SUBSTRATES EVALUATION AND EFFECTS OF pH AND NUTRITIONAL SUPPLEMENTATION ON PRODUCTION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

BY

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other University.

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DEDICATION

Especially to my late loving son **Mavin Olulo**

My Dear Wife Agnes Olulo

and

My Loving Children,

Michael Olulo and Melissa Olulo

My Parents: Joyce Odero and Isaac Odero

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LIST OF ABBREVIATION/ACRONYMS

ABE	-	Average Biological Efficiency
ARV	-	Antiretroviral
CD4	-	T-lymphocyte cell with CD4 protein molecule on their surface
C/N RATIO	-	Carbon Nitrogen Ratio
FAO	-	Food and Agriculture Organization
HIV/AIDS	-	Human Immune Virus/Acquired Immune Deficiency Syndrome
RCBD	-	Randomized Complete Block Design
CRD	-	Completely Randomized Design
SAS	-	Statistical Analyses Systems

ABSTRACT

Mushrooms have been grown all over the world for many years because of their culinary, medicinal, bioremediation and biodegradation properties. Lack of locally generated information on substrates for oyster mushroom cultivation has led to over reliance on wheat straw. This is one of the major challenges facing the mushroom sector in Kenya today. Ten different substrates were tested using plastic bag technology in a randomized complete block design (RCBD) experiment to determine their effect on time to pinning, number of caps, average biological efficiency (ABE), pileus diameter, stipe length and flushing interval. Substrates tested were water hyacinth (*Eichhornia crassipes*), maize cobs (*Zea mays*), coconut fibre (*Cocos nucifera*), finger millet straw (*Seteria microcheata*), banana fibre (*Musa sp*), sugarcane bagasse (*Saccharum officinarum*), sawdust (*Eucalyptus sp*), rice straw (*Oryza sativa*), bean straw (*Phaseolus vulgaris*) and wheat straw (*Triticum aestivum*). The pH of maize cobs, coconut fibre and sugarcane bagasse were adjusted by liming using calcium carbonate to 6.0, 6.5 and 7.0 in order to determine the effect of pH on their productivity. Supplementation with maize germ, wheat bran and rice bran was done on bean, finger millets, rice and wheat straws at 3% dry weight basis to determine their effect supplementation on the productivity of these substrates. Substrates and pH had significant ($P \leq 0.05$) effect on average biological efficiency while supplementation had no effect. The average biological efficiency (ABE) varied between the ten substrates from 4.0% on sawdust to 106.2% on bean straw and the time to pinning was from 19.6 days on maize cobs to 39.9 days on water hyacinth. Adjusting the pH of maize cobs, coconut fibre, eucalyptus sawdust

and sugarcane bagasse, increased their ABE to between four to tenfold. Supplementation increased the average biological efficiency marginally. Choice of substrate and correct pH adjustment are very important to profitable oyster mushroom cultivation as was observed from the results of this study.

Key words: *Average biological efficiency, mycelia, pinheads, pinning, primodia, spawn run, pileus*

CHAPTER ONE

1.0 Introduction

Worldwide production of cultivated mushrooms is estimated at 5 million tons. The leading countries are China, United States of America, Netherlands and France (Chang, 1999A; FAO, 2002). In Africa, mushroom farming for either the local or external markets is at its infancy in most countries. It is only South Africa, Zimbabwe and Kenya which have been reported to produce mushrooms at a commercial scale (FAO, 2002).

1.1 Economic importance of mushrooms

Overall world production of cultivated edible and medicinal mushroom was recorded as 4,909.3 x 10^3 tons in 1994 increasing to 6,158×10^3 tons in 1997 with an estimated value in excess of US $ 14 billion (Chang, 1999a). The ten leading species make up 92% of total world production and of this: *Agaricus bisporus* account for 31.8% of total production whilst *Lentinus edodes*, *Pleurotus sp*ecies, *Auricularia amicula*, *Fammulina velutipes*, *Volvariela volvaceae* contribute 25.4%, 14.2%, 7.9%, 4.6%, 7.9%, respectively (Chang,1999b). By late 1994, only *Agaricus* and *Pleurotus species* were cultivated worldwide and this was followed by *Lentinus edodes* in 1997. The rest of the major species are grown exclusively in Asia (Chang, 1999b).

1.2 Nutritional value of mushrooms

Mushrooms contain varying amounts of carbohydrates, proteins, vitamins, fats and minerals (Oriyo, 2002; Jiskani, 2001; Isikhuemhen *et al.*, 1999). Fresh mushrooms are approximately 90% water, while dried one is about 15%. White button mushroom contains more protein than kidney beans while shiitake (*Lentinus edodes*) though less nutritious, is a good source of protein (Chang, 1999b). Vitamins like riboflavin (B_2), pantothenic acid (B_5) biotin, thiamine, thiamine and niacin are found in mushrooms (Oriyo, 2002). They contain minerals such as potassium, phosphorus, calcium magnesium iron and sodium (Park, 2001). All essential amino acids are present in mushrooms, as well as water soluble vitamins and all the essential minerals (Buigut, 2002)

The protein value of mushrooms is twice that of asparagus and potatoes, four times that of tomatoes and carrots, and six times that of oranges. Their energy value also varies according to species, which is about equal to that of an apple (Jiskani, 2001).

Nutritional analysis showed that mushrooms are a more valuable source of protein than either cattle or fish on dry weight basis, and are good sources of almost all the essential amino acids when compared with most vegetables and fruits (Mattila *et al.*, 2002). Proximate analysis showed that oyster mushrooms contained 25.24 to 36.35 % protein, 1.53 to 1.95 % fat (dry matter basis).

1.3 Medicinal value of mushrooms

The medicinal uses of *Pleurotus* include their potential to act as antitumour agents (Kawamura *et al.*, 2000; Zhang *et al.*, 2001; Jiskani, 2001), antifungal and antiviral (Gunde-cimerman, 1999), ability to lower cholesterol (Wasser and Weis, 1999). They are also recommended to diabetic and anemic persons, owing to their low carbohydrate and high folic acid content. Some mushrooms are reputed to possess anti-allergic, anticholesterol, and anti-cancer properties (Jiskani, 2001).

Pleurotus tuber-regium is a common species in the western part of Kenya and it is useful, in some combinations, in the cure of headache, stomach ailments, colds and fever, asthma, smallpox and high blood pressure while *Lentinus tuber-regium* and *L. tigrinus* are used for treating dysentery and blood cleansing, respectively. *Auricularia* species have been traditionally used for treating hemorrhoids. *Boletus edulis* and *Lactarius* spp. are used for killing flies, while the puffballs are used for healing wounds (Delena, 1999).

Pharmaceutically, mushroom functions through the stimulation of blood lymphocytes, muscle relaxation and disease resistance. HIV/AIDS patients whose CD4 counts were between 100-200 showed increased CD4 count and haemoglobnin level when *Ganoderma lucidum* was added on their diet prior to ARV treatment. No negative effects of *Ganoderma lucidum* were detected when added to ARV. It seems to activate a protease inhibitor which stops the virus from

developing. *Ganoderma lucidum* also improves the healing process, and improves skin smoothness (Zeri, 2005)

1.4 Importance of mushrooms to the environment

Mushrooms are actively involved in the re-circulation of carbon at a global level because they are an active lignin-degrader. Lignin is the second most abundant biopolymer on earth, after cellulose. It is found in wood and grassy plants, and its breakdown is necessary for making cellulose accessible to degradation which could eventually lead to biofuel production (Stamets, 2000).

Mushrooms act like sponges, holding water for the forest. Mushrooms make micro-nutrients available as food for the trees and other plants with which they symbiotically coexist. It is also a model for studying the carbon dioxide cycle (the main greenhouse effect gas) and has a great potential for use in biodegradation of contaminants (Stamets, 2000).

1.5 Constraints to mushroom cultivation

Mushroom growers have used inappropriate housing which lowers the production due to sub-optimal lighting, poor aeration, non-conducive temperatures and poor relative humidity.

Spawn quality, availability and cost are constraints to mushroom cultivation. Due to the high cost of spawn, farmers resort to using lower spawning rates that leads to

delayed pinning and more exposure of substrate to contamination by competitor microorganisms like green mold. Delayed pinning lengthens the crop cycle and so lowers the profitability of mushroom cultivation. Mushroom cultivation skills and extension services are lacking among the farmers. Lack of market due to low local consumption is a constraint to adoption of mushroom production.

Diseases and pests are among the major constraints that reduce the mushroom yield. Bacterial blotch, which presents itself as an orange discoloration and brittleness of the basidiocarp, is caused by *Pseudomonas tolaasi* and is the most common bacterial problem encountered by growers. Green mold is caused by *Trichoderma viride*, and is noticed as green surface of the substrate. The two diseases can be controlled by improved sanitation and fly control. House flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*), sciarid (*Lycoriella mali*), phorid (*Megasellia halterata*) are among the flies that spread diseases, feed on the mycelia and reduce yield and quality of the mushroom. The flies can be reduced by timely disposal of spent substrates, exclusion from growing rooms, complete pasteurization and minimizing the breeding sites.

1.6 Problem statement

One of the major inputs in mushroom cultivation enterprise is the substrate. The oyster mushroom is a primary decomposer which is able to grow on a wide range of substrates and therefore farmers can use many different kinds of readily available plant biomass. However, the yields vary greatly from one substrate to

another. Unpredictable yields due to use of unsuitable substrate has discouraged most small-scale farmers who are often, unable to keep on with mushrooms cultivation. In order to maximize mushroom yields, use of the right substrate, is imperative. This study was therefore done to evaluate the productivity of locally available substrate and establish possible ways that can be used to improve their suitability to oyster mushroom cultivation.

1.7 Justification of the study

Mushrooms are grown for their culinary value, income generation, medicinal values, ability to act as bioremediators and agricultural waste utilizers. Mushroom cultivation is labour intensive and, therefore, creates employment. These attributes make mushroom cultivation ideal for Kenya where employment creation, improvement of nutrition level and income generation is a priority. Mushroom cultivation faces many challenges one of which is lack of knowledge on substrates, their suitability and how to optimize their productivity. This study sought to generate information on different substrates and establish the effects of adjusting the pH and nutritional supplementation on the yields of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm.

1.8 Research questions

The study had the following research questions:

(a). what is the effect of locally available substrates on oyster mushroom *Pleurotus ostreatus* production?

(b). what is the effect of pH and supplementation on mushroom yield?

1.9 Hypotheses

(a) Oyster mushroom *(Pleurotus ostreatus)* performance does not depends on the kind of substrate used.

(b) The productivity of acidic substrates can be improved by liming and supplements with rice bran, wheat bran and maize germ.

1.10 Overall objective

The main goal is to optimize oyster mushroom production by identifying a high yielding growth substrates for *P.ostreatus*.

1.11 Specific objectives

(a). To evaluate the suitability of different kinds of substrates in production of *Pleurotus ostreatus*.

