COMPARATIVE STUDIES ON GROWTH AND YIELD OF *Pleurotus ostreatus* ON DIFFERENT TYPES OF SUBSTRATES

BY

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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(BSc. AGRICULTURE)

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DECLARATION

I hereby declare that this submission is my own work towards the MSC and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

So many agricultural wastes such as coconut coir and bean straw can be turned into useful products to increase their biomass use in Ghana. Proper composting and effective management of these wastes can render them useful in mushroom production. The project was carried out to compare the response of *Pleurotus ostreatus* on three types of substrates at three different composting periods. The substrate types used included sole bean straw, bean straw mixed with coconut coir at 2:3 ratio and sole coconut coir substrate. These were composted at different time periods and different parameters such as rate of mycelia formation; number of bags fully colonized; time of first flush; length, width and perimeter of fruits; yield of mushroom; dry matter content of the mushroom; biological efficiency and cost benefit analysis of using the different substrate types were determined. The sole coconut coir substrates gave the highest C:N ratios ranging from 97. 75 for the sole coir composted for 21 days to 106. 85 for the same substrate composted for a day. The sole bean straw and the mixed substrates produced similar C:N ratios of between 33.70 – 53.48. The substrates with the least C:N ratio gave the highest yield of mushrooms more especially in the sole coir and in the mixed substrates. Composting period did not affect the rate of mycelia formation, however, it affected the yield of the mushrooms. Substrates composted for longer periods in the sole coir gave the highest yield while the substrate composted for just a day in the mixed substrates produced the highest yield with the highest profit margin. The mixed substrates gave the greatest biological efficiencies ranging between 11.0 to 22.90 % as well as the highest returns. The results indicated that the effectiveness of substrates to produce higher returns depends on the initial C:N ratio of the substrates. As a result, substrates with high C:N ratio need longer periods of composting, while substrates with initial C:N ratio equal or below 55 do not need any composting.

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DEDICATION

This work is solely dedicated to Almighty God for His marvelous blessings upon me through the course work and the research work.

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GLOSSARY

*	Significantly Different
AOAC	Association of Official Analytical Chemists
APCAEM	Asian and Pacific Centre for Agricultural Engineering and Machinery
C:N	Carbon to Nitrogen Ratio
cm	Centimeters
CSIR	Centre for Scientific and Industrial Research
CT	Compost Time
DAC	Days after Cropping
DAS	Days after Spawning
FAO	Food and Agriculture Organization
GB	Gross Benefit
IMA	International Mycological Association
$Lsd_{(0.05)}$	Least Significant Difference at P = 0.05
NB	Net Benefit
NHB	National Horticulture Board
ns	Not Significantly Different
OECD	Organization for Economic Co-operation and Development
pH	Hydrogen ion Concentration
RCBD	Randomized Complete Block Design
RR	Rate of Return
T	Trials
S.e	Standard Error
SAMFA	South African Mushroom Farmers' Association
ST	Substrate Types
TNAU	Tamil Nadu Agricultural University
TVC	Total Variable Cost
UHDP	Upland Holistic Development Project
USCC	Unite State Composting Council
WPR	World Population Review

CHAPTER ONE

INTRODUCTION

1.1 Background

The growth of the population coupled with inadequate supply of food, diminishing quality of health, high rate of unemployment and increasing environmental degradation are some of the key underlying problems affecting the future well-being of humankind (Chang, 2007). The magnitude of these problems is said to increase as the world's population continues to grow (Chang, 2007). The population of Ghana is estimated to be 26,652,762 in 2014, an increase from 2013's estimate of 25,961,452 (WPR, 2014) on a land area of 230,020sq km. With a population growth rate of 2.2%, and a population density of about 78 – 114 persons per square kilometer (Ghana Embassy, 2012; World Bank, 2013), coupled with high unemployment rate of 25.6%, for the proportion of the population of the ages between 15 – 24, who are mainly youth (African Economic Outlook, 2012), a developing country like Ghana needs an intervention to help sustain the economy and to feed the many mouths expected to occupy her territory.

One way of doing this is to increase the work force by providing many opportunities for all and sundry especially in the area of small scale enterprise where every lay person can partake. One important aspect of production to help sustain the country and to create more employment is mushroom farming. Mushroom cultivation involves providing a medium and the right environment for the fungi (mushrooms) to expand their mycelia to the point that the mycelia mass will transform into fruiting bodies (the mushroom). Mushroom farming is a non-farm enterprise that can be integrated into small farms to increase incomes and enhance livelihoods.

Mushroom cultivation can help reduce vulnerability to poverty and strengthen livelihoods through the generation of a fast yielding and nutritious source of food and a reliable source of income (Anon Biotech, 2010). Since it does not require a large access to land area, mushroom cultivation is a viable and attractive activity for rural farmers and peri-urban dwellers as well as reducing the pressure on land as the population is growing.

Small-scale production of mushrooms does not require any huge capital investment: mushroom substrate can be prepared from any clean agricultural waste material, and mushrooms can be produced in temporary clean shelters. They can be cultivated on a part-time basis, and require little maintenance (Elaine and Nair, 2009). Mushroom production can be done anywhere at any time. It is an enterprise for both men and women and it is especially an excellent enterprise for women since it does not demand much labor and energy for production. Mushroom production indirectly provides materials that are used to improve the soil structure for production of other crops (FAO, 2009). The substrate used for the production of mushroom can be used as animal feed (Gianotti *et al.*, 2009). Through the provision of income and improved nutrition, successful cultivation and trade in mushrooms can strengthen livelihood assets, which can not only reduce vulnerability to shocks, but also enhance an individual's and a community's capacity to access other economic opportunities (Elaine and Nair, 2009). Mushroom production can contribute to the development of Ghana's economy in nutritional enhancement, thereby reducing some diseases such as Kwashiorkor, beriberi etc. as well as its use for medicinal purposes.

Mushrooms belong to the Kingdom Fungi, subdivision of Basidiomycotina, and the class Hymenomycetes (Orson, 1994). The word *mushroom* is derived from the Gallo-

Roman *mussiro* which evolved to *mussereroun* in Middle English (Filippone, 2006). A mushroom is the fleshy, spore-bearing fruiting body of the fungus, typically produced above ground on soil or on its food source. True mushrooms typically look like umbrellas. They consist of a stalk topped by a flat or cup-shaped cap (Fergus and Fergus, 2003). Mushrooms are sometimes referred to as 'toadstool', but this word usually refers to a poisonous mushroom (Lincoff, 2011). It was earlier thought that mushroom was used by the Egyptians over 4600 years ago and it was preserved as food for the royals and as such no commoner was supposed to eat mushroom (Florida Center for Instructional Technology, 2012).

Mushroom farming is the growing and caring for mushrooms. Mushroom cultivation can be traced back to around the 1600s in Europe, but it was not until the 18th century in France that modern mushroom cultivation techniques began (Fresh, 2000). Earlier, around the third century, the Japanese were known to have placed fresh logs next to mushroom growing logs. In one or two years' time the other log was also known to have produced Shiitake mushrooms (McCoy, 2014). Mushroom picking from the wild has been the custom of Ghanaians before its farming. Ghanaians took the advantage of picking mushrooms during the mushroom season from dead plants such as palm trees and other special trees. Places such as ant hills also provide a right source of picking of mushrooms when in season. Various species of mushrooms picked from the wild include *Volvariella volvacea* (growing mostly on palm trees) and *Termitomyces* species (growing mainly on termite hills). Other species include *Pholiota* species, *Lepiota* species and *Coprinu*s species (Dzomeku, 2009; Osemwegie *et al.*, 2014; Tiimub *et al.*, 2015).

Mushroom production in Ghana really started in the 1990s when a Ghanaian scientist, Leslie Sawyer, introduced some interested people to the cultivation of oyster mushroom, which he

learnt during his trips to Belgium and China (Galandzy, 2006). Currently there are about 350 mushroom cultivation centres in the country, all depending on the use of sawdust as substrates (Galandzy, 2006). The introduced mushroom was the lignocellulolytic type, *Pleurotus* species, which is more economical and easier to produce than the local ones. The qualities of *Pleurotus ostreatus* such as high yielding, ability to grow on a wide variety of substrates such as sawdust or other organic wastes make it a preferred choice for cultivation than the local ones. The nutritional content of *Pleurotus ostreatus* also makes it outstanding for its acceptability by consumers (Garcha *et al.*, 1993).

1.2 Problem Statement

Mushrooms in general lend themselves to many different growing systems from simple and inexpensive to highly sophisticated and expensive (Barney, 1997). Mushrooms and other fungi grow almost everywhere, on every natural material imaginable (Fogel and Rogers, 1997). Some fungi grow only in association with certain trees (Fogel and Rogers, 1997) while others grow on large logs. Mushrooms are also found in soil, on decomposing leaves, in dung, mulch and compost. For small scale farming there is the need for an effective substrate for the growth of mushroom, more especially *Pleurotus ostreatus* which is the most cultivated mushroom in Ghana. The main substrate used for the production of this mushroom is sawdust. Is sawdust the only efficient substrate for the production of the mushroom? How can areas with limited supply of sawdust also venture into such lucrative business with the available and easily accessible farm wastes? There are so many organic wastes that can be experimented upon for their efficient use for the production of this and other mushrooms in Ghana.

Coconut coir is a major waste in the coconut producing regions in the country such as the Western region. Its disposal is a big problem to the producers therefore to recycle the waste will be an innovative way of preventing environmental pollution. Meerow (1994) reported that growth index, top and root dry weights of both crops were significantly better in coir-based medium than sedge peat-based medium when he researched into the growth of two subtropical ornamentals using coir (coconut mesocarp pith) as a peat substitute study. Many researchers (Shashirekha and Rajarathnam, 2007; Thomas *et al.*, 1998; Giménez, and Pardo-González, 2008) have confirmed the use of coconut coir for the growth of mushroom, but failed to indicate the right composting time for the maximum production of mushroom.

In their research on suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya, Kimenju *et al.* (2009) observed that bean straw (*Phaseolus vulgaris*) produced the highest yield of oyster mushroom as compared to nine other substrates which included sawdust (*Eucalyptus* sp.), 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 fiber (*Musa* spp.). Musieba *et al.* (2012) also found that that bean straw is the best substrate for *Pleurotus citrinopileatus* production. These researchers also did not indicate the right composting period of the bean straw that would give a high yield. Bean (Cowpea) is one of the major crops produced in areas such as Northern, Brong Ahafo, Upper East, Upper West as well as parts of Ashanti regions. The straw can be an effective substrate for the production of mushroom if the right composting time is established or if it is used as a supplement to other substrates such as coconut coir or sawdust.

1.3 Objectives

General Objective: To compare the growth and yield response of *Pleurotus ostreatus* to coconut coir and bean straw as substrates for oyster mushroom cultivation in Ghana.

Specific Objectives

- To investigate the effect of C:N ratio, pH and moisture content of bean straw and coconut coir on mushroom growth
- To investigate the effect of composting period on mycelia growth and yield of the mushrooms
- To identify the substrate type that influences the fastest mycelia formation, flushes appearance and mushroom yield
- To determine the profit level of using the different types of substrates for mushroom production

1.4 Justification

Areas such as the Northern Regions of Ghana produce a lot of bean with the straw being a waste. This is not different when one visits the Western Regions of Ghana where coconut production is one of the main occupations of the natives. The farmers normally burn the wastes with the smoke being environmental nuisance. The biomass of these crops can be enhanced when used in mushroom production. Additionally, if the right composting time is established for the wastes of these crops, the efficiency of the materials in the production of mushroom can be promoted. This work is to study the effectiveness of these substrates for the promotion of the growth of *P. ostreatus* in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.1 What are Mushrooms?

Mushrooms are very special in the scientific classification. They are neither plants nor animals, but are still organisms because they perform all the life processes of other organisms. They belong to their special group called the fungi which are microscopic. Notwithstanding, there are other members in the group that are macroscopic and mushrooms can be described as one of the macroscopic groups. What then is a mushroom? The word 'mushroom' has many meanings in different parts of the world. Many researchers have tried to explain or more still define the word 'mushroom'. According to Ganopedia (2011), a mushroom is a fungus that has a stem, a cap and gills or pores on the underside of the cap. Cho and Kang (2004) defined mushroom as "a macrofungus with a distinctive fruiting body which can be either epigeous (growing on or close to the ground) or hypogeous (growing underground). The word mushroom refers only to the fruit and must be large enough to be seen with the naked eye and to be picked by hand (Cho and Kang, 2004). The word mushroom actually refers to the fungi that is seen with the naked eyes and that is picked by other organisms and sometimes used as food. In a broad sense "Mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Mills, 1992). Mushrooms are not only basidiomycetes, they can also be ascomycetes, grow underground, have a non-fleshy texture and could be inedible (Chang, 2007). All the poisonous and the non-poisonous fungi that can be seen with the naked eye and can be picked with the hand are described as mushrooms. The various types and shapes of mushrooms that can be picked from the wild include the most common type of umbrella shape with a pileus (cap) and a stipe (stem) i.e. *Lentinula edodes* (Chang, 2007).

There are other species that have different shapes such as volva (cup) in *Volvariella volvacea* or an annulus (ring) in *Agarius campestris* and some even, like the human ear such as *Plearotus ostreatus* (Chang, 2007). The life cycle of a mushroom may be traced from - a spore which under favorable conditions germinates to form a mass of branched hyphae of mycelia which colonize a substrate (Dike *et al.*, 2011). Mushrooms go through two stages, the vegetative stage and the reproductive phase. The vegetative stage ceases when the hyphae fully colonize its substrate. The reproductive phase starts when the hyphae develop primordia. The mushroom is a fruit that results from fully matured primordia of the fungi (Dike *et al.*, 2011).

2.2 Classification of Mushrooms

Classification is the arrangement of things or organisms into classes according to common features shared by the organism. Mushroom classification is therefore the arrangement of mushrooms based on their common characteristics. Mushrooms can be classified by their tropic pattern as saprophytes, parasites or mycorrhizae (Cho and Kang, 2004). The saprophytes are decomposers growing on organic matters like wood, leaves and straw in nature. They produce enzymes to digest the organic waste outside their body before they absorb them into their body (Austin, 2004). The parasites on the other hand grow, feed and are sheltered on or in a different organism while contributing nothing to the survival of their host, while mycorrhizas form a symbiotic association of their mycelia with the roots of certain plants (Cho and Kang, 2004).

There are three groups of mushrooms according to their economic importance; these are edible mushrooms, toxic mushrooms and medicinal mushrooms (Ganopedia, 2011). Edible mushrooms are mushrooms that have desirable taste and aroma without poisonous effect and

are used extensively in cooking; toxic mushrooms produce toxin, mind altering substances, antibiotics and antiviral substances, therefore, ingestion of toxic mushrooms may cause harmful effects that vary from mild symptoms such as gastric upset to severe life-threatening organ-failure which may result in death (Ganopedia, 2011). Medicinal mushrooms on the other hand have extracts that are possibly used for treatment of diseases. Oyster mushroom belongs to the edible type.

On the other hand, Chang (2007) had earlier reported that mushrooms can be grouped into four main categories, these include "(1) those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus*; (2) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (3) those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., *Amanita phalloides*; and (4) a miscellaneous category which includes a large number of mushrooms whose properties remain less well defined, which may tentatively be grouped together as 'other mushrooms'."

Mushrooms can also be classified according to the substrates they grow on (Dzomeku, 2009; Oei 1991). These include cellulolytic mushrooms, lignocellulolytic and termitomyces. The cellulolytic mushrooms grow mainly on cellulose such as straws; examples include *Vovariella volvacea*, *Agaricus bisporus* etc. The lignocellulolytics grow well on both straws and decaying wood such as sawdust; examples include *Pleurotus ostreatus*. The termitomyces grow mainly on anthills and their life cycles are completed by the help of ants or termites; examples include the Termitomyces family.

2.3 The Basidiomycota as Fungi

According to Ganopedia (2011), mushrooms, yeasts and molds belong to the kingdom fungi. Fungi are separated from plants as the fungal cells have cell walls that contain chitin instead of cellulose in the cell walls of plants. Fungi absorb their food after digesting it with secreted enzymes and this mode of nutrition combined with a filamentous growth form, nuclear mitosis, and other traits make them highly distinctive (Raven and Johnson, 1999). Christianson (2007) used phylogenic tree to illustrate that fungi are more closely related to animals than to land plants. From the phylogenic diagram, fungi and animals share closely related ancestors and therefore are grouped in the Unikonta subgroup and these two also share a common ancestry with the Archaeplastida subgroup which includes land plants and Red and Green Algae (Appendix 1). The fact that fungi are heterotrophic and store sugar as glycogen (like animals) and have cell walls made of chitin (unlike cellulose in land plants) further supports this discovery (Christianson, 2007). Most of the mushrooms belong to the ascomycetes and basidiomycota with most of them being in the *basidiomycota* group (Thakkar, 2010).

Mushrooms belong to Kingdom Fungi and Phylum Basidiomycota. The phyllum basidiomycota are distinguished from the other phyla within the same kingdom by the possession of sexual spores known as basidia which are produced outside by a club-shaped structure called a basidium (Christianson, 2007). Syngamy (the process by which two haploid cells fuse to form a diploid zygote) occurs within the basidium, giving rise to the zygote, the only diploid cell of the life cycle (Raven and Johnson, 1999). The basidiomycetes include mushrooms, toadstools, puffballs, jelly fungi and some plant pathogens such as rhizoctonia, rusts and smuts (Raven and Johnson, 1999). Kuo, (2011) illustrated that the subphyllum of

Basidiomycota; phylum Agaricomycotina has 3 classes (Class: Dacrymycetes, Class: Tremellomycetes, and Class: Agaricomycetes), 15 orders (with the order Agaricales possessing 34 families). The Basidiomycota phylum has about 16,000 named species (Raven and Johnson, 1999).

2.4 Description and Classification of Oyster Mushroom

Oyster mushroom belongs to the class Agaricomycetes, order Agaricales, family Pleurotaceae *or* Tricholomataceae, genus *pleurotus* and species *ostreatus*. Scientifically, oyster mushroom is known as *Pleurotus ostreatus* (Kuo, 2011). The Latin word *Pleurotus* means 'beside the ear' and *ostreatus* means 'oyster shaped' (Cohen *et al*., 2002). Oyster mushrooms include many species such as *P. flobellotus P. sojar - caju, P. eryngii, P. osfreafies, P. floride* and *P. sapidus* etc (Dike *et al*., 2011). There are over 70 species of *Pleurotus* for which new species still being discovered (Kong, 2004). All the varieties or species of oyster mushrooms are edible except *P. olearius* and *P. nidoformis* (Agridaksh, 2011).

Oyster mushroom is an edible, saprophytic and lignocellulolytic type of mushroom. The fruiting bodies of oyster mushroom are usually flat with the cap offset from the stalk, or the stalk hardly present at all (Woller, 2007). *Pleurotus ostreatus* (roughly translating to "beside-the-ear oyster-shaped") predominantly grows on hardwoods such as stumps, logs, and trunks of deciduous trees. It has a pale lilac-grey spore print and a soft fleshy fruiting body that ranges in color from white to grey, brown or even blackish (Woller, 2007). There is some variability among the species due to the wide distribution and reproductive isolation between continents. The caps of *Plearotus ostreatus* are shell shaped, semicircular to elongate. The margins are smooth and sometimes wavy and are whitish to grayish to tan; the texture is

velvety, the flesh is thick and white, gills are narrow, the stalk is short, thick and white and the base being hairy (Mdconline, 2013). The spores look narrowly elliptical, smooth and colorless when magnified. On average, the cap width ranges between 2 – 15 cm, stalk length is around 4 cm and stalk width is around 2 cm (Mdconline, 2013). *P. ostreatus* fruits year-round, especially after a good rain, if the weather is mild (Mdconline, 2013)

2.5 The Oyster Mushroom Life Cycle

The oyster mushrooms are basidiomycetes that bear their spores externally on basidia. The basidia grow to release the reproductive basidiospores. The oyster bears so many spores. The spores on landing in a favourable environment germinate into haploid mycelia. When the mycelia meet other haploid mycelia, they mate and then undergo plasmogamy. This results in the fusion of their cell membrane to create a dikaryotic cell, one with two genetically different haploid nuclei (Woller, 2007) (Appendix 2). The oyster mushroom has four mating types to ensure the chance of successful mating. The new dikaryotic cell multiplies and divides to live as a multi-cellular dikaryotic organism. This is the dominant stage for growing and gathering of nutrients. The dikaryotic mycelia then mature to a mushroom. The mushroom develops dikaryotic basidia within the gills (Woller, 2007). The nuclei in the basidia finally undergo karyogamy, fusion of the nuclei, and at last form a diploid nucleus that quickly undergoes meiosis. Each diploid nucleus yields four haploid nuclei of different mating types that develop into a basidiospore to repeat the cycle (Woller, 2007). The oyster really begins its life with a spore, from the spore the oyster grows in a thread-like, branching formation known as a hypha. The hyphae continue to elongate and branch repeatedly to form a network of vegetative hyphae which is known as mycelia. The spores and the initial hyphae are haploid (contains only one copy of each chromosome) (IMA-Fungus, 2002). A haploid mycelia meeting

another haploid mycelia of the same species join together and exchange nuclear materials. The process is called diploidization (IMA-Fungus, 2002). This is normally done before meiosis and spore production – Karyogamy. The two nuclei duplicate themselves, fuse, mix-and-match their genes, divide, and divide again to produce four new sexual nuclei which become the nuclei of the four spore produced on a "typical basidium" (IMA-Fungus, 2002).

2.5.1 Mode of Feeding of Oyster Mushroom

Oyster mushrooms are unable to manufacture their own food; they feed by excreting digestive enzymes through the tips of their hyphae (Woller, 2007; Volk, 2001). The hyphae branch to form thick mass mycelia to increase their surface area through which feeding can be maximized. The oyster mushrooms feed by secreting a range of enzymes such as peroxidases, laccases, cellulases, hemicellulases and xylanases (Cohen *et al.*, 2002). This makes the oyster mushroom well adapted on lignin and cellulose containing substrates such as sawdust, rice straw etc. These mushrooms, as a result of their saprophytic and the lignocellulotic ability, are able to grow on lignocellulosic substrates with little composting. This is because oyster prefers the lignin that makes up the secondary cell walls of hard woods from angiosperm trees (Woller, 2007). The oyster mushrooms take their protein by secreting a potent toxin to kill nematodes or roundworms that may be present in the rotten wood (Mdconline, 2013; Woller, 2007). The hypha of the fungi then secretes enzymes to digest these microorganisms, and then the mushroom absorbs the nutrients within them. That is how oyster mushrooms solve their nitrogen problem and defend themselves from a potential predator (Woller, 2007).

2.5.2 Nutritional Requirement of Oyster Mushroom

The ability of a fungus to synthesize enzymes to degrade the substrate is influenced by the strain, substrate composition and nitrogen concentration in the cultivation medium

(Elishashvili *et al*., 2008; Stajic *et al*., 2006).The substrate on which the mushroom is grown should supply specific nutrients required for oyster cultivation and the main nutritional sources for oyster mushrooms are cellulose, hemicelluloses and lignin (Kang, 2004). Cellulose and hemicelluloses which are the main sources of carbohydrates for oyster mushrooms are often incrusted within lignin, which forms a physical seal around cellulose and hemicelluloses and the proportion of these three structural components along with nitrogen content of residues affect mycelia growth, mushroom quality and crop yield (Philippoussis and Diamantopoulou, 2011). The strategy of the oyster mushroom is to decompose the lignin in wood so as to gain access to the cellulose and hemicelluloses embedded in the lignin matrix (Philippoussis and Diamantopoulou, 2011). Oyster mushrooms require more carbon and less nitrogen, however most of the substrates must be supplemented with nitrogen source to reach optimal Carbon: Nitrogen (C:N) ratio for the mushroom (Philippoussis and Diamantopoulou, 2011). Different researchers have suggested different C:N ratios for the growth of oyster mushroom. According to Srivastava and Bano (1970) C:N ratio between 30:1 and 117:1 was appropriate for *Plearotus fabellatus*. Wu *et al.,* (2004) recommended C:N ratio of 18:1 to 36:1 to be appropriate for *Pleurotus tuber-regium,*, while Rajarathnam and Bano (1989) suggested a C:N ratio around 85:1 as appropriate for *Pleurotus ostreatus* which falls within a C:N ratio ranging from 40:1 to 90:1 which was suggested by Quimio and Sardsud (1981). Shroomery (2011) on the other hand lowered the optimum range of C:N ratio for *P. ostreatus* to between 40:1 and 60:1.

Oyster mushrooms do not require more nitrogen for their growth. Excess nitrogen may cause stratum degradation when nitrogen is excessively added (Rajarathan and Bano, 1989). The activities of Laccase which is the main enzyme used by oyster mushroom to degrade the lignin

content of the substrate is reduced when excess nitrogen is added to the substrate, although excess nitrogen increases mycelia growth (D'Agostini *et al.,* 2011). When working on "Effect of Organic Nitrogen Supplementation in *Pleurotus* species", Upadhyay *et al.* (2002) concluded that substrates supplemented with 1% de-fatted soybean meal performed better than those supplemented with 2.5%, 5%, 7.5% and 10% of the same material or cotton seed cake and that substrates with higher supplementation gave lower yields of the mushroom.

The pH of the substrate is also a contributing factor to the growth of oyster mushrooms. Most edible mushrooms do well within a pH range of 3 to 7 at a temperature of 20 ^{o}C to 25 ^{o}C (childhopecambodia, 2010). Ibekwe et *al.* (2008) recorded optimum mycelia yield at a pH of 6.5. Nwokoye *et al.* (2010) reported that a pH range of 3 – 10 is required for mushroom growth in the tropics and thus the ability of the mycelia to tolerate the high temperature of 28^{o}C and the pH range of 3 – 10 enable them to flourish on agro wastes in the tropics.

2.6 Cultivation of Oyster Mushroom

2.6.1 World History

Mushrooms were used as food even before man understood the nature of other organisms (Quimio, 2004). Mushroom cultivation started in the ancient times for their nutritional value and flavor (Chakravarty, 2011). Oyster mushroom was collected as wild specimen from the forest of Florida and later actively cultivated in several countries around the world. Out of the over 200 species of fungi being reported as edible, 20 species are cultivated for edible purposes in different parts of the world (Ramachandraiah, 2000). Barney (1997) on the other hand reported that 300 mushroom species are edible but only 30 have been domesticated and 10 are grown commercially. Thus there is no exact information on the number of mushrooms that are edible as some are discovered daily. *Pleurotus ostreatus* is the second most cultivated

edible mushroom worldwide after *Agaricus bisporus* (Sanchez, 2010). Cultivation of mushrooms in Western cultures was first recorded in 1650 and *Agaricus bisporus* was the first species to be cultivated on compost. The production of the mushroom started in Germany on experimental bases by Flack in 1917 (NHB, 2005). The main materials for the production of the mushroom were tree stumps and wood logs by then. In the 1950s the innovation of the cultivation of mushroom after the successful cultivation of *Agaricus bisporus* paved the way to the cultivation of *Pleurotus ostreatus* on wood. By the late 1970s the cultivation of *Pleurotus ostreatus* became common in the various western cultures; hence, the marketing of the mushroom was promoted in the various supermarkets (NHB, 2005). Today the market for mushrooms continues to grow due to interest in their culinary, nutritional, and health benefits (Beetz and Kustudia, 2004).

2.6.2 Oyster Mushroom Cultivation Process

The process for the cultivation of oyster mushroom is simple because its cultivation does not lend itself to complex scientific procedures. Mediocre farmers can cultivate the fungi with less supervision. The cultivation of the fungi relies on the interaction of a particular set of physical, chemical and biological factors (Rangel *et al.*, 2006). The important areas to note in the cultivation of oyster mushroom in order to balance the three factors include substrate selection, composting, pasteurization, spawn running, fruiting and harvesting (Rangel *et al*., 2006).

2.6.3 Substrate Selection

A substrate is any material that serves as a medium of growth for a living thing in which enzymes can act upon and break it to release nutrients for the growing organism. Rouse (2010) defined a substrate as any solid substance or medium to which another substance is applied

and to which that second substance adheres. The use of a supportive substrate to enhance the growth and the development of sporophores is a fundamental requirement for the production process of mushrooms (Hayes, 1981). Mushroom is to a substrate as a plant is to a soil (Kwon and Kim 2004). A good substrate should be rich in nutrients, have good aeration and water holding capacity (Wightman, 1999). Diego *et al.* (2011) grouped mushroom substrates into three; thus bulky (e.g., sawdust, straw bagasse etc.), concentrates (e.g., meals, brans, urea, etc.) and conditioners (e.g., Gypsum and calcium carbonates). Oyster mushrooms have multilateral enzyme system that helps to grow on a wider variety of agricultural wastes (Fogel, and Rogers 1997). These agricultural wastes can be maize cob, rice straw, bean straw, coconut coir, sawdust etc., (Kwon and Kim, 2004). One significant point about the agricultural wastes is that these agrowastes are easy to come by and inexpensive to own. Over 500 billion kg agricultural wastes and 100 billion kg forestry wastes are generated yearly and 360 billion kg of fresh mushrooms can be produced on the total of 600 billion kg of dry waste producing an annual mushroom crop of 60kg per head per year, all containing 4% protein of fresh mushroom (Poppe, 2004).

The oyster mushroom, being lignocellulotic in nature, has enzymes that have the ability to break down the lignin and the cellulose in the agrowaste to release nutrients for the developing mycelia. Lignocellulose consists of lignin, hemicelluloses and cellulose and large amounts of lignocellulose wastes are often disposed of by burning, particularly in developing countries including Ghana (Howard *et al.*, 2003). A range of about 200 different wastes is available as oyster mushroom substrates (Poppe, 2004). Every grower producing oyster mushrooms can make their own best substrate choice from among all those wastes and this makes the cultivation of the fungi more satisfactory leading to a renewed appreciation for what is called a

waste (Poppe, 2004). Two of the most important mentioned substrates are the bean straw and the coconut coir or fiber.

Kimenju *et al.* (2009) tried several agricultural wastes as suitable substrate for oyster mushroom production and reported that all the substrates can produce mushroom but the straws which included bean straw, rice straw, finger millet straw and wheat produced the highest yield with bean straw topping the table. After trying seven substrates for the production of oyster mushroom which included bean (*Phaseolus vulgaris*) straw, sawdust of African mahogany (*Khaya anthotheca*), rice straw (*Oryza sativa*), maize cobs (*Zea mays*), wheat straw (*Triticum aestivum*), sugarcane bagasse (*Saccharum officinarum*) and banana leaves (*Musa* sp.), Musieba (2012) concluded that bean straw (*Phaseolus vulgaris*) gave the best performance for the production of oyster mushroom. Bean straw from all the genera can serve as the best substrate for the production of oyster mushroom (Poppe, 2004). In his research on cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization, Siqueira *et al.* (2012) observed that bean straw was a unique substrate suitable for the cultivation of oyster mushroom. They reported this after they had observed high productivity and biological efficiency with very little contamination after using the bean straw.

Thomas *et al.* (2012) reported that coir pith when treated well can be used as a soil conditioner.

The coir pith has many distinguishing characteristics that make it a better choice for use as rooting medium and substrate for mushroom production. The coir pith has high moisture retention capacity of 500 – 600%, high cation exchange capacity varying from 38.9 to 600m

eq/100g, which make it retain large amounts of nitrogen, and the absorption complex has high content of exchangeable K, Na, Ca and Mg (Thomas *et al.,* 2012). The coir pith is also valued for its high potassium content, low bulk density and particle density (Thomas *et al.,* 2012). The high C:N ratio of around 100:1, lignin content of around 37% and polyphenol content of around 100 mg per 100g makes the coir pith sometimes not a better choice (Thomas *et al.,* 2012). Notwithstanding, Rangel *et al.* (2006) used coconut coir which is a lignocellulotic agrowaste in place of black soil for the production of a mushroom and reported that the performance of the agro-waste with 15% of black soil resulted in a very high yield of the mushroom. Rangel *et al.* (2006) reported that porosity and texture (particle size distribution in the mixture), as well as the water holding capacity of the black soil, are markedly improved by addition of coconut coir, while pH, electrical conductivity, and the contents of soluble cations of the black soil remained statistically unaltered. These changes altogether provided an enhanced environment for the development and growth of the fruiting bodies. Hundred percent (100%) coconut coir however resulted in a low yield of the mushroom. This could be due to improper composting time of the coconut fiber which did not pave way for the release of the nutrients within the coir for the development of the mushroom (Rangel *et al.,* 2006). Coconut coir has one of the highest concentrations of lignin and therefore good resistance to microbial action (FAO, 2013). This perhaps contributed to less yield of the mushroom in the 100% coconut coir composition. Barkley (2002) performed an experiment on the evaluation of coconut coir as compared to sawdust for tomato crop production and concluded that coconut coir is a suitable growing medium for the production of tomatoes. Since coir has more lignin content (about 48% lignin) and amorphous powdery nature, its support to mushroom growth is very low (Shashirekha and Rajarathnam, 2007). Supplementing the coir with rice straw/horse gram plant residue increased the activities of cellulose, hemicelluloses and protease enzyme

from inoculation till the end of fructification whilst laccase activity decreased during frutification, in consonance with decreased lignin degradation during frutification (Shashirekha and Rajarathnam, 2007). Coconut leafstalk and bunch waste are superior to leaflets and coir pith in producing significantly more edible biomass of mushrooms (Thomas *et al.,* 1998). The yield of sporophores was positively related to cellulose content and the cellulose: lignin ratio of the substrates. Oyster mushrooms respond better to sawdust than to coconut coir (Vetayasuporn, 2007). Vetayasuporn (2007) observed that the percentage of mycelia colonization on a sawdust substrate reduced when percentage of coconut residue supplemented in sawdust substrate was increased.

All these researchers however failed to study the composting period of the coir that enhances enzymatic activity to release nutrients (Thomas *et al.*, 1998; Vetayasuporn, 2007; Shashirekha and Rajarathnam, 2007) in order to utilize its benefits in mushroom production

2.6.4 Composting

"Composting is the biological decomposition and stabilization of organic substrates under conditions which allow development of thermophilic temperatures as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land without adverse environmental effects" (Haug, 1980). Composting is the process by which various aerobic micro-organisms decompose raw organic materials to obtain energy and materials they need for growth and reproduction (Graves and Hattemer, 2000). Composting can also be explained as the controlled biological decomposition of organic material that has been sanitized through the generation of heat and stabilized to the point that it is beneficial to serve as a substrate for the growth of plants or mushrooms (USCC, 2008). Compost which is a

product that comes out from composting bears little characteristic with the raw materials used for its production and they have unique ability to improve the chemical, physical, and biological characteristics of the growing media (USCC, 2008). Composting is a natural process that employs the use of micro- and macro-organisms to break down the raw materials to release the nutrients for the growth of mushroom (Washington Organic Recycling Council, 2008). Good composting comes about through a process involving microorganisms, organic matter, air, moisture, and time (Coleman, 2013). In composting, micro and macro organisms such as bacteria, fungi, insects, worms, mites, protozoans, actinomycetes etc., in an aerobic reaction, convert the carbon from dead plants into energy for their own growth and in so doing release nutrients from the decaying plants into their body and later into the soil (Cogger and Sullivan, 2009).

The composting process starts with the activities of macro organisms such as mites, ants, earthworms, and beetles which break the bulk organic material into smaller particles. This increases the exposure and the surface area of the materials for microbial attack (Chen *et al.*, 2011). After the attack of macro-organisms, micro-organisms such as bacteria, fungi, actinomycets, and protozoa colonize the organic material and initiate the composting process (Chen *et al*., 2011). Since the oxygen (O_2) remaining in the pile is quickly consumed by the resident microorganisms, the compost pile must be regularly aerated by turning to prevent anaerobic conditioning. The composting process is done by several microorganisms of specific temperature requirements; mesophilic microbes which function best at 24^{o}C to 47^{o}C initiate the composting process. Their activities raise the temperatures which cause their inactivation and therefore paving the way for thermophilic microorganisms which love to work in the high temperature such as 47^{o}C. Decomposition at this phase is very active until most of the nutrients are used up by the thermophiles. Decomposition then slows down and

therefore decreasing the temperature. Mesophiles then take over again and complete the decomposition process (Chen *et al.,* 2011) (Appendix 3).

Factors that affect the composting include nutritious food for the microbes, suitable moisture, pH, oxygen and temperature (Chen *et al.,* 2011).

Micro-organisms need nutrients such as nitrogen, phosphorus, sulfur etc. to grow and to reproduce and these nutrients occur in the raw materials used in the compost mix and therefore additional fertilizer is often not needed (Cogger and Sullivan, 2009). Good compost must have a balance of carbon-rich (woody material) and nitrogen rich (green leafy matter or manure) material. Organisms that decompose organic matter use carbon as a source of energy and nitrogen for building cell structure. Too much carbon slows decomposition because nitrogen will be used up and some organisms may die, but other organisms may form new cell material using their stored nitrogen. Thus in the process more carbon is burned and therefore reducing the carbon content while nitrogen is recycled (Chisholm *et al.,* 2014). Generally a C: N ratio of 30:1 is ideal for microbial activity (Jenkins and Zwieten, 2003). Thus bean straw which has high content of nitrogen will serve as good compost. To ensure effective and active work of the microbes on the substrates so as to release the nutrients, Cogger and Sullivan (2009) suggested that the particle size of the materials can be reduced through grinding, chopping or cutting. Small particles have more surface area for microbial activity and are easier to break down for the release of the nutrients.

Moisture is one of the important factors for composting. Microbes need water for their activity and therefore must be supplied. All materials in the pile must be moist, but not soaking wet (Cogger and Sullivan, 2009). The mixed material should be moist but water should not be

squeezed out of it when handled with the hand. For aerobic composting, the maximum moisture content should be kept at a level that allows the whole composting process to be aerobic (Chen *et al*., 2011). The optimal water content of composting should be between 50 and 60% by weight (Jenkins and Zwieten, 2003). Too low water will slow down the activities of the microbes whilst too much water will lead to anaerobic conditions (Chen *et al.*, 2011).

Since microbes work within a specific pH medium, there is the need to monitor the pH of the compost to ensure proper microbial activity. The optimal pH for the activities of the microbes ranges from 6.0 to 7.5 for bacteria and 5.5 to 8.0 for fungi (Chen *et al*, 2011). This means fungi have a wide range of pH for operation of which oyster mushroom is not an exception.

Oxygen is one of the most important factors for effective composting. This is because the microorganisms need oxygen for their growth and multiplication. As microbial activity in the compost increases, more oxygen is consumed and must therefore be replaced by regular turning (Chen *et al.*, 2011). Without sufficient oxygen, anaerobic process can set in and will produce undesirable odor. The pile needs to be porous to pull air into it from outside (Cogger and Sullivan, 2009). To ensure air circulation within the pile, it is important to turn the pile regularly and it is also important to include a range of different sized and shaped materials (Jenkins and Zwieten, 2003). To turn the pile, the temperature of the pile should be checked first and a shovel is used to dig the middle of the pile to notice the appearance of a steam (Jenkins and Zwieten, 2003). The materials outside should then be turned in and the materials within should be turned outside.

Temperature as the amount of heat dissipated by the body is also important for composting. Temperature affects microbial growth, microbial activity and hence the rate at which the raw materials decompose (Chen *et al*, 2011). Higher temperatures favor faster breakdown of the

organic materials. However extreme temperature can inhibit the activities of the microbes. The optimal temperature range is between 130^{o}C to 150^{o}C. To ensure effective microbial activities temperature must be constantly monitored using thermometer (Chen *et al.*, 2011). The pile must be turned after the correct temperature has been established (Jenkins and Zwieten, 2003). One of the principal elements in planning a compost facility is site investigation, (Graves and Hattemer, 2000). The area for the composting must be flat and free from stones, tree stumps, drainage lines and weeds. The materials can also be covered if there is excessive rainfall (Jenkins and Zwieten 2003).

2.6.5 Fungi and Composting

A variety of microorganisms bring about composting. One of the most important microorganisms is fungi. Fungi have the ability to degrade decay-resistant materials such as waxes, proteins, hemicelluloses, lignin and pectin (Graves and Hattemer, 2000). This shows that fungi can degrade a variety of agro-wastes since these composed of complex compounds. Although fungi have the ability to degrade most of the resistant materials, fungi cannot survive in high temperatures (above 60^{o}C) as bacteria do and also have low tolerance for low oxygen environments (Graves and Hattemer, 2000). This is clear as fungi give way to only bacteria at the peak of the composting where only thermophiles can survive. The fungal activity is inhibited as a result of the intense heat during the thermophilic stage of composting (Thambirajah *et al.*, 1995). This observation was supported by Graves and Hattemer (2000) when they reported that fungi tend to be present in the later stages of composting because of the nature of the material they decompose. During the maturation stage of the composting i.e. during the later stage of the composting, bacteria activity paved way for the activities of fungi (Fuchs, 2010). Thus the bacteria first of all degrade the substrates and then produce

metabolites thereby creating a new physio-chemical environment for the fungi to continue the work (Ryckeboer *et al.*, 2003; Fuchs, 2010). Fungi spread and grow vigorously by producing many cells and filaments and in so doing, attack organic residues that are too dry, acidic, or low in nitrogen and then give chance to bacterial decomposition when the cellulose in the substrate is consumed (Trautmann and Olynciw, 1996). The activity of the fungi is impeded after the maturation stage when all the cellulose in the substrate is used up by the microbial culture. Since the fungi have a right time to effect composting, monitoring the fungal population in compost is important to determine their quality and field of application (Anastasi *et al.*, 2005; Peters *et al.*, 2000). Fungi mostly take over after bacteria have started the composting process and therefore degrade the cellulose and the lignin in the compost (University of Illinos, 2014). In mushroom production the composting time is important to ensure the effectiveness of the mushroom which is a macro-fungus. There is therefore the need to study the composting time before inoculation to enhance easy colonization of the mycelia for fruiting.

2.6.6 Effect of Time on Composting

Substrate production is one of the most critical stages of cultivation of mushrooms because it has a dramatic consequence on the yield and quality of the crop and consequently the economic viability of the crop (Diego *et al.*, 2011; Cormican and Stauton, 1991; Dhar, 1994). The main purpose of composting, to a mushroom grower, is to prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of other microorganisms (Obodai *et al.*, 2010) and also to ensure the timely release of the nutrients to the crop. In nature, the fleshy fungi which are cultivated for their mushrooms usually exist in organic debris which has been worked over by worms, insects and other fungi (Oei, 2003). Thus the fungi do not

grow on fresh substrates, hence, for species such as *Agaricus*, and sometimes *Volvariella*, *Shiitake* and *Pleurotus*, the substrate must be fermented in order to become suitable. A good substrate must be suitable chemically, physically and must have suitable conditions for microbial activities. A good chemical condition is required for the release of some nutrients from the substrate during the mushroom cultivation process (Afriza, 2009).

Required time needed for composting depends on factors such as C: N ratio, moisture content, weather, type of operation, management and the types of waste being composted (Saskatchewan Agriculture, 2008). Low C: N ratio, optimum moisture content, and regular turning of substrate increase microbial activity (Nutongkaew *et al.,* 2014). If the materials in the pile are turned regularly, e.g. at two days interval, it takes two weeks or a little longer to compost (FAO, 2007). The longer the interval between turnings, the longer it takes for the compost to be ready.

There are three methods of composting with respect to time and these include; standard, slow and fast methods (Home Composting, 1999). The standard method is required when there are varieties of organic waste materials and this method produces compost in six to eight weeks; the slow method is used when a steady supply of organic materials are not available and it takes barely six months to two years (Home Composting, 1999). In the slow method, time is not spent checking for the proper mix or moisture and since composting is a natural process, it occurs with little or no attention. The fast method requires much more time and energy to chop all the organic materials into pieces and watch the composting process closely to guarantee ideal conditions at all times (Home Composting, 1999). The compost pile must also be turned every three days interval to regulate aeration and moisture content.

There is no fixed time to produce finished compost. Duration depends on feedstock, composting method used and management (Cooperhand, 2002). Compost maturity is usually defined as the degree of humification (conversion of organic compounds to humic substances, which are most resistant to microbial breakdown) (Cooperhand, 2002). This gives an indication that the oyster mushroom which is also a microorganism that takes part in the composting process has a working limit. Furthermore, oyster mushroom has two phases of growth; the vegetative phase and the reproductive phase and the change from vegetative phase to reproductive phase is influenced by some environmental factors such as nutrient availability, temperature, light and changes in concentration of atmospheric gases (Afriza, 2009). It is therefore imperative that the composting time should be managed to know exactly when to inoculate the spawns to ensure proper mycelia formation and fruiting. The fungi are not good starters of the composting process but good finishers (Graves and Hattemer, 2000; Fuchs, 2010; Ryckeboer *et al.*, 2003). When the material is fully composted by the fungi and the nutrients are fully used, they cannot continue to degrade the material to release nutrients for their growth (Cooperhand, 2002). By this way they produce new spores packed in their fruits in order to escape the nutrient deficiency and the unfavourable environment. The fruits then mature to release the spores which will escape the unfavourable environment to colonize new area and start another life cycle (Woller, 2007). With this idea it is good that the substrate for the mushroom production be composted partially to serve as a starter for the mycelia formation of the mushroom and later fruiting when the substrate is fully composted and nutrients are fully exhausted. Formation of the humus will restrict the continuous decomposition of the fungi (oyster mushroom) and this will trigger fruiting of the fungi with the idea of producing spores to escape or to disperse to a new environment for survival. Oei (2003) reported that when the spawns are placed in fresh substrate, the heat that will be

produced from the decomposition of the substrate can kill the mycelia of the fungi; it is therefore important to compost the substrate to make it more selective and suitable for the growth of the fungi of interest

Different substrates have different composting periods for oyster mushroom production. When working on assessment of growth support potential of different substrates for the cultivation of *Volvoriella vovalceae*, Markson *et al*. (2012) composted sawdust for thirty (30) days and was turned regularly at 7 days interval, however, the rest of the substrates including coconut coir, banana leaves and dead palm trunk were just soaked in water for 12 hrs. After the experiment Markson *et al.* (2012) reported that palm trunk fibre and coconut coir did not support the growth of the test mushroom and observed that the reason was due to the acidity level of those substrates. Obodai *et al*. (2010) composted sawdust from *Triplochiton scleroxylon* K. for 28 days and a turning of 4 days interval to cultivate *Pleurotus sajor – caju* (Fr.).

Composting coconut coir pith reduces its bulkiness and converts plant nutrients to the available form (TNAU, 2008). Coconut coir is also composted to reduce its wider C: N ratio from 112:1 to 24:1, thus reducing the carbon content and increasing the nitrogen content. The coir must be composted for 60 days with 10 days interval for turning (TNAU, 2008). The chemical observations of composted coconut coir include narrower C:N ratio of about 20:1, less oxygen uptake, less number of microorganisms, more amount of available nutrient and high cation exchange capacity; some physical observations including volume reduction of the heap and waste particle size reduction (TNAU, 2008). Thomas *et al.* (2012) composted coir pith with poultry manure to achieve compost maturity at 45 days and then concluded that the

composting process facilitated by poultry manure amendment brought bioconversion of coir pith to a final product which possessed physico-chemical characteristics required for quality organic manure. Vetayasuporn (2007) tested the feasibility of using coconut residue as a substrate for oyster mushroom cultivation and he composted the residue for seven days. The coconut residue alone did not ensure complete mycelia colonization within the time used, but the mass of whitish mycelia was thick, dense and comparatively compact when compared with sawdust. Combination of coir residue and sawdust accelerated mushroom growing process and gave a high mushroom yield and percentage biological efficiency (Vetayasuporn, 2007).

A lignocellulytic mushroom such as *Pleurotus spp* can be grown on raw lignocellulosic material without composting but other mushrooms such as *Agaricus* and *Lentinula* species need composting for their production (Diego *et al.*, 2011). To use straw as a substrate, the straw must first be chopped into reasonable sizes and then soaked in water for about 1-2 hours, and then it is rinsed 2 to 3 times in clean water and left for 3 to 4 hours before spawning can be done (Kwon and Kim, 2004). When evaluating lignocellulosic biomass from coconut palm as substrates for cultivation of *Pleurotus sajor-caju* (Fr.) Singer, Thomas *et al.* (1998) reported that the coir pith was soaked in water for 16 hours after sun drying. Bagging and pasteurization then followed and then later the substrates were inoculated without composting. The results showed that all the different components of the coconut used gave high yield of the mushroom, but the yield of the coconut coir was very low as compared to the other substrates.

De Siqueira *et al*. (2012) composted bean straw for 7 days with a turning interval of 2 days when trying two methods of pasteurizing substrates (axenic verses composting/steam pasteurization) for the growth of three species of Pleurotus. He observed that *P ostreatus* and

P.pulmonarius had excellent results in the two pasteurization methods as against *P. eryngii*. De Siqueira *et al*. (2012) concluded that the results obtained in the study indicate the suitability of bean straw as a unique substrate for *P. ostreatus* and *P. pulmonarius* cultivation, using the composting process for substrate preparation for mushroom cultivation. In Vietnam straws are fermented for 7 - 10 days with turning intervals of three days before they are used as substrate for mushroom production (Kwon and Kim, 2004).

2.6.7 Pasteurization

Pasteurization is a partial sterilization of substrates at a temperature that destroys harmful microorganisms without major changes in the chemistry of the substrate. It is a term used to apply to the process of heating mushroom substrates, in order to reduce weeds, diseases and pests (Kurtzman, 2010). Pasteurization is used to reduce competitors in a substrate, thereby giving the mycelia an advantage over harmful organisms, allowing it to take over the substrates and eventually producing mushrooms (Mushroom Appreciation, 2008). Pasteurization removes competitive fungi thereby permitting faster and better uniform spawning as well as ensuring resistance to future infections (Ficior *et al*., 2006)

Pasteurization occurs between 71^{o}C and 82^{o}C and anything more than that risks the proliferation of healthful microorganisms (Mushroom Appreciation, 2008). The compost must be pasteurized for at least 6 hours at 60^{o}C to kill possible pathogenic fungi and harmful bacteria (Kang *et al.*, 2004). As it is imperative that a soil must be prepared for the growth of plants, so it is for the growth of mushroom in order to destroy harmful microorganisms that might compete with the healthful microorganisms during the mushroom growth (Kurtzman, 2010).

The methods available for mushroom substrate pasteurization include; hot water treatment with boiling water for 30 minutes; chemical sterilization with formalin and pasteurization through steam by using steam drum where the plastic bags are kept in the steam drum filled with 4-5 inches bottom layer of water and heated at 80^{o}C for one hour (Ali *et al*., 2004; Dias, 2012). Steam pasteurization was found to be the best method for substrate pasteurization since substrates from steam pasteurization produced the fastest mycelia formation among the three methods (Ali *et al*., 2004). Materials that are not pasteurized normally produce low yields of mushroom (Ficior *et al*., 2006; Ali *et al.*, 2007).

2.6.8 Spawn and Spawn Running

Mushrooms are to spawns as plants are to seeds; however, spawns are only pure mushroom mycelia (the vegetative part of the fungus) which are growing on a sterilized grain medium (Maheshwari, 2013). By nature fungi such as mushrooms produce spores as a means of reproduction, but these spores are too tiny to hold and to work with, therefore the technology of spawn (the vegetative method of mushroom propagation) production makes cultivation of mushrooms easier (Mushroominfo, 2010). Additionally, the spores are likely to yield a new strain and performance would be unpredictable (Thakkar, 2010). Mushroom spawns serve as growing material for mushroom production and they are prepared in a sterilized medium with a substrate for mushrooms to grow around. Mostly grains are used to produce spawns due to their faster ramification of the substrate and their ease of planting (Stanley and Awi-Waadu, 2010). The first stage in the production of the spawn is the culturing of the mushroom mycelia on nitrified agar media before the starter culture is used to make the grain spawn. The grain spawn is then used to make the final fruiting substrate (Ogden and Prowse, 2004), (Appendix 5). After the mycelia is added to the grain, the grain and the mycelia are shaken 3 times at 4

days intervals over a 14 day period for active mycelia growth (Mushroominfo, 2010). Stanley and Awi-Waadu (2010) performed experiments on using different grains to produce mushroom spawns and then concluded that the best grain to use for mushroom spawn was white maize grain. The maize grain ensured faster mycelia growth than the red sorghum that was normally used. The factors that affect spawn preparation include CO_2, oxygen, light, pH, temperature and humidity (Nwanze *et al*., 2005).

After the spawns are made or are bought, they are then mixed with the pasteurized compost for the mycelia to colonize the compost. The time needed for the mycelia to colonize the compost depends on the rate and distribution of the spawns, the moisture content, quality and nature of the compost and temperature; as such, a completed spawn run usually requires 14 to 21 days for the mostly used substrates (Mushroominfo, 2010).

Spawn run is a term used to describe the situation whereby the mycelia colonize the substrate and use the available nutrients (Thakkar, 2010). The mycelia take about 21 – 22 days to fully colonize sawdust substrate (Randive, 2012). During spawn running, the required temperature is 20 - 25^{o}C with a humidity of 65 -70% and water content of substrate of 65% (Thakkar, 2010). Many factors such as mushroom cultivar used, compost factors, sanitation etc. can determine the proper spawn growth (Royse, 2014). Compost factors such as ammonia content, nitrogen content, lipid content, moisture, temperature, salt concