Z., Musimba, N.K.R., Nyangito, M.M., Nyariki, D.M., Mwang'ombe, A.W. (2010). Improving hydrological properties of degraded soils in semi-arid Kenya. *Journal of Environment Science Technology*, 4(3), 217-225

Mohamed F., Dalia M.T Nassef, Esmat A. Waly and Amira M. Kotb, (2014). Production of oyster mushroom (*Pleurotus* spp.) intercropped with field grown faba beans (*Vicia faba* L.),*Asian journal of crop science,2014.pp. 1-11.*

Musieba F., Okoth S., Mibey R.K., Wanjiku S. and Moraa K., (2012). Suitability of locally available Substrates for Cultivation of the Kenyan Indigenous Golden Oyster Mushroom (*Pleurotus citrinopileatus* Singer).Kenya Industrial Research Development Institute (KIRDI). *Agricultural Journal 7 (4): 240-244, 2012.*

Mwami B.M., (2017). Effects of Foliar Fertilizer Application Rates on Productivity of Selected Bean (*Phaseolus Vulgaris L*.) Varieties. Thesis. Department of Dryland Agriculture, South Eastern Kenya University

National Farmers Information service (Kenya) (2015). www.nafis.go.ke /vegetables/mushroom production/importance of mushroom-cultivation.

Nazir ,W., Iqbal S.M, Bhatti, M.S., Khalid N.,* And Riaz A., (2012). Evaluation of Yield Parameters and Nutritional Aspects of Different Strains of Oyster(*Pleurotus* ostreatus) Mushroom. J. Chem .Soc. Pak., 34(6), 1491-1497.

Odendo M., Kirigua V., Kimenju J.M., Wasila L., Musieba F., Orina M., (2011) Analysis of Mushroom value chain in Kenya. *East African Agricultural Journal. Vol 76 No. 3&4.*

Odero, G.O.M., (2009). Suitability of locally available substrates for oyster mushroom (*Pleurotus osteaus*) cultivation in Kenya. *Thesis, University of Nairobi.*

Onuoha, C.I., (2007) Cultivation of the mushroom (*Pleurotus tuber regium*) using some local substrates. Department of Plant Science and Biotechnology, Imo State University. *Life Science Journal. 2007; 4(4): 58 – 61] (ISSN: 1097 – 8135).*

Onyango B.O., Palapala V.A., Arama P.F., Wagai S.O., & Gichimu B.M., (2011) Suitability of selected supplemented substrates for cultivation of Kenyan Native wood Ear Mushrooms (*Auricularia auricular*). American Journal of food Technology 6(5): 395-403.

Orwa C, A Mutua, Kindt R, Jamnadass R, S Anthony., (2009) Agroforestree Database:a tree reference and selection guide version 4.0. (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp)

Pathania S., Sharma N., and Gupta D., (2017). A Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus* ostreatus) on Wheat Straw Mixed with Horticultural Waste (Apple Pomace) in Different Ratio and their Nutritional Evaluation. University of Horticulture and Forestry, Nauni, Solan, H.P., India. Int.J.Curr.Microbiol.App.Sci (2017) 6(8): 2940-2953.

Philippoussis A.N (2009), Production of Mushrooms Using Agro-Industrial Residues as substrates:https://www.researchgates.net/publication/226917053.

Piepho, H.P., (2018). Letters in mean comparisons: What they do and don't mean. J. Agron. Vol.110: 431–434 Crop Sci. Univ. of Hohenheim, Institute of Crop Science, Fruwirthstr, Germany. 204.

Poppe, J., (2000). Use of agricultural waste materials in the cultivation of mushrooms. (L. vanGriensven, Ed.) Science And Cultivation Of Edible Fungi, Vol1. and 2, 3-23.

Prince B., Arif A., Kamal U.A., Abir Hossain M.D., Amir Hamja M.D., Rabin M.H. (2016). Yield of Oyster Mushroom (*Pleurotus Ostreatus*) Influenced by Different Sawdust Substrate. International Journal of Scientific and Research 6 (12: 291:2250-3153 www.ijsrp.org. Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Ragupathi V., Kumerasan S., Selvaraju S., Karthikeyan V., (2016). Optimizing the growth conditions and adopting new methods growing oyster and milky mushrooms in same conditions, Thiagarajar College, Madurai 9, Tamil Nadu, India. International Journal of Herbal Medicine 2016; 4(3): 01-04.

Rinker, D. L., ZERI, K. S., & Kang, S. W. (2004). Recycling of oyster mushroom substrate. *Mushroom Growers' Handbook-1. Post-harvest Manag*, *9*, 187-191.

Ruegger, M.J., Tornisielo S. M., Bononi, V. L., Capelari M., (2001). Cultivation of the edible mushroom oudemansiella canarii (jungh.) höhn. in lignocellulosic substrates. Brazilian journal of microbiology 32:211-214.

Ruiz-vega J., Nuñez-Barrios A., Cruz-Ruiz M.A, Ciidir Ipn., (2010). Decomposition Rates of Intercropped Green Manure Crops In Oaxaca, Mexico . Universidad Autónoma de Chihuahua, Chihuahua, Chih. CP 31000, Mexico. Anadolu J. Agric. Sci., 2010,25(S-3):212-26.

Sitaula H.P., Dhakall R., Geetesh, D.C.I., Kalauni D., (2018). Effect of Various Substrates on Growth and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*), Agriculture and Forestry University (AFU), Chitwan, Nepal. Int. J. Appl. Sci. Biotechnol. Vol 6(3): 215-219.

Tisdale T.E., (2004). Cultivation of the mushroom (*Pleurotus* sp.) on wood substrates in Hawaii. Unpublished Thesis, University of Hawaii.

Uddin M.N., Yesmin S., Khan M.A., Tania, M., Moonmoon M., & Ahmed S., (2011). Production of Oyster Mushrooms in Different Seasonal Conditions of Bangladesh. Journal of Scientific Research 3 (1), 161-167.

Wachira P.M., (2003). Cultivation of *Pleurotus* (fr) kummer mushroom species using selected agricultural and industrial wastes. Unpublished Thesis, University of Nairobi.

Wasser S.P., (2014). Medicinal Mushroom Science: Current Perspectives, Advances, Evidences, and Challenges. Biomed J Vol. 37 No. 6 pp.345-356.

Yang, W., Guo, F., & Wan, Z. (2013). Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi journal of biological sciences*, *20*(4), 333-338.

Yehia, R. S. (2012). Nutritional value and biomass yield of the edible mushroom *Pleurotus ostreatus* cultivated on different wastes in Egypt. *Innovative Romanian Food Biotechnology*, *11*, 9.

Zhao, L., Huo, C., Shen, L., Yang, Y., Zhang, Q., & Shi, Q. (2010). Chemical constituents of plants from the Genus*Melia*. *Chemistry & Biodiversity*, *7*(4), 839-859.

APPENDICES

APPENDIX I: MUSHROOM SUBSTRATES

Treatment	**Substrate**	**Composition**	**Dry weight per bag (g)**
T1	Substrate 1	Maize stalks, 1kg	314
T2	Substrate 2	Bean straw, 1kg	351
T3	Substrate 3	Maize cobs, 1kg	317
T4	Substrate 4	Rice straw, 1kg	333
T5	Substrate 5	Melia volkensii leaves, 1kg	382
T6	Substrate 6	Combination between maize stalks (500gms) and bean straw (500gms)	358
T7	Substrate 7	Combination of maize stalks (500gms) and Maize cob (500gms)	316
T8	Substrate 8	Combination of maize stalks (500gms) and Rice straw (500gms)	324
T9	Substrate 9	Combination of maize stalks (500gms) and *Melia volkensii* leaves, (500gms)	348
T10	Substrate 10	Combination of bean straw (500gms) and Maize cobs (500gms)	334
T11	Substrate 11	Combination of bean straw (500gms) and Rice straw (500gms)	342
T12	Substrate 12	Combination of bean straw (500gms) and *Melia volkensii* leaves, (500gms)	367
T13	Substrate 13	Combination of Maize cobs (500gms) and rice straw (500gms)	325
T14	Substrate 14	Combination of maize cobs (500gms) and *Melia volkensii* leaves, (500gms)	350
T15	Substrate 15	Combination of rice straw (500gms) and *Melia volkensii* leaves, (500gms)	358

APPENDIX II: LAYOUT OF THE EXPERIMENT (RANDOMIZED COMPLETE BLOCK DESIGN (RCBD)

Plot#	Entry	Block	Treatment
108	1	1	T1
214	16	2	T1
313	31	3	T1
103	6	1	T6
202	21	2	T6
307	36	3	T6
115	7	1	T7
207	22	2	T7
311	37	3	T7
114	8	1	T8
212	23	2	T8
310	38	3	T8
109	9	1	T9
215	24	2	T9
301	39	3	T9
111	2	1	T2
210	17	2	T2
308	32	3	T2
105	10	1	T10
205	25	2	T10
312	40	3	T10
104	11	1	T11
209	26	2	T11
315	41	3	T11
112	12	1	T12
201	27	2	T12
306	42	3	T12
106	3	1	T3
204	18	2	T3
304	33	3	T3
107	13	1	T13
211	28	2	T13
303	43	3	T13
110	14	1	T14
213	29	2	T14
305	44	3	T14
101	4	1	T4
203	19	2	T4
309	34	3	T4
102	15	1	T15
206	30	2	T15
302	45	3	T15
113	5	1	T5
208	20	2	T5
314	35	3	T5

APPENDIX III: ANOVA TABLES

ANOVA table 1: Effects of rice straw, maize cobs, maize stalks, bean straw on days from mushroom spawning to first harvest

Duncan[a,b]		Variable: Days to first harvest	
		Subsets	
Treatment	N	1	2
Rice straw	15	40.5333	
Maize stalks	15	41.7333	41.7333
Bean straw	15		43.8667
Maize cobs	15		44.1333
Sig.		.449	.155

ANOVA table 2: Effects of rice straw, maize cobs, maize stalks, bean straw on days from mushroom spawning to second harvest

Duncan[a]		Variable: Days to second harvest	
		Subsets	
Treatment	N	1	2
Rice straw	15	49.9333	
Maize stalks	15	53.5333	53.5333
Maize cobs	15	54.7333	54.7333
Bean straw	15		56.2667
Sig.		.110	.362

ANOVA table 3: Effects of rice straw, maize cobs, maize stalks, bean straw on days from mushroom spawning to third harvest

Duncan [a,b]		Variable: Days to third harvest
		Subset for alpha = 0.05
Treatment	N	1
Rice straw	15	60.1333
Maize cobs	13	60.8462
Maize stalks	13	63.5385
Bean straw	11	66.1818
Sig.		.066

ANOVA table 4: Effects of rice straw, maize cobs, maize stalks, bean straw on days from mushroom spawning to fourth harvest

Duncan[a,b]		Variable: Days to fourth harvest	
		Subset for alpha = 0.05	
Treatment	N	1	2
Rice straw	10	61.9000	
Maize stalks	7	66.7143	66.7143
Maize cobs	12	70.0833	70.0833
Bean straw	5		72.8000
Sig.		.074	.181

ANOVA table 5: Effects of rice straw, maize cobs, maize stalks, bean straw on mushroom stipe length

Duncan[a]		Variable: Mean mushroom stipe length	
		Subset for alpha = 0.05	
Treatment	N	1	2
Bean straw	15	4.9472	
Maize stalks	15	5.2591	5.2591
Rice straw	15		5.6574
Maize cobs	15		5.7567
Sig.		.217	.064

ANOVA table 6: Effects of rice straw, maize cobs, maize stalks, bean straw on mushroom cap diameter

Duncan[a]		Variable: Average cap diameter (cm)	
		Subset for alpha = 0.05	
		1	2
Treatment	N		
Maize stalks	15	7.3133	
Rice straw	15		8.0733
Maize cobs	15		8.3356
Bean straw	15		8.3978
Sig.		1.000	.333

ANOVA table 7: Effects of rice straw, maize cobs, maize stalks, bean straw on number of fruitbody

Duncan[a]		Variable: Mean mushroom number of fruitbodies	
		Subset for alpha = 0.05	
Treatment	N	1	2
Maize stalks	15	6.6322	
Maize cobs	15	7.6811	7.6811
Bean straw	15		8.5878
Rice straw	15		9.3144
Sig.		.241	.086

ANOVA table 8: Effects of rice straw, maize cobs, maize stalks, bean straw on total mushroom weight per 1kg wet substrate

Duncan[a]		**Variable: Total mushroom weight**		
		Subset for alpha = 0.05		
Treatment	N	1	2	3
Maize stalks	15	223.0667		
Bean straw	15	284.1333	284.1333	
Maize cobs	15		336.1333	
Rice straw	15			434.8667
Sig.		.128	.194	1.000

ANOVA table 9: Effects of rice straw, maize cobs, maize stalks, bean straw on biological Efficiency (%)

Duncan[a]		**Variable: Biological Efficiency (%)**		
		Subset for alpha = 0.05		
Treatment	N	1	2	3
Maize stalks	15	71.0403		
Bean straw	15	80.9497		
Maize cobs	15		106.0358	
Rice straw	15			130.5906
Sig.		.415	1.000	1.000

ANOVA table 10: Interaction effects of mushroom substrates on days to first harvest

		Variable: Days to first harvest				
Duncan[a,b]		Subset for alpha = 0.05				
Treatment	N	1	2	3	4	5
Maize cobs + rice straw	30	35.1333				
Maize stalks + maize cobs	30	36.4000	36.4000			
Maize stalks + bean straw	30		37.9333			
Bean straw + rice straw	30			42.8000		
Maize stalks + rice straw	30			44.8000	44.8000	
Bean straw + maize cobs	30				45.7333	
Bean straw + *Melia volkensii*	28					48.0714
Sig.		.279	.190	.088	.425	1.000

ANOVA table 11: Interaction effects of mushroom substrates on days to second harvest

Duncan[a,b]		Days to second harvest		
		Subset for alpha = 0.05		
Treatment	N	1	2	3
Maize stalks + maize cobs	30	48.2000		
Maize stalks+ bean straw	30	49.0000		
Maize cobs + rice straw	30	50.3333	50.3333	
Bean straw + rice straw	30		53.1333	
Bean straw + *Melia volkensii*	22			56.4545
Bean straw + maize cobs	30			57.2667
Maize stalks + rice straw	30			57.5333
Sig.		.208	.079	.526

ANOVA table 12: Interaction effects of mushroom substrates on days to third harvest

Duncan[a,b]	Days to third harvest				
		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Maize stalks + maize cobs	30	61.7333			
Maize stalks + bean straw	30	62.6000	62.6000		
Bean straw + rice Straw	30	65.1333	65.1333	65.1333	
Maize cobs + rice straw	28		66.4286	66.4286	66.4286
Maize stalks + rice Straw	28			67.1429	67.1429
Bean straw + maize cobs	30			68.2667	68.2667
Bean straw + *Melia volkensii*	12				69.8333
Sig.		.108	.070	.155	.121

ANOVA table 13: Interaction effects of mushroom substrates on days to fourth harvest

Duncan[a,b]		Days to fourth harvest	
		Subset for alpha = 0.05	
Treatment	N	1	2
Bean straw + *Melia volkensii*	4	67.0000	
Maize cobs + rice straw	12	69.0000	69.0000
Maize stalks + bean Straw	20	69.6000	69.6000
Maize stalks + maize cobs	24	70.4167	70.4167
Bean straw + rice Straw	14	70.8571	70.8571
Maize stalks + rice straw	10	72.0000	72.0000
Bean straw + maize cobs	12		73.6667
Sig.		.099	.125

ANOVA table 14: Interaction effects of mushroom substrates to average stipe length

Duncan[a,b]		Average stipe length			
		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Bean straw + *Melia volkensii*	28	3.7677			
Maize cobs + rice Straw	30		5.2322		
Maize stalks + rice straw	30		5.2840		
Bean straw + rice straw	30		5.3322		
Maize stalks + bean straw	30		5.5274	5.5274	
Maize stalks + maize cobs	30			5.9043	5.9043
Bean straw + maize cobs	30				6.0559
Sig.		1.000	.187	.063	.453

ANOVA table 15: Interaction effects of mushroom substrates to average cap diameter (cm)

Duncan[a,b]		Variable: Average cap diameter (cm)		
		Subset for alpha = 0.05		
Treatment	N	1	2	3
Bean Straw + *Melia volkensii*	28	7.5905		
Bean Straw + rice straw	30		8.2533	
Maize cobs + rice straw	30		8.3222	
Maize stalks + bean Straw	30		8.3489	
Maize stalks + rice straw	30		8.4578	
Maize stalks + maize cobs	30		8.4867	
Bean straw + maize cobs	30			9.1911
Sig.		1.000	.488	1.000

ANOVA table 16: Interaction effects of mushroom substrates on number of mushrooms fruitbody

Duncan[a,b]		Variable: Number of fruitbodies			
		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Bean straw + *Melia volkensii*	28	6.1714			
Maize stalks + rice straw	30	6.7067	6.7067		
Bean straw + maize cobs	30	6.9278	6.9278	6.9278	
Bean straw + rice straw	30		7.4067	7.4067	
Maize cobs + rice straw	30			8.0489	
Maize stalks + maize cobs	30				9.2644
Maize stalks + bean straw	30				9.4667
Sig.		.206	.243	.059	.719

ANOVA table 17: Interaction effects of mushroom substrates on total fresh weight

Duncan[a,b]		**Variable: Total mushroom weight**			
		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Bean straw + *Melia volkensii*	28	136.2143			
Bean straw + rice straw	30		260.2000		
Maize stalks + rice straw	30		273.4000	273.4000	
Maize cobs + rice straw	30		295.6667	295.6667	
Bean straw + maize cobs	30			303.1333	
Maize stalks+ maize cobs	30				374.2000
Maize stalks + bean straw	30				403.6667
Sig.		1.000	.084	.149	.129

ANOVA table 18: Interaction effects of mushroom substrates on biological Efficiency (%)

Duncan[a,b]		**Variable: Biological Efficiency (%)**			
		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Bean straw + *Melia volkensii*	28	37.1156			
Bean straw + rice straw	30		76.0819		
Maize stalks + rice straw	30		84.3827	84.3827	
Bean straw + maize cobs	30			90.7585	
Maize cobs + rice straw	30			90.9744	
Maize stalks + bean straw	30				112.7561
Maize stalks + maize cobs	30				118.4177
Sig.		1.000	.150	.283	.326