(b). To determine the effect of pH and nutritional supplementation on oyster mushroom production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical background

Historically, mushrooms were gathered from the wild for consumption and for medicine. China has been the source of many early consumption of mushrooms, like *Auricularia auricular* (600AD), *Flamulina velutipes* (800AD), *Lentimus edodes* (1000AD) and *Tremella fuciformis* (1800AD). *Agaricus bisporus* was first cultivated in France in 1960. *Pleurotus ostreatus* (Jacq: Fr.) Kumm. was first grown in the United States of America in 1900 (Chang, 1999b). While mushroom cultivation now spans many centuries, it is only over the last 2-3 decades that there have been a major expansion in basic research and practical knowledge leading to the creation of major worldwide industries (Deanna, 2001).

2.2 Mushroom species

There are at least 2000 species of fungi that can be considered as mushrooms with at least 200 species of mushrooms showing various degrees of edibility (Chang, 1999a). Further, over 200 species of mushroom have been collected from the wild and utilized for various traditional medical purposes mostly in the Far East. To date, about 38 mushroom species have been cultivated commercially and of these, about 20 are cultivated on industrial scale. The majority of these cultivated species are both edible and possess medicinal properties. However, two major medicinal mushrooms namely *Ganoderma incidium* and trametes (*Coriolus* spp) are distinctly inedible (Oriyo, 2002).

2.3 Mushroom biology

Mushroom is the popular name for any large fleshy fungi, most of which are in *Basidiomycetes* class (Staunton, 2002). A mushroom is not a complete fungus but a spore producing body that develops from an extensive mass of fine threads present in the ground or substrates on which they grow. Most mushrooms, comprising many hundreds of species, are gill fungi and *boletes (order agaricales)*. Sometimes, the name mushroom is applied only to edible fungi, while inedible kinds – especially those with umbrella shaped cap – are called toadstools. Usually, the name mushroom covers both edible and poisonous kinds. Like other fungi, Mushrooms does not contain chlorophyll and cannot manufacture their own food. Most of them live in decaying organic matter such as dead woods, humus, manure, but some are capable of living, at least in part as parasites on trees (Dabbour *et al.*, 2002)

Mushrooms are heterotrophs and belong to the kingdom Fungi, most of which are in sub-phyla Basidiomycota and Ascomycota. Mushroom is a fruiting body or a reproductive structure of a fungus some of which are edible while others are extremely poisonous (Kivaisi and Magingo, 1999). They breed by spores which germinate into hyphae which collectively form primary mycelia and then secondary mycelia through plasmogamy. When stimulated, mycelia colonies form pins which grow into fruit bodies (MushWorld, 2004).

Mushrooms produce millions of spores in their gills. These spores, which are tiny, powdery, and barely visible to the naked eye, are carried and scattered by the wind. If conditions are favorable, they germinate and develop into full blown mushrooms. This is how nature propagates mushrooms.

The mushroom has both vegetative and reproductive phases. The vegetative phase shows linear growth of fungal mycelia dissolving complex substrate components into simpler molecules and absorbing them as nutrients. Lignocellulolytic enzymes of *P. ostreatus* help in biodegradation processes and other biotechnological processes (Litchfield, 2002). Vegetative growth ceases and reproductive phase begin when the mycelia is subjected to low temperature, high humidity, increased oxygen and sometimes light (Khan *et al.*, 2004).

2.4 Substrates used for mushroom cultivation

Mushrooms may be grouped into saprophytes, parasites and mycorrhizae depending on their trophic patterns. The most commonly grown mushrooms are saprophytes and decomposers in an ecosystem growing on organic materials like wood, leaves and straw in nature. Plant materials can be used as substrates for primary decomposers such as oyster which have lignocellulosic enzymes. Secondary decomposers like button mushrooms or straw mushrooms require substrate degraded by bacteria or other fungi. Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose or lignin, thus, most of organic

matters containing these components can be used as mushroom substrates. However, demanded amount of each nutritional source differ according to mushroom species. Button mushroom (*Agaricus bisporus*) requires relatively high nitrogen sources. The optimum C/N ratio for button mushroom is 17:1 while oyster and shiitake mushrooms require less nitrogen and more carbon. Mushroom mycelia secrete digestive enzymes into the substrate and absorb the dissolved nutrients. Cellulose, the main nutritional source for mushrooms, is one of the most abundant organic matters on earth and its digestive enzyme; cellulase is produced by several microorganisms including fungi. Mushroom is important in the sense that it is the only one by which cellulose is dissolved, absorbed and transformed into food for mankind (MushWorld, 2004).

The mycelial growth makes use of soluble carbohydrates, glucose, molasses, organic nitrogen sources like wheat bran, oat, maize, Soya bean crust and sunflower cake as well as mineral sources such as ammonium sulphate (Chang, 1999b)

According to worldwide survey, over 90 different kinds of wastes have been proved to be useful for oyster mushroom growing. Evaluation of all these wastes leads to renewed perception of what we call wastes. (Litchfield, 2002). Several agricultural residues have been used to produce the edible mushroom. Among these residues, the use of sugarcane bagasse allows the byproduct to be utilized in the production of a food of high nutritional value, with a protein content of up to 40% in

dry matter (Kivaisi *et al.*, 1999). The abundant availability of these agricultural byproducts makes Kenya a country with a great mushroom-producing potential.

Rice husks mixed with cotton residues is used for the production of *Pleurotus sajor-caju* (Fr.) Singer while cassava residues or banana leaves mixed with sugarcane bagasse for the production of *Pleurotus ostreatus* (Chang, 1999 b). Agricultural residues, which are mostly locally available, are used as substrates for mushroom production. Whereas some of these materials are readily available in all areas, others are not. The most commonly used bulk substrates in all areas are banana fiber, rice straw, wheat straw and sugarcane bagasse (Kivaisi *et al.*, 2003). Oyster mushroom can grow on several substrates but generally a good substrate should be of high lignocellulose content, correct water content (water activity), good microbial activity, a pH of 6 to 7, selective and high nutrient availability (Nandi and Mukherjee, 2006).

Paul Stamets was an early advocate of integrating a variety of mushrooms into a permaculture system (Stamets, 2000). In his design, agricultural wastes like corn stalks, wheat straws or rice straw can be used as growing media for oyster mushrooms production. After harvest, the spent substrates can be recycled as fodder or mulch for garden soils (Stamets, 2000).

Oyster mushroom is grown on sterile straw (wheat, rice). They also grow on a wide variety of high-cellulose waste materials. Some of these materials do not

require sterilization, only pasteuralization which is less expensive. Another advantage of oyster mushroom is that, its high biological efficiency increases potential profitability. Many types of organic wastes from crop production or food processing industry can be used to support oyster mushroom production (MushWorld, 2004).

Mushrooms can produce fruit bodies on rice straw (*Oryza sativa),* wheat (*Triticum aestivum)*, ragi (*Elucine coracana)*, bazra (*Pennisetum typhoides)*, sorghum (*Sorghum vulgare)* and maize (*Zea mays*) (Krisnamurthy *et al.*, 2000). Woods of poplar (*Populus robuster*), oak (*Quercus leucotricopora*), horse chestnut (*Aesculus indica*), *Acassia spp*, chopped banana pseudostem, cotton stalk, pea shells and poplar sawdust support mushroom production (Philippoussis *et al.*, 2001).

Water hyacinth (*Eichhornia crassipes*) is prominent for growing *Pleurotus* in the Philippines, Indonesia, Africa and Bangladesh. In India, it had an average biological efficiency of 50% when used to grow *Pleurotus sajor-caju* (Gujral *et al.*, 1989).

Banana pseudostem gave better results compared to rice straw and sawdust (Jandiak *et al.*, 1974). Coconut fibres have been composted and used for *Pleurotus* production in India (Shah *et al.*, 2004).

2.5 Nutrient contents in substrates

Wheat straw is a basic component for fermented *Agaricus* compost and contains 1% protein, 13% lignin, 39% hemicellulose and 40% cellulose (Mamiro, 2003) while Heltay et al., (1960) documented that it contained 48% cellulose, 20% lignin, 0.5% total nitrogen, 0.04% phosphate (P_2O_5), 0.1% potash (K_2O), 4.1% silica, carbon/nitrogen ratio of 104 and a pH of 6.9. Dry banana leaves contain 1.45% nitrogen and it is very productive for the oyster mushroom cultivation (Chan-Ho *et al.*, 1979).

Corn cobs which were hummer-milled gave variable yields. The cobs contain 40% cellulose, 15% lignin, 0.4% total nitrogen, 0.1% phosphate (P_2O_5), 0.25% potash (K_2O), 0.5% silica, and carbon/nitrogen ratio of 129, with a pH of 7 (Heltay *et al.*,1960).

Rice straw contains 41% cellulose, 13% lignin, 0.8% total nitrogen, 0.25% phosphate (P_2O_5), 0.3% potash (K_2O), 6% silica and a carbon/nitrogen ratio of 59 with a pH of 6.9 (Heltay *et al.*,1960). Some authors in India reported a composition of 14% lignin, 37% cellulose, 0.4% phosphate (P_2O_5), 0.55% total nitrogen, 1.6% potash (K_2O), 12% silica and a C/N ratio of 70 (Kaul *et al.*, 1981). The sawdust of beech or oak contains 44% cellulose, 26% lignin, 0.2% total nitrogen, 0.01% phosphate (P_2O_5), 0.03% potash (K_2O), 0.9% silica and a carbon/nitrogen ratio of 244 (Heltay *et al.*, 1960).

2.6 Research work done on substrates

A substrate evaluation study involving sawdust, talahib, sitaw, corn, ipil-ipil, guava, and water lily carried out by a team of researchers in July-Sept. 2003 at Bautista National High School, Pangasinan in The Philippines found that sawdust and sitaw leaves were the most productive and profitable substrates to produce oyster mushroom, followed by talahib straw, corn, ipilipil, and water lily leaves whose yield was significantly lower than sawdust and sitaw leaves but significantly higher than guava leaves and paper (Zervakis *et al.*, 2001).

Another study revealed that the heaviest fresh fruit bodies were obtained by *Pleurotus ostreatus* followed by *Pleurotus v. florida* being 233 and 215 grams/wet kilogram substrate, respectively. Fiber fractions analysis showed that cellulose and hemicellulose content in cornstalks decreased from such treatment due to the growth of fungi. *Pleurotus ostreatus* and *Plerotus v. florida* were the best strains for cellulose and hemicellulose degradation and the highest protein content increase (90%) over the untreated cornstalks (Okhuoya, 2000).

A recent study conducted showed that rice straw, mango pods, banana leaves, and sugar cane leaves can be used as substrates for oyster mushroom production. It was found out that mango pods as substrate gave the highest yield followed by rice straw, sugar cane leaves, and banana leaves, respectively (Goswami *et al.*, 1987).

An investigation on cultivation of *P. ostreatus* on dry weed plants (*Leonotis spp*, *Sida acuta*, *Parthenium argentatum*, *Ageratum cornizoides*, *Cassia sophera*, *Tephrosia purpurea* and *Lantana camara*) showed that *Leonotis spp* was the best in fruit production of *Pleurotus ostreatus* when mixed with rice straw (1:1 wet weight). Fruiting time for *Pleurotus ostreatus* was also less with *Leonotis spp* than any other weed substrates tested in the investigation. The main problem of oyster cultivation on weed substrates was low yields in second flush that could be overcome by blending with rice straw. The protein content of mushroom obtained from *Cassia sophera, Parthenium argentatum* and *Leonotis spp* were not only better than rice straw but also from weeds supplemented with rice straw (Mukherjee *et al.*, 2004).

Fasidi and Kadiri (1993) reported the successful growth of mushrooms on lignocellulose wastes such as banana, plantain leaves, cereal straw, cassava peels, coconut core, cotton waste (kapola) and paper wastes, which provided the essential nutrients required for its growth.

Rice straw was found to be a better substrate than rice husk because it gave 29 to 48% higher yield by different oyster mushroom species. Maximum yield of 51.5 to 72.3% was obtained when an optimum amount of rice straw i.e. 1.5 kg/tray (52x38x10 cm) was used for cultivation of different species of oyster mushrooms. Further increase in the amount of substrate to 2 kg/tray decreased the average yield by 11-16%. Different carbohydrate sources have different effect on mycelia growth. The highest mycelia growth of 9.71 mg ml^{-1} was achieved using millet

extract while rye gave the least mycelial growth of 9.47 mg ml^{-1} (Ibekwe *et al*, 2008). Among the different carbohydrate sources (millet, rice, maize and rye) studied, millet proved the best carbohydrate source for the production of mushroom mycelia.

2.7 Cultivation Modes

Oyster mushrooms cultivation modes include bag, shelf, log and bottle cultivation methods. In bag cultivation, the spawned substrate is put in plastic bags for incubation and subsequent growing. Using the shelf method, the growing medium is filled into shelves in the culture room where pasteurization, incubation and subsequent production take place. In log technology, inoculation holes are drilled into the logs of approximately one meter long and 15 to 20 cm diameter. After the introduction of spawn plugs into the holes, the logs are incubated and when the spawn run is complete, they are transferred to the culture room for ultimate production. In the bottle technology, spawned substrate is put in the bottles from where the mushrooms are produced (MushWorld, 2004).

The cultivation of mushrooms in Ghana is basically by the plastic bag method, with the use of decomposed sawdust mixed with cereals (rice or millet) to produce *Pleurotus* species of mushrooms (Obodai *et al.*, 2001).

2.8 Mushroom Substrate Supplementation

Substrate supplementation is a practice that has been used to produce *Pleurotus* sp. in order to increase productivity as evaluated through biological efficiency (Tagwira *et al.*, 1998). Among various tested supplements, mulberry leaves and stalks were used in rice hull supplementation for the production of *Pleurotus sajor-caju* (Madan *et al.*, 1987), while wheat bran and calcium carbonate were used in sugarcane bagasse supplementation for the production of *Pleurotus* sp.

Supplementation with 15% pigeon pea on sugarcane bagasse gave an ABE of 97.9% Tagwira *et al.*, (1998). Wheat straw supplemented with soya bean and alfalfa increased the production of *P. sajor-caju* by 300% but when ammonium nitrate was used, production increased by 50% (Tagwira *et al.*, 1998).

According to Tagwira *et al.* (1998), addition of nitrogen to an alkaline substrate stimulated mycelium formation and production of mushrooms. However, excessive addition of inorganic nitrogen can inhibit the synthesis of the lignin degrading enzyme, thus causing decrease in productivity of the substrate.

2.9 Substrates pH and its Effect on Mushroom Growth

The optimal substrate's pH ranges between 6 and 8 depending on mushroom species (MushWorld, 2004).

The optimum pH value for mycelial growth is 5-6.5, though mycelia can survive between 4.2 and 7.5 while the growth reduces as the pH lowers and stops growing at pH 4. If the pH is higher than optimal, mycelial growth accelerates but produces abnormal structures. Optimal pH value for primodial initiation and fruit formation is between 5-5.5 though it is possible at 5.5-7.8. The pH can be adjusted by addition of gypsum (Mukherjee *et al.*, 2004).

2.10 Spawning rates

Surveys indicate that whereas some farmers are over-spawning the substrates by using concentration of 10-20% which is uneconomical, others are under spawning by using a concentration of less than 1% (Magingo *et al.*, 2004). Under spawning, resulting into delayed colonization exposing the substrate to contamination for a longer time while over spawning delays pinning (Royse, 2002). However, the main hindrance to mushroom cultivation in small scale farms has been low level of hygiene, specifically at the substrate disinfestations and bagging stages. This has been brought about by ineffective substrate disinfestations. The use of unsuitable substrates results into low production, making mushroom cultivation unprofitable (MushWorld, 2004).

Growers have sought, in the past, to optimize the amount of spawn used to inoculate their substrate. Increasing the amount of spawn used (up to 5% of the wet weight of the substrate) has resulted in increased yields. Increasing spawn rates from 1.25% substrate wet weight to 5% may result in yield increases of

nearly 50%. Yield increases may be due to several factors. First, the increased level of nutrient available in higher levels of spawn used would provide more energy for mycelial growth and development. Second, more inoculum points, available from increased spawn levels, would provide faster substrate colonization and thus, more rapid completion of the production cycle. Finally, a more rapid spawn run would reduce the time non-colonized substrate is exposed to competitors such as weed molds and bacteria (Beyer, 2003).

There is a negative correlation between spawn rate and days to production. As the spawn rate increases, the number of days to production decreases. By using a spawn rate of 5 percent of the wet substrate weight, it is possible to reduce the time to production by more than 7 days compared to a spawn rate of 1.25 percent. Thus, growers could complete the crop cycle faster, minimizing the exposure of the production substrate to pest infestations, especially sciarid flies. (*Lycoriella mali* [Fitch]). Research has shown that the sciarid fly may complete its life cycle in 25 days at 21°C, while 35 to 38 days are required at 18°C. (Penn State research, 2003)

2.11 Utilization of spent mushroom substrate

Spent mushroom substrates improve soil physical properties such as reducing soil bulk density and increasing soil water content. Soils that received mushroom substrate applications tended to measure lower in surface hardness. Phosphorus, potassium, magnesium, and calcium levels are generally increased with increasing

applications of spent mushroom substrate (McNitt *et al.*, 2002). Spent mushroom substrate can improve the structure of clay soils, reduce surface crusting and compaction, promote drainage, increase microbial activity, and provide nutrients for plant growth. These improvements promote faster crop establishment and color, increased rooting, and less need for fertilizer and irrigation.

The pH of most spent mushroom substrate products is between 6.0 and 8.0, a range which is favorable for turf grass root growth and the electrical conductivity is below injury levels to crops (less than 18mmhos/cm) (Penn State's Agricultural Analytical Services Lab, 2008). Test results of spent mushroom substrate products typically indicate 1.5 to 3% total nitrogen on a dry weight basis. Other nutrients found in spent mushroom substrate include phosphate (0.5 to 2.0 %), potash (1.0 to 3.0%), calcium (3 to 6%), and magnesium (0.4 to 1.0%) (Penn State's Agricultural Analytical Services Lab, 2008).

After mushroom harvest, the spent substrates can be used as a soil conditioner, fodder or mulch for garden soils. Substrate from bags can be reused to make new bags but must be well pasteurized and mycelium removed (Stamets, 2000).

2.12 Oyster mushroom

The oyster mushroom, scientifically known as *Pleurotus spp*, is so-called because of its flavor and which is coupled with a sweet smell. *Pleurotus* species is also known as "oyster mushroom", "hiratake", "shimeji", or "houbitake" (Alberto *et al.*,

2002). There are many species in this genus among them *P.ostreatus*, *P.palmonarius*, *P.sajor-caju*, *P.erygii*. In this experiment, the white oyster mushroom (*P.ostreatus)*, was cultivated. Oyster mushroom varies in texture from very soft to very chewy, depending on the strain and the time of the year of picking. Unlike any common mushrooms with umbrella-like structure, oyster mushroom grows transversal that seems like a flower. Its cap begins to open at the start of pinheads and grows to form fruiting bodies with an oyster-like structure. It is commonly found in overlapping clusters on logs and dead or dying hardwood trees. The fruiting body has short laterally attached stalks and caps that are white to ashy gray, with white gills underneath. This mushroom species is an edible spore producing fungus with a stipe and pileus proverbial for its rapid growth and usually produced by means of natural method or tissue culture (Kivaisi *et al.*, 2003).

2.13 Requirements for oyster mushroom cultivation

Temperature, relative humidity, pH of the substrate, moisture content of the substrate, nutrient level in the substrate, C/N ratio, ventilation, smoke, chemical vapours and light, are the key factors that affect oyster mushroom growth. Higher substrate temperatures above 35°C may injure mushroom spawn, reduce mycelial growth rates, and leave the substrate vulnerable to competitors such as *Coprinus* spp. (ink caps) and *Trichoderma* spp. (green mold) (Kadiri and Kehinde, 1999). Mushroom mycelia can survive between 5-40^{0}C depending on species but can grow well in temperature range of 20-30^{0}C. Pins form at 10-20^{0}C, a temperature

lower than that of mycelia growth by 10^0C. Oyster mushroom primordia are very sensitive to chemical vapors. Being aerobic fungi, mushrooms need fresh air for growth, but ventilation is more required for reproductive stage. Deformed mushrooms may be traced to insufficient ventilation, smoke, chemical vapors.

Most mushroom mycelial growth is better yielding in the absence of light while fruit bodies give better yields during light and darkness alternation. Though mycelia can grow without light, some species require light for fruit body formation (Zandrazil, 1982).

The substrate moisture content should be 60-75% and log moisture content, 35-45%. During fruiting, different relative humidity levels, ranging from 80-95% are needed at early, mid and late stage. It has been established that oyster mushrooms require darkness during spawn run, some ventilation and a relative humidity of 65-70%, and for fruit induction, a relative humidity of about 90% with good ventilation are required by most oyster mushrooms. On the other hand, fructification requires that the relative humidity be lowered to 80-85% with adequate light after pinhead formation (MushWorld, 2004).

CHAPTER THREE

3.0 EFFECT OF LOCALLY AVAILABLE SUBSTRATES ON OYSTER MUSHROOM (*Pleurotus ostreatus*) GROWTH PARAMETERS AND BIOLOGICAL EFFICIENCY

3.1 Abstract

Lack of information on suitable substrates for oyster mushroom cultivation leading to use of any available material is one of the major challenges facing mushroom sector in Kenya today. Ten different substrates namely, 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 (*Triticum aestivum*) were screened for their suitability in mushroom production. A plastic bag technology in a randomized complete block design (RCBD) experiment was used to determine the effect on time to pinning, number of caps, average biological efficiency (ABE), pileus diameter, stipe length and duration between flushes. While the effect of substrates was significant ($P \leq 0.05$) on time to pinning, number of caps and average biological efficiency, it was not on cap diameter, stipe length and pinhead abortion. The time to pinning ranged from 19.6 to 39.9 days in maize cobs and water hyacinth, respectively. The number of caps ranged from 4.0 to 54.3 in sawdust and bean straw, respectively per 250g of dry substrate. The average biological efficiency (ABE) ranged from 4.0% to 106.2% on sawdust and

bean straw, respectively. The flushing interval varied between 12 to 22 days. In descending order of their positive differences, bean, rice, finger millet and wheat straws can be recommended for oyster mushroom production. Low-mycelial density and high contamination was recorded in low producing substrates with 93% of the sawdust bags failing to produce the third flush due to contamination

Key words: Average biological efficiency, pileus, pinning, spawn run, spawning, stipe

3.2 Introduction

Pleurotus ostreatus (Jacq.: Fr.) Kumm. is a saprophytic fungus that is able to draw its nutrients from dead plant material with the aid of lignocellulytic enzymes. The mushroom exists in the wild and has the ability to utilize cellulose, hemicellulose and lignin for their growth (Zhang *et al.*, 2003). It is, therefore, able to survive on plant materials with varying biological efficiency. Two hundred substrates have been used to grow oyster mushroom (Chang, 1999). Some species of *Pleurotus* are able to colonize different types of vegetable wastes, increasing their digestibility (Mukherjee and Nandi, 2004). The mycelial growth makes use of soluble carbohydrates, glucose, molasses, organic nitrogen sources like wheat bran, oat, maize, soya bean crust and sunflower cake as well as mineral sources such as ammonium sulphate.

Ninety kinds of wastes have been proven to be useful for oyster mushroom growing (Zhang *et al.*, 2001). Evaluation of all these agricultural residues, leads to renewed perception of what we call wastes since they can be used in growing mushrooms (Stamet, 1999a).

Nutritional substances for oyster mushroom can be classified into staples or base nutritional materials and additives like proteins which are nitrogen sources. Staples that are rich in cellulose and hemicellulose include wheat straw, barley, hardwood chips and many other agricultural residues. These can be utilized alone, as is done in 90-98% of cases, or mixed with other materials as in the example of 55% wheat straw and 38% corn cobs (Okhuoya, 2000).

Several agricultural residues have been used to produce the edible mushroom *Pleurotus* sp., also known as "oyster mushroom", "hiratake", "shimeji", or "houbitake" (Mizuno *et al.*, 1995). Among these residues, is the sugarcane bagasse which can be used to produce mushrooms with a protein content of up to 40% in dry matter (Rajarathnam and Bano, 1999). The abundant supply of these agricultural residues makes Kenya a country with a great mushroom-production potential.

Among the agricultural substrates used to produce *Pleurotus* sp., are rice hulls mixed with cotton residues for the production of *Pleurotus sajor-caju* (Fr.) Singer (Chang, 1999), banana leaf mixed with sugarcane bagasse or maize cobs and also cassava residues with sugarcane bagasse for the production of *Pleurotus ostreatus* (MushWorld, 2004). Oyster mushroom can grow on several substrates but, generally a good substrate should be of high lignocellulose content (Nandi and Mukherjee, 2006).

Oyster mushroom can produce fruit bodies on straws of rice (*Oryza sativa)*, wheat (*Triticum aestivum*), ragi (*Eucine coracana)*, bazra (*Pennisetum typhoides)*, sorghum (*Sorghum vulgare)*, and maize (*Zea mays*) (Bano *et al.*, 1987; Goswami *et al.*, 1998). It can also grow on, woods of poplar (*Populus robuster*), oak (*Quercus leucotricopora*), horse chestnut (*Aesculus indica*), *Acassia spp* (Pant *et al.*, 1998), chopped banana pseudostem (Singh and Tandon, 1987), cotton stalk, pea shells and poplar sawdust (Philippoussis *et al.*, 2001).

Water hyacinth (*Eichhornia crassipes*) is prominent for growing *Pleurotus* in The Philippines, Indonesia, Africa and Bangladesh. In India, it gave an ABE of 50% when used to grow *Pleurotus sajor-caju* (Gujral *et al.,* 1999). Wheat straw is a basic component for fermented *Agaricus* compost and contains 1% protein, 13% lignin, 39% hemicellulose and 40% cellulose (Heltay *et al.*, 1960). Locally available organic materials which are mostly agricultural residues can be used for oyster mushroom cultivation. These wastes are specific to regions (Kivaisi, 2003).

From the literature cited, it is evident that many researchers have done studies on mushroom substrates in different parts of the world but none of them have compared water hyacinth, maizecobs, coconut fibre, banana fibre, sugarcane bagasse, sawdust, rice straw, bean straw, finger millet straw and wheat straw in a single study. Lack of locally generated information on substrate suitability to oyster mushroom cultivation, has led to use of any available substrate which are sometimes unsuitable leading to low production, farmer disappointment and even abandonment of mushroom cultivation.This study, therefore, was done to determine the suitability of the ten substrates for oyster mushroom cultivation.

3.3 Materials and Methods

Ten substrates studied were namely, bean straw (*Phaseolus vulgaris)*, sawdust (*Eucalyptus spp)*, sugarcane baggase (*Saccharum officinarum)* (control), coconut fibre (*Cocos nucifera)*, finger millet straw (*Seteria microchaeta)*, water hyacinth (*Eichhornia crassipes)*, rice straw (*Oryza sativa)*, maize cobs (*Zea mays*), wheat straw (*Triticum aestivum)* and Banana fibre (*Musa spp)*. The experiment was laid

out in a completely randomized block design (CRBD) with four replications per treatment. The experiment site was at Kabete campus, University of Nairobi between September, 2007 and January, 2008. Oyster mushroom spawn GSP1POK2 from Kabete laboratory was used.

The plastic bag technology was used in this experiment. Substrates were air-dried to a constant weight and chopped. Autoclavable polypropylene transparent bags size 15×18 cm were filled with 250g of substrate and soaked overnight. The substrates were sterilized in an autoclave at a temperature of 121°C and a pressure of 15 psi for 20 minutes. The substrates were left to cool to 25±3 °C, wet-weight of the bags recorded and spawned at a rate of 4±1% w/w. Spawned bags were placed in a dark room at 23±3°C until spawn run was complete. Upon completion of spawn run, two 10mm holes were made on each bag. The temperature and relative humidity were kept at 22±3°C and 75±10% respectively by spraying the culture room with clean water two to three times per day depending on weather conditions. Insects were controlled by using insect screens on doors and windows and using sticky traps. Three to four days after the emergence of pinheads, mature mushrooms were harvested. Measurements taken on marketable mushrooms were weight of fresh mushrooms, pileus diameter, stipe length, number of mature mushrooms and aborted pinheads. Times to pinning and flushing interval were recorded per bag.

Average biological efficiency (ABE) was obtained by average biological efficiencies of the bags of each treatment using the expression:

ABE% = Total wet mass of mushroom×100 / dry mass of the initial substrate (Chang, 1999b). Total wet mass of mushroom was obtained by the sum of yields recorded from four flushes. The pileus diameter was obtained by taking two perpendicular measurements across the pileus and getting the average since the oyster mushrooms pileus is not perfectly round. The pinhead abortion ratio was obtained from the average number of pinheads from bags of each treatment using the expression:

Percent pinhead abortion (%) = aborted pinheads × 100/total number of pinheads

The moisture content of the substrates was calculated using the expression:

Moisture content (%) = substrates' dry-weight ×100/substrates' wet-weight

The data was analyzed using Statistical Analyses System (SAS Institute, 2000) and means separated using Tukey's test.

3.4 Results

The number of marketable mature caps harvested from each bag varied significantly ($P \leq 0.05$) among the ten substrates tested (Table 1). The sawdust had the lowest (4.0) while bean straw gave the highest (54.3) average number of pileus per bag of 250g of dry substrate among the ten substrates. Effect of maize cobs, water hyacinth, coconut fibre and sawdust on the number of mature caps was not significantly ($P \leq 0.05$) different from sugarcane bagasse (control) while banana fibre, bean straw, finger millet straw rice straw and wheat straw had significant

effect on the same parameter. Banana fibre was not significantly different from wheat straw but different from finger millet straw.

The effect of the bean straw, finger millet straw, rice straw and wheat straw on average biological efficiency was significantly ($P \leq 0.05$) different from the sugarcane bagasse while the others were not (Table 1) and it varied, from 4.0% to 106.2% on sawdust and bean straw respectively among the substrates tested. Water hyacinth, maize cobs, coconut fibre, and sugarcane bagasse were not significantly ($P \leq 0.05$) different in terms of average biological efficiency.

The pileus diameter varied significantly ($P \leq 0.05$) among the treatments with the mean diameter varying between 34.6mm to 48.4mm on sawdust and sugarcane bagasse, respectively. The results also showed that seven out of the ten substrates were not significantly different in terms of cap diameter. Substrates had significant ($P \leq 0.05$) effect on the stipe length with the longest and the shortest stems being observed on beans straws and sawdust, respectively among the ten substrates tested (Table 1). There was no significant ($P \leq 0.05$) effect on percent pinhead abortion, among the ten substrates tested, although it varied from 49.9-63.9% on sawdust and bean straw, respectively (Table 1).

It was observed that cap diameter is very much dependent on the length of time taken between pinhead emergence and harvesting. Sugarcane baggase produced muhrooms with the largest pileus, 48.3mm, while sawdust produced the smallest,

34.6mm. The tallest and the highest number of mushrooms was obtained from bean straw although it had the highest percent pinhead abortion (63.9%)

Table 1: Experiment I, Suitability of various substrates for oyster mushroom growth as determined by various yield parameters

Substrate	Number of pileus	ABE (%)	Pileus diameter (mm)	Stipe length (mm)	Pinhead abortion (%)
Banana fibre	30.9^{c}	65.1^{c}	45.5^{a}	36.7^{ab}	63.3
Bean straw	54.3^{a}	106.2^{a}	45.8^{a}	40.6^{a}	63.9
Coconut fibre	15.8^{d}	22.8^{d}	45.2^{a}	26.7^{d}	59.4
Finger millet straw	41.7^{b}	85.4^{b}	48.3^{a}	38.9^{a}	59.0
Maize cobs	12.9^{de}	25.2^{d}	47.2^{a}	30.4^{bd}	61.3
Rice straw	45.4^{ab}	92.1^{ab}	41.9^{ab}	38.0^{a}	61.9
Sawdust	4.0^{e}	4.0^{e}	34.6^{b}	18.6^{d}	49.9
Sugarcane bagasse	12.2^{de}	22.5^{d}	48.4^{a}	28.2^{e}	62.0
Water hyacinth	12.3^{de}	21.9^{d}	43.2^{ab}	34.4^{abc}	61.8
Wheat straw	36.5^{bc}	77.1^{bc}	46.4^{a}	39.4^{a}	60.3
LSD (P=0.05)	**9.4**	**16.4**	**6.3**	**7.2**	**NS**
CV (%)	**31.3**	**27.6**	**17.8**	**19.0**	**19.7**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

The same trend was observed when the experiment was repeated (Table 2). The substrates had significant (P≤0.05) effect on number of pileus, ABE, pileus diameter and stipe length but not on pinhead abortion ratio.

Table 2: Experiment II, Suitability of various substrates for oyster mushroom growth as determined by various yield parameters

Substrate	Number of pileus	ABE (%)	Pileus diameter (mm)	Stipe length	Pinhead abortion (%)
Banana fibre	31.1^{c}	73.5^{b}	47.9^{a}	36.7^{a}	62.9
Bean straw	56.1^{a}	106.0^{a}	43.4^{ab}	30.8a^{bc}	66.9
Coconut fibre	14.6^{d}	19.8^{cd}	37.3^{ab}	23.9^{de}	58.5
Finger millet straw	42.9^{bc}	96.0^{a}	42.9^{ab}	30.6^{abcd}	61.0
Maize cobs	14.8^{d}	26.7^{c}	40.3^{ab}	28.1^{cd}	61.4
Rice straw	48.5^{ab}	93.4^{a}	40.5^{ab}	29.5b^{cd}	63.3
Sawdust	3.5^{d}	3.6^{d}	35.8^{b}	19.7^{e}	51.3
Sugarcane bagasse	13.5^{d}	24.6^{c}	46.5^{ab}	26.1^{cde}	62.6
Water hyacinth	13.0^{d}	25.7^{c}	44.7^{ab}	28.1^{cd}	70.9
Wheat straw	37.4^{bc}	76.4^{b}	46.7^{a}	36.0^{ab}	59.6
LSD (P=0.05)	**12.7**	**16.7**	**10.9**	**6.8**	**NS**
CV (%)	**28.2**	**18.7**	**15.7**	**14.3**	**19.6**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Spawn run was observed from third day after spawning. Primodia induction occurred between 19-39 days (Table 3) depending on substrates. Treatment effect on days taken between spawning to primodia appearance was highly significant ($P \leq 0.05$) among the substrates tested, with maize cobs taking the shortest and water hyacinth the longest. Wheat, rice, finger millet straws and banana fibre were the same in terms of days to pinning while sugarcane bagasse and wheat straw were not.

There was a significant difference in flushing intervals among the substrates (Table 3). The mean length of time taken between the first and the second flushes varied between 12.6 days in saw dust to 24.7 days in bean straw. The duration from the second to third was 13.9 days in finger millet straw to 21.6 days on water hyacinth (Table 3).

Green mold (*Trichoderma* spp.) contamination was observed just after the first flush, rendering 20% of the maize cobs, 30% of sugarcane bagasse and 80% of saw dust substrate bags not to produce the second flush, while 93% of sawdust failed to produce third flush.

Table 3: Experiment I, Effect of substrate on duration to pinning and flushing intervals in *Pleurotus ostreatus*

Substrate	Time to pinning	Flushing interval (days)	
		1-2	2-3
Banana fibre	32.3^{bc}	16.9	18.5
Bean straw	29.8^{c}	12.6	14.8
Coconut fibre	22.6^{de}	18.6	19.2
Finger millet straw	31.1^{bc}	17.5	13.9
Maize cobs	19.6^{e}	20.2	19.5
Rice straw	36.3^{ab}	15.6	15.0
Sawdust	22.4^{de}	24.7	15.3
Sugarcane bagasse	28.2^{cd}	17.3	15.6
Water hyacinth	39.9^{a}	20.3	21.6
Wheat straw	28.8^{cd}	18.4	18.1
LSD (P=0.05)	**6.31**	**NS**	**NS**
CV (%)	**19.12**	**19.69**	**39.66**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

The substrates had significant (P≤0.05) effect on time to pining but not on flushing interval (Table 4). The same was observed from the first crop.

Table 4: Experiment II, Effect of substrate on duration to pinning and flushing intervals in *Pleurotus ostreatus*

Substrate	Time to pinning	Flushing interval (days)	
		1-2	2-3
Banana fibre	36.3ab	16.9	19.0
Bean straw	33.9bc	10.8	13.5
Coconut fibre	27.8dc	21.0	19.8
Finger millet straw	42.6a	22.1	13.3
Maize cobs	24.9d	17.1	17.1
Rice straw	41.1a	16.5	13.8
Sawdust	28.9cd	14.5	13.0
Sugarcane bagasse	33.1bc	15.3	14.6
Water hyacinth	41.4a	19.5	21.3
Wheat straw	32.6bc	18.3	17.4
LSD (P=0.05)	**6.5**	**NS**	**NS**
CV (%)	**11.6**	**40.6**	**31.9**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Table 5 shows that the substrates had significant ($P \leq 0.05$) effects on yields, first, second and third flushes. The yields varied from 254.6g in bean straw to 6.5g in sawdust per 250g dry substrate. The highest percentage of the harvest was picked during the first flush with the substrates of low productivity giving the highest. Sawdust had the highest percentage yield (92.0%) in the first flush while rice straw gave the lowest (48.2%). Finger millet straw had more evenly spread production over the three flushes compared to the other substrates which were tested.

Table 5: Experiment I, Effect of substrate on yield per flush and percentage of yield per flush

Substrate	Total yields (g)	Yield per flush (g)			Percentage yield per flush		
		1st	2nd	3rd	1st	2nd	3rd
Banana fibre	159.9^{c}	83.8^{c}	49.9^{b}	26.2^{bc}	54.0^{cd}	31.1^{a}	14.9^{abc}
Bean straw	254.6^{a}	143.5^{a}	66.4^{ab}	44.7^{ab}	57.2^{cd}	25.6^{a}	17.3^{abc}
Coconut fibre	53.7^{d}	25.7d^{e}	13.8^{c}	14.3^{cde}	49.3^{d}	27.2^{a}	23.5^{a}
Finger millet straw	195.3^{bc}	96.5^{bc}	48.4^{b}	50.3^{a}	49.6^{d}	24.6^{a}	25.8^{a}
Maize cobs	60.8^{d}	40.4^{d}	14.0^{c}	6.4^{de}	67.0^{bc}	23.1^{a}	9.9^{bcd}
Rice straw	220.3^{ab}	101.7^{bc}	69.4^{a}	49.2^{a}	48.2^{d}	31.2^{a}	20.6^{ab}
Sawdust	6.5^{e}	5.5^{e}	0.8^{c}	0.1^{e}	92.0^{a}	5.8^{b}	2.2^{d}
Sugarcane bagasse	55.8^{d}	41.5^{d}	11.2^{c}	3.1^{de}	74.6^{b}	20.0^{a}	5.4^{cd}
Water hyacinth	54.7^{d}	32.2^{d}	17.1^{c}	5.5^{de}	69.6^{bc}	23.6^{a}	6.8^{cd}
Wheat straw	192.0^{bc}	115.4^{b}	55.1^{ab}	21.5^{dc}	60.0^{bcd}	29.0^{a}	11.0^{bcd}
LSD (P=0.05)	**36.5**	**20.2**	**19.5**	**19.8**	**16.2**	**13.1**	**12.1**
CV (%)	**25.6**	**25.9**	**49.6**	**78.6**	**22.9**	**47.9**	**77.6**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

During the second crop the same trends were observed as in the first crop (Table 6). The substrates had significant (P≤0.05) effects on yields and percentage yields per flush. The lowest (6.5g) and the highest (256.1g) yields were obtained from sawdust and bean straw, respectively. No harvestable produce was recorded from sawdust after the second flush (Table 6).

Table 6: Experiment II, Effect of substrate on yield per flush and percentage of yield per flush

Substrate	Total yield (g)	Yield per flush (g)			Percentage yield per flush (%)		
		1st	2nd	3rd	1st	2nd	3rd
Banana fibre	175.1^{b}	92.8^{b}	53.2^{ab}	29.2^{abc}	54.0^{bc}	30.8^{a}	15.2^{abc}
Bean straw	256.1^{a}	136.7^{a}	66.7^{a}	52.7^{a}	53.8^{bc}	25.7^{a}	20.5^{ab}
Coconut fibre	53.0^{cd}	23.5^{cd}	11.9^{c}	17.6^{bc}	47.5^{c}	25.3^{a}	27.2^{a}
Finger millet straw	196.2^{b}	98.6^{b}	50.0^{ab}	47.6^{ab}	52.2^{c}	26.1^{a}	21.5^{ab}
Maize cobs	62.1^{c}	41.2^{c}	13.1^{c}	7.7^{c}	67.6^{bc}	21.3^{ab}	11.1^{abc}
Rice straw	219.0^{ab}	96.2^{b}	75.8^{a}	47.0^{ab}	46.6^{c}	34.3^{a}	19.1^{ab}
Sawdust	7.5^{d}	6.7^{d}	0.8^{c}	0.0^{c}	94.1^{a}	5.9^{b}	0.0^{c}
Sugarcane bagasse	59.1^{cd}	43.4^{c}	11.7^{c}	4.1^{c}	74.4^{ab}	18.9^{ab}	6.6^{bc}
Water hyacinth	69.5^{c}	33.3^{cd}	25.3^{bc}	10.9^{c}	52.7^{c}	33.6^{a}	13.7^{abc}
Wheat straw	178.4^{b}	108.1^{b}	51.3^{ab}	19.5^{bc}	60.2^{bc}	29.1^{a}	10.8^{abc}
LSD (P=0.05)	**54.4**	**27.3**	**29.3**	**32.6**	**21.4**	**18.0**	**18.6**
CV (%)	**26.1**	**24.6**	**49.9**	**84.5**	**21.7**	**44.0**	**78.2**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

3.5 Discussion

The average biological efficiency was significantly different among the substrates tested in this study. This finding is in conformity with (Das and Mukherjee 2006) who reported the same while studying use of different weed plants as substrates for *P. ostreatus* production. The hypothesis that the treatments had no effect on biological efficiency, number of caps and duration taken to pinning was not sustained. The results show that the days to pinning greatly depends on the substrate and the highly productive substrates like bean and rice straws came into production later than the less suitable substrates. The tendency of the poorer substrates to pin earlier is attributed to nutritional stress that mycelium is subjected to (Oei, 2003). The number of mushrooms harvested and time taken to pinning varied greatly as has been reported by other researchers like Nageswaran *et al.* (2003), indicating that the two variables are substrate dependent. Water hyacinth pinned much later than has been recorded by other researchers Nageswaran et al., (2003). This is because its moisture content was higher (83%) than optimum (75%) as reported by MushWorld (2004). The high water content hindered mycelial development thereby delaying the pinning.

Cap diameter and stipe length depends on amount of aeration and light (Kivaisi *et al.*, 2003). During the study, it was observed that the two parameters also depend on the length of time taken from pinhead appearance to harvesting in addition to the substrate type. It was observed that pileus diameter was very much dependent on the number of caps per cluster. The fewer they were, the wider the diameter due to lower competition for space and available nutrients.

Pinhead abortion was in most substrates more than 50% which means that more than half of the pinheads that emerged did not grow to marketable produce. This phenomenon was not dependent on the substrates. Reducing this abortion rate therefore means more produce and by extension more earnings from mushroom cultivation. It was observed that the most suitable substrate exhibited shorter flushing interval which implies shorter cropping cycles and therefore more crops in a given length of time.

Mycelial density was high on sugarcane bagasse but most of the bags were lost due to contamination. The high levels of contamination in sugar cane baggase have been attributed to traces of sugar (Oei, 2003). Only one to two flushes could be harvested making them even lower producers. Slow colonization of the relatively poorer substrates makes them prone to contamination by competing fungi especially *Trichoderma* sp.

3.6 Conclusion and Recommendation

Since the average biological efficiency is substrate dependant, choice of the right substrate for oyster mushroom cultivation is important to growers. From the results of this study, the ten substrates can be arranged in order of decreasing suitability as follows bean straw, rice straw, finger millet straw, wheat straw, banana fibre, maizecobs, sugarcane bagasse, coconut fibre, water hyacinth and sawdust. It is recommended the choice of substrates for oyster mushroom cultivation be made in this order.

CHAPTER FOUR

4.0 EFFECT OF pH AND NUTRITIONAL SUPPLEMENTS ON SUITABILITY OF SUBSTRATES FOR OYSTER MUSHROOM (*Pleurotus ostreatus*) CULTIVATION

4.1 Abstract

One of the key factors that affect mycelial growth and, subsequently, the sporophore yields in oyster mushroom cultivation is the pH of the substrate. The effect of pH variations on the growth of *Pleurotus ostreatus* cultivated on maize cobs (*Zea mays*), coconut fibre (*Cocos nucifera*), sugarcane bagasse (*Saccharum officinarum*) and sawdust (*Eucalyptus sp*) was determined. The substrates were acidic with basal pH of 3.9, 4.0, 4.5 and 4.9 for coconut fibre, sawdust, sugarcane bagasse and maize cobs, respectively. The pH of the four substrates was adjusted to 6.0, 6.5 and 7.0 using calcium carbonate. Bean, finger millet, rice and wheat straws were supplemented with wheat bran, rice bran and maize germ at 3% on a dry weight basis to evaluate their effect on yields of oyster mushroom. The pH had a significant effect ($P \leq 0.05$) on yield, average biological efficiency (ABE), number of pileus, time to pinning and flushing among all the four substrates tested while supplementation had no effect on total number of pileus, yield, pileus diameter and stipe length. The average biological efficiency of maize cobs increased from 18.4% to 68.3% when the pH was adjusted to 6.5. However, the efficiency decreased to 31.8% when the pH was raised to 7.0. Coconut fibre recorded the shortest duration to pinning and a 5 times increase in ABE when pH was adjusted to 6.0. Among the substrates tested, sawdust recorded more than 10 times increase in ABE at pH

7.0. Sugarcane bagasse recorded the shortest duration to pinning and a 3 times increase in ABE at pH 6.5. The fresh mushroom yields increased by between 1.7 to 10.9 times in maize cobs and sawdust, respectively. During the crop's cycle, no mushroom bags were lost through contamination when the pH was adjusted beyond 6.0. Finger millet supplemented with maize germ gave an average biological efficiency of 131%, which was the highest, while rice straw supplemented with maize germ gave 49.6%, which was the lowest.

Key words; Average biological efficiency, mycelia, pinning, primodia.

4.2 Introduction

The effect of pH as a key determinant of mushroom substrate productivity has been documented by several researchers and it is among the most important parameters for the hyphal growth (Kadiri and Kehinde, 1999; Mukherjee *et al.*, 2004). Various mushrooms show varying responses to pH but, for the majority of isolates, hyphal growth, which is very sensitive to the pH of the medium, was found to be optimal at pH 5.

Ground limestone is recommended for making substrate for elm oyster and in casing soil, as it buffers the pH upward. The pH of the casing soil should not be driven so low as this interferes with the interaction between the metabolites of the mycelium which lowers the ultimate yield of mushrooms Randall, 2002). When selecting additives for ameliorating eucalypt sawdust their influence on substrate pH and hence hyphal growth should be considered Stott *et al.*, (2004). Because sugarcane bagasse is slightly acidic, lime is added to adjust the pH (MushWorld, 2004).

The maximum mycelia yield and weight was recorded at pH 6.5. The mycelia yield decreased at pH above 6.5 while poor mycelia growth and the least mycelia weight was recorded at pH 2.0 (Akinyele and Adetuyi, 2005).

Fasidi and Kadiri (1996) reported that mushroom mycelium was able to tolerate pH range of 3 – 10. This probably explains the ability of the mushroom to flourish very well on various agricultural wastes in the tropics. The pH range for the growth of straw mushroom was found to be 5.5 – 8.5, while the optimum pH was found to be 6.5. Kuforiji and Fasidi (1998) obtained an optimal pH range of 5 to 7 for *Pleurotus tuberregium* Jonathan and Fasidi (2000) reported appreciable growth of *Psathyrella atroumbonata* at pH 6.5. Similar observations were made by Anyakorah *et al.*, (1998) on the cultivation of *Lentinus squarrosulus.*

From the foregoing literature, it is evident that the mycelia can grow within a pH range of 2-10. However, of importance is the optimal pH level and amendments to be made in order to achieve the optimal pH. This study seeked to establish the liming levels on coconut fibre, maizecobs,eucalyptus sawdust and sugarcane bagasse in order to achieve the optimal pH for *Pleurotus ostreatus* (Jacq.: Fr.) Kumm.

4.3 Supplementation

When incorrectly formulated or incorrectly supplemented, other organisms referred to as weed molds grow in mushroom substrate. Fungal infestation may be more of a problem when substrates are supplemented with nitrogen-rich nutrients, especially if the supplements are not commercial delayed-release nutrients. At the time of spawning, a commercial delayed-release supplement consisting of paraffin-coated whole soybean or formaldehyde-denatured soybean and feather meal may

be added at rates of 3 to 6 percent of dry substrate weight, to stimulate yield of the mushroom (Staunton and Dunne, 1995).

Yield increases of up to 90 percent have been observed when 6 percent (dry weight) supplement is added to the substrate at the time of spawning. Delayed-release nutrient supplements have also been shown to decrease the number of days to harvest. The addition of 3 percent nutrient at time of spawning may reduce time to production by 2 to 3 days. Thus, growers wishing to hasten the production process may do so by supplementing with only small quantities of supplement. Use of supplements, however, may cause overheating of the substrate if growers are unable to anticipate and control air temperatures to maintain a steady substrate temperature. Additional cooling capacity is required when higher levels of supplement are used (Hafiz *et al.*, 2003). Rise in temperature was observed, during incubation, when fish waste was used as a supplement. This was due to the increase in the nutrient content (carbohydrates and nitrogen), such that resident bacteria and competitive moulds in the substrate increased in numbers to cause the high temperature (Lelley and Janben, 1993).

Button mushrooms naturally grow on materials with relatively high nitrogen content such as horse manure (1.8% nitrogen) and wheat straw (0.65% nitrogen). The optimal carbon/nitrogen ratio for button is 17:1 while oyster and shiitake grow from wood with relatively low nitrogen source of C/N ratio which range from 350 to 500:1. The optimal C/N ratio varies from mushroom species to another

(MushWorld, 2004). The main substrate alone may not supply the optimal nitrogen required and hence the need for supplementation with rice and wheat bran as nitrogen sources (MushWorld, 2004).

The best mycelial development and sporophore yield was observed on sawdust supplemented with wheat bran at 5%. Rice bran and cassava peels as additives stimulate both mycelial extension and sporophore yield of *Lentinus subnudus* on various agricultural wastes (Fasidi and Kadiri, 1993). High production rate was reported when wheat bran was used as supplement to shiitake mushroom substrates Royse and Schisler, (1986), Han *et al.*, (1981), working on the growth of *Lentinus edodes,* found that nutrient supplementation of sawdust with wheat bran, soya bean cake, sesame cake and peanut cake increased the mycelial growth with optimum concentration at 5%. The observed stimulatory effect of wheat bran may be due to carbohydrates, amino acids and mineral elements present in wheat bran (Fasidi and Kadiri, 1993). The lowest yield was observed on sawdust supplemented with urea at 0.5%. This low yield may be due to carbon to nitrogen imbalance in the sawdust. Supplemented sawdust can produce at least an 8-10 fold greater yield than the natural log (Stamets, 2000).

The yield of *P. atroumbonata* on some agro industrial wastes was enhanced using supplements such as wheat bran and nitrogen, phosphorous and potassium fertilizers (Stamet, 2000). Supplementation of rice straw with different nitrogen sources like *Vigna cajan*, corn gluten meal and leaf protein concentrate increased

the yield of mushrooms between 72.3 to 83.5 % whereas ammonium sulphate, ammonium nitrate, urea and mustard seed meal suppressed or inhibited the growth of mushroom sporophores. This experiment was carried to determine the effect of pH and supplementation on oyster mushroom (*P. ostreatus*) production.

4.4 Materials and methods

The substrates used in this experiment were water hyacinth (*Eichhornia crassipes*), maize cobs (*Zea mays*), coconut fibre (*Cocos nucifera*), finger millet straw (*Seteria microcheata*), sugarcane bagasse (*Saccharum officinarum*), banana fibre (*Musa sp*), sawdust (*Eucalyptus sp*), rice straw (*Oryza sativa*), bean straw (*Phaseolus vulgaris*) and wheat straw (*Triticum eastivum*). The pH was adjusted to 6, 6.5 and 7 using calcium carbonate (lime). A completely randomized experimental design (CRD) was used with four replications per treatment.

The basal pH of the ten substrates was established by measuring the pH of the leachate of presoaked and sterilized substrate using a pH-meter. These measurements were taken three different times, in order to counter check the outcomes and the basal pH for coconut fibre, sawdust, sugarcane bagasse, maize cobs, wheat straw, finger millet straw, water hyacinth, bean straw, banana fibre and rice straw was found to be 3.9, 4.0, 4.5, 4.9, 6.3, 6.4, 6.4, 6.6, 7.0 and 7.0, respectively. Based on their basal pH, coconut fibre, maize cobs, sugarcane bagasse and sawdust were selected to be used to determine the effect of pH on substrate productivity while wheat, bean, rice, finger millet straws were utilized at

their basal pH to determine the effect of nutrient supplementation on yield and other growth parameters of *P. ostreatus.*

4.5 Effect of pH on yield and growth parameters of *P. ostreatus*

The amount of lime needed to adjust the pH was determined by, measuring 100g dry substrate, soaking it overnight then adding lime at a rate of 2% dry weight. The substrates were then sterilized using the autoclave then letting it cool to room temperature. The leachate was drawn and the pH measured using a pH-meter and from the pH change caused by unit addition of lime, the amount of lime required to adjust pH to 6.0, 6.5 to 7.0 was calculated. The basal pH was used as the control in this experiment.

The materials were chopped and 100g of dry substrate put in autoclavable polypropylene bags. Calcium carbonate was weighed and added to the substrate. The amount of water, enough to achieve 70±5% moisture content was measured and used to soak the mixture overnight. The mixture was then sterilized in an autoclave at 121^{0}C for 20 minutes. The sterilized bags were allowed to cool to 25±4^{0}C and then spawned at 4±1% (w/w). A breather neck (0.5 inch diameter, 1inch long plastic pipe plugged with cotton wool) was installed to allow gaseous exchange. The spawned bags were incubated in the dark until spawn run was complete. After the spawn run, the bags were exposed to light and holes opened to allow in more air for of pinhead induction.

4.6 Effect of nutrient supplementation on yield and growth parameters of *P. ostreatus*

The substrates were chopped and measured out and maize germ. rice bran and wheat bran added to wheat straw, finger millet straw, rice straw and bean straw at 3% (dry weight basis) then soaked overnight. The substrates were supplemented at their basal pH. The amount of water, enough to achieve 70±5% moisture content was measured and used to soak the mixture overnight. The mixture was then sterilized in an autoclave at 121^0C for 20 minutes. The sterilized bags were allowed to cool to 25 ± 4^0C and then spawned at 4±1% (w/w). A breather neck (0.5 inch diameter, 1inch long plastic pipe plugged with cotton wool) was installed to allow gaseous exchange. The spawned bags were incubated in the dark until spawn run was complete. After the spawn run, the bags were exposed to light and holes opened to allow in more air for of pinhead induction.

The data taken were weight of fresh marketable mushrooms, time taken to pinning, time taken between flushes, pileus diameter, stipe length, number of mature caps and number of aborted pinheads. The data collected were analyzed by statistical analyses system (SAS Institute, 2000).

4.7 Results

Effect of pH on oyster mushroom yield and other growth parameters

During the second crop, the yields were lower but the same trends were observed. The yields were increased by more than four times when the pH of coconut fiber, maize cobs, sawdust and sugarcane bagasse were adjusted to levels ranging from

6.0 to 7.0. The mycelial density was much higher and contamination of bags by green mold (*Trichoderma viride)* was decreased.

When the pH of maize cobs was adjusted, there were significant ($p \leq 0.05$) effects on number of pileus, yields, average biological efficiency, flushing intervals and duration to pinning. There was an improved mycelial density in all the substrates whose pH was adjusted compared to the controls. There was no contamination on bags whose pH were adjusted. Higher yields were observed at pH 6 (Table 7) with 3% liming level.

Table 7: Experiment I, Effect of pH on productivity of maize cobs

pH level	Duration to pinning (days)	Pinhead abortion ratio (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
4 9 (Control)	48.3^{a}	53.5	22.5	7.8^{b}	54.5	24.5^{ab}	22.5	18.4^{c}	18.4^{c}
6.0	23.3^{c}	57.6	21.3	12.3^{ab}	52.0	21.3^{bc}	26.3	68.3^{a}	68.3^{a}
6.5	29.8^{b}	65.7	23.1	16.8^{a}	50.6	20.8^{c}	22.8	51.8^{ab}	51.8^{ab}
7.0	27.5^{b}	73.2	20.8	9.8	41.7	25.5^{a}	23.3	31.8^{bc}	31.8^{bc}
LSD (P=0.05)	**2.52**	**NS**	**NS**	**6.78**	**NS**	**3.4**	**NS**	**24.88**	**24.88**
CV (%)	**3.72**	**19.17**	**19.65**	**27.76**	**12.50**	**7.04**	**5.35**	**27.85**	**27.85**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Liming had a significant ($p \leq 0.05$) effect on yield and average biological efficiency but not on number of pileus, cap diameter, stipe length, days to pinning or flushing intervals (Table 8). The same trend was observed during the first crop.

Table 8: Experiment II, Effect of pH on productivity of maize cobs

pH level	Duration to pinning (days)	Pinhead abortion ratio (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
4.9 (Control)	52.8	80.2	19.3	6.3	38.2	26.5	25.0	19.6^{b}	19.6^{b}
6.0	36.3	78.5	19.9	9.8	40.2	23.0	23.3	38.0^{a}	38.0^{a}
6.5	49.3	88.0	21.7	9.8	39.6	25.0	23.0	46.3^{a}	46.3^{a}
7.0	54.5	79.3	21.6	9.8	40.5	28.0	27.0	38.7^{a}	38.7^{a}
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**12.8**	**12.8**
CV (%)	**40.3**	**7.2**	**20.7**	**17.5**	**5.3**	**29.6**	**13.6**	**15.8**	**15.8**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

When the pH of coconut fibre was adjusted to 6, 6.5 and 7.0 there was a significant ($p \leq 0.05$) effect on number of pileus, yield, average biological efficiency, pinhead abortion ratio, date to pinning and flushing intervals. There was no significant ($p \leq 0.05$) effect on cap diameter and stipe length. The yields improved by upto five times at pH 7.0 (Table 9). The liming level to achieve pH 7.0 was 5.5%.

Table 9: Experiment I, Effect of pH on productivity of coconut fibre

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
3.9 Control	47.5^{a}	61.8^{bc}	19.5	5.8^{c}	42.9	25^{ab}	23.5^{ab}	7.9^{d}	7.9^{d}
6.0	24.3^{b}	54.2^{c}	26.1	17.0^{a}	47.0	23.8^{ab}	24.5^{a}	45.0^{a}	45.0^{a}
6.5	34.3^{b}	70.8^{a}	19.6	10.5^{b}	49.1	28.8^{a}	19.8^{b}	29.1^{c}	29.15^{c}
7.0	59.5^{a}	66.2^{ab}	21.9	5.0^{c}	39.8	23.0^{b}	21.5^{ab}	35.8^{b}	35.8^{d}
LSD (P=0.05)	**12.8**	**8.4**	**NS**	**2.1**	**NS**	**5.4**	**4.6**	**5.7**	**5.7**
CV (%)	**14.7**	**6.3**	**18.5**	**10.4**	**10.2**	**10.3**	**9.8**	**9.2**	**9.2**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

The pH level of coconut fibre had significant ($p \leq 0.05$) effect on the yield and average biological efficiency but not on number of pileus, cap diameter, stipe length, flushing intervals or days to pinning (Table 10). The highest yield (13.7g) was observed at pH 6.0 which was in conformity with the finding during the first crop. The days to pinning increased during the second crop compared to the first.

Table 10: Experiment II, Effect of pH on productivity of coconut fibre

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
3.9 (Control)	58.3	74.2	12.7	4.5	27.9	19.8	19.8	5.5^{b}	5.5^{b}
6.0	69.3	75.4	14.1	6.3	31.1	23.5	23.5	13.7^{ab}	13.7^{ab}
6.5	48.5	82.2	14.7	6.0	33.4	23.3	23.3	11.5^{ab}	11.5^{ab}
7.0	63.5	80.4	14.0	4.3	32.7	33	19.8	8.7^{ab}	8.7^{ab}
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**8.0**	**8.0**
CV (%)	**23.4**	**7.3**	**14.7**	**32.3**	**11.2**	**31.0**	**19.9**	**38.8**	**38.8**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Sawdust showed marked improvement of yields, up to ten times. There was a significant ($p \leq 0.05$) effect on number of caps, yields, average biological efficiency and stipe length but there was no significant ($P \leq 0.05$) effect on pileus diameter, pinhead abortion ratio, date to pinning and flushing interval. The highest yields were observed at pH 6.5 (Table 6).

Table 11: Experiment I, Effect of pH on productivity sawdust

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
4.0 (Control)	52.8	61.3	18.8^{c}	3.8^{b}	44.3	21.5	22.3	4.1^{b}	4.1^{b}
6.0	57.5	66.5	22.7^{b}	11.0^{a}	49.2	26.8	24.8	42.2^{a}	42.2^{a}
6.5	62.8	70.5	25.0^{ab}	9.8^{a}	52.3	22.0	22.0	44.5^{a}	44.5^{a}
7.0	67.3	77.7	27.1	7.3^{ab}	49.1	25.8	25.0	36.9^{a}	36.9^{a}
LSD (P=0.05)	**NS**	**NS**	**3.6**	**4.7**	**NS**	**NS**	**NS**	**18.2**	**18.2**
CV (%)	**12.4**	**12.0**	**7.3**	**28.2**	**9.0**	**10.5**	**7.9**	**27.2**	**27.2**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

When the experiment was repeated, the supplements had significant (P≤0.05) effects on yield and average biological efficiency but not on cap diameter, stipe length, pinhead abortion ratio, days to pinning or flushing intervals.

Table 12: Experiment II, Effect of pH on productivity sawdust

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
4.0 (Control)	62.8	73.2	15.7	3.5	27.7^{b}	23.5	26.3	5.6^{b}	5.6^{b}
6.0	59.8	70.3	15.0	8.3	$34.8a^{b}$	22.8	22.8	21.2^{a}	21.2^{a}
6.5	80.0	75.7	18.6	5.5	43.2^{a}	24.5	24.0	21.8^{a}	21.8^{a}
7.0	68.0	77.7	19.3	6.3	35.9^{ab}	26.0	22.0	18.8^{ab}	18.8^{ab}
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**10.5**	**NS**	**NS**	**13.5**	**13.5**
CV (%)	**29.1**	**22.4**	**20.7**	**25.2**	**13.8**	**7.6**	**9.2**	**38.3**	**38.3**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

When the pH of sugarcane bagasse was adjusted, there was a significant (P≤0.05) effect on number of pileus, yield, average biological efficiency, date to pinning and flushing interval. There was no significant effect on pileus diameter, stipe length and pinhead abortion ratio. The average biological efficiency improved by up to four times. The yields were upto 64.0g per 100g of dry substrate when pH was adjusted to 6.5.

Table 13: Experiment I, Effect of pH on productivity of sugarcane bagasse

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1 (days)	Flushing interval 2 (days)	Yield (g)	Average biological efficiency (%)
4.5 (Control)	51.0	60.0	23.0	10.8^{ab}	49.0	22.5	22.8	19.0^{b}	19.0^{b}
6.0	25.0^{b}	70.0	23.0	12.3^{a}	51.9	23.5^{b}	26.0	44.6^{ab}	44.6^{ab}
6.5	30.0^{b}	70.0	25.7	13.8^{a}	51.7	21.0	21.0	64.0^{a}	64.0^{a}
7.0	53.0^{a}	60.3	24.6	6.5^{b}	48.1	40.0	30.0	37.2^{ab}	37.2^{ab}
LSD (P=0.05)	**7.9**	**NS**	**NS**	**4.3**	**NS**	**14.1**	**NS**	**27.2**	**27.2**
CV (%)	**9.5**	**13.9**	**13.9**	**19.1**	**12.4**	**24.8**	**18.5**	**31.4**	**31.4**

- The value in each cell represents means from four replications each bag containing 100g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang. (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Table 14 shows that liming had a significant (P≤0.05) effect on number of pileus, yield and average biological efficiency but not on cap diameter, stipe length, days to pinning and flushing intervals. The highest yield of 53.6g was recorded at pH 6.5 a trend that was also observed during the first Crop.

Table 14: Experiment II, Effect of pH on productivity of sugarcane bagasse

pH level	Duration to pinning (days)	Pinhead abortion (%)	Stipe length (mm)	Number of pileus	Cap diameter (mm)	Flushing interval 1(days)	Flushing interval 2(days)	Yield (g)	Average biological efficiency (%)
4.5 (Control)	63.8	79.2	16.5	7.5^{b}	39.8	29.8	24.3	23.2^{b}	23.2^{b}
5.0	54.4	84.2	17.9	10.5^{ab}	44.2	23.8	24.3	40.5^{a}	40.5^{a}
6.5	56.8	83.7	20.0	11.8^{a}	44.7	24.0	22.5	53.6^{a}	53.6^{a}
7.0	42.8	78.2	18.8	11.0^{ab}	44.4	24.0	22.5	47.5^{a}	47.5^{a}
LSD (P=0.05)	**NS**	**NS**	**NS**	**3.8**	**NS**	**NS**	**NS**	**16.7**	**16.7**
CV (%)	**16.3**	**8.3**	**11.5**	**17.6**	**6.0**	**16.9**	**12.8**	**22.4**	**22.4**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Effect of supplementation with maize germ, wheat bran and rice bran on oyster mushroom yields and other growth parameters

Bean straw supplementation with wheat bran, rice bran and maize germ had no significant (P ≤0.05) effects on total number of pileus, yield, average biological efficiency, stipe length and pinhead abortion ratio. The mycelial growth was denser in the initial stages. There was no loss of bags through contamination and slight improvement an average biological efficiency compared to the control treatment (Table 8).

Table15: Experiment I, Effect of supplementation on productivity of bean straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	Cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	31.0	162.0	81.0	51.8	25.0	72.1
Wheat bran	44.8	183.9	91.9	48.6	24.5	70.5
Rice Bran	33.8	182.4	91.2	51.3	57.2	77.1
Maize germ	34.0	173.2	86.6	49.5	22.4	76.3
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	**28.0**	**26.9**	**26.9**	**13.4**	**101.4**	**5.8**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Supplementation had no significant (P≤0.05) effects on number of pileus, yield, average biological efficiency cap diameter stipe length and pinhead abortion ratio (Table15), a slight increase in quantity of mushroom yield was observed, these trends were observed from the first experiment.

Table16: Experiment II, Effect of supplementation on productivity of bean straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	Cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	98.0	19.5	48.9	52.7	24.3	73.1
Wheat bran	133.4	25.0	66.7	53.6	25.2	69.1
Rice bran	135.3	26.0	67.8	51.5	26.3	70.5
Maize germ	123.6	26.5	61.9	54.3	29.8	78
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	**21.8**	**31.7**	**21.8**	**13.7**	**11.0**	**8.0**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Supplementation had no significant ($p \leq 0.05$) effect on wheat straw production of oyster mushroom. There was insignificant effects on total number of pileus, yield, average biological efficiency, pileus diameter and stipe length. There was significant ($P \leq 0.05$) effect on pinhead abortion ratio. There was slight improvement on fresh mushroom yields and no contamination was observed (Table 9).

Table 17: Experiment I, Effect of supplementation on productivity of wheat straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	25.5	141.1	70.5	52.2	26.5	74.1^{ab}
Wheat bran	32.5	166.3	83.2	47.9	27.8	69.9^{b}
Rice Bran	28.0	171.6	85.8	52.9	25.4	75.6^{ab}
Maize germ	22.8	159.9	79.9	54.1	23.5	80.8^{a}
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**8.7**
CV (%)	**28.6**	**22.0**	**22.0**	**7.5**	**13.8**	**5.5**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Supplementation had no significant (P ≤0.05) effects on the yield, number of pileus, average biological efficiency, cap diameter, stipe length and pinhead abortion ratio of oyster mushroom (Table 18). Results from the first experiment (Table 17) also show the same trend.

Table18: Experiment II, Effect of supplementation on productivity of wheat straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	Cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	32.0	175.0	87.5	52.0	29.8	77.6
Wheat bran	24.5	166.3	83.2	53.2	31.1	73.1
Rice bran	29.5	175.7	87.9	50.8	28.1	77.9
Maize germ	27.0	169.5	84.8	52.2	29.0	75.0
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	**25.2**	**20.9**	**20.9**	**8.9**	**13.6**	**12.0**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

With the exception of pinhead abortion, supplementation had no significant effect on the other growth parameters when oyster mushroom was grown on finger millet straw although marginal improvement in yields was recorded (Table 19).

Table 19: Experiment I, Effect of supplementation on productivity of finger millet straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	cap diameter (mm)	Stipe length (mm)	Pinhead abortion (%)
Control	30.3	167.1	81.0	55.6	25.5	66.9^{b}
Wheat bran	35.3	189.5	99.9	55.6	26.0	57.8^{c}
Rice Bran	41.5	195.7	97.7	50.4	27.3	59.8^{bc}
Maize germ	35.0	207.8	137.7	56.4	24.0	80.3^{a}
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**8.7**
CV (%)	**35.2**	**24.9**	**37.3**	**13.1**	**13.4**	**6.2**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

Table 20 shows that supplementation had a significant (P≤0.05) effect on yields and average biological efficiency but not on number of pileus, cap diameter, stipe length and pinhead abortion ratio. The same trend was observed in the first season (Table 19).

Table 20: Experiment II, Effect of supplementation on productivity of finger millet straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	Cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	27.5	140.9^{b}	70.3^{b}	51.4	34.6	62.3
Wheat bran	28.5	154.3^{ab}	77.1^{ab}	50.0	33.5	71.7
Rice bran	33.0	217.6^{a}	108.8^{a}	50.4	34.6	62.3
Maize germ	30.5	179.8^{ab}	89.9^{ab}	53.1	37.2	71.8
LSD (P=0.05)	**NS**	**71.2**	**35.6**	**NS**	**NS**	**NS**
CV (%)	**32.7**	**19.6**	**19.6**	**5.7**	**10.3**	**12.9**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang. (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

When rice straw was supplemented with maize germ, there was no significant ($p \leq 0.05$) on the number of caps, yield, average biological efficiency, pileus diameter, stipe length and pinhead abortion ratio (Table 21).

Table 21: Experiment I, Effect of supplementation on productivity of rice straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	27.5	147.7	73.9	51.3	28.1	59.6
Wheat bran	22.5	138.4	69.2	61.6	22.9	66.6
Rice Bran	25.8	134.9	67.5	53.4	24.8	74.9
Maize germ	16.5	99.1	49.6	54.7	27.5	64.0
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	**34.2**	**27.9**	**27.9**	**13.2**	**10.0**	**26.8**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly ($P \leq 0.05$) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

The repeat experiment (Table 22) showed that there was no significant (P≤0.05) effects on the yields, ABE, cap diameter, stipe length and pinhead abortion ratio. The same trend was observed during the first crop.

Table 22: Experiment II, Effect of supplementation on productivity of rice straw

Supplement	Number of pileus	Yield (g)	Average biological efficiency (%)	cap diameter (mm)	Stipe length(mm)	Pinhead abortion (%)
Control	25.8[ab]	149.4	74.7	50.9	28.6	67.3
Wheat bran	20.0[b]	127.9	64.0	59.0	23.8	65.3
Rice bran	27.5[a]	145.5	72.8	52.3	24.7	74.1
Maize germ	20.3[ab]	114.4	57.3	56.3	29.0	52.6
LSD (P=0.05)	**6.4**	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	**13.0**	**20.6**	**20.6**	**7.4**	**13.1**	**28.8**

- The value in each cell represents means from four replications each bag containing 250g dry weight of substrate
- ABE (average biological efficiency) = Total wet mass of mushroom/dry mass of the initial substrate×100 Chang, (1999b)
- NS means not significant at 0.05 level
  Values in each column bearing same letter/s are not significantly (P≤0.05) different while those with different letter/s are significantly different
- LSD (P=0.05) means least significant difference at 0.05 significance level
- CV (%) means percent covariance ratio

4.8 Discussion

This study has revealed that the sporophore yield of *Pleurotus ostreatus* was significantly affected by the substrates' basal pH. The yields and by extension ABE of substrates increased when the pH was adjusted to 6.0 and 6.5 but decreased when pH was adjusted to 7.0. Due to lower temperatures during the second crop, lower yields were observed. The same phenomenon was observed by Ibekwe et al. (2008) who found that the optimum pH for mycelial yield was 6.4. On maize cobs the highest yield was observed at pH 6.0 which corresponded to a liming level of 3% on a dry weight basis. When the pH was increased to 7.0, there was a decrease in ABE. On coconut fiber the highest yield was observed pH 6. The ABE decreased when pH was raised to 7.

The study indicated that the optimal liming level for coconut fibre is 3.2% on dry weight basis. Sawdust gave the highest ABE at pH 6.5 which corresponded to a liming level of 4.4% dry weight basis. Sugarcane bagasse gave the highest yield at pH 6.5 which corresponds to a liming level of 4.5% on a dry weight basis. This finding is in agreement with a previous report by Chang and Miles (1989) which attributed the lower yields to toxicity of low pH to hyphal growth. Bilgranii and Verma (1992) reported that mycelia are more tolerant to acidic media than basic media. Zadrazil (1978) reported that the growth of *Pleurotus ostreatus* and *Pleurotus erygii* mycelia were positively affected by pH range 4 to 6.

Liming improved the fresh mushroom yields by more than 300%. The highest were obtained between pH 6.0 and 6.5 this confirms Ibekwe (2008) finding that optimum mycelia yield was observed at pH 6.5.

In all the supplemented substrates tested, the mycelial density was higher compared to control. This was attributed to the readily available nutrients provided by the additives. There was a slight improvement in average biological efficiency though not significant among the different cereal-based supplements. This is in conformity with the findings by Fasidi and Kadiri (1993) who observed stimulatory effect of wheat bran which they attributed to carbohydrates, amino acids and mineral elements present in wheat bran. The contamination of mushroom bags was not observed on supplemented substrates. This was attributed to the increased mycelial growth that warded off other possible competitors.

In this study, no significant effect on average biological efficiency and other growth parameters was observed; this was attributed to low level of supplementation.

4.9 Conclusion and Recommendation

The results indicate that liming is very important if sawdust, sugarcane bagasse, coconut fiber or maize cobs are to be used for oyster mushroom cultivation and that the optimal liming levels are 4.4%, 4.5%, 3.2% and 3.0% for sawdust, sugarcane bagasse, coconut fiber and maize cobs respectively. It is also evident that different substrates require different liming levels depending on their basal pH. Since over liming leads to drastic drop in yields, it is recommended basal pH of the substrates be established and adjusted before using them on mushroom cultivation.

It is recommended, that any of the three cereal based supplements can be used depending on whichever is available without compromising on the yields of oyster mushroom.

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Appendix

Appendix 1
Percentage moisture content of the substrates used in suitability experiment

Substrate	Water hyacinth	Maize cobs	Coconut fiber	Finger millet straw	Banana fiber	Sugarcane bagasse	sawdust	Rice straw	Bean straw	Wheat straw
Moisture content(%)	83	65.3	76	79.3	78.3	76.4	67.5	73.5	72.4	76.4

Appendix 2
Percentage lime used to adjust the pH

substrate	Basal pH	pH		
		6.0	6.5	7.0
Maize cobs	4.9	3.0	4.4	5.5
Coconut fiber	3.9	3.2	4.4	5.5
Sugarcane bagasse	4.5	3.5	4.5	5.7
Sawdust	4.0	3.4	4.4	5.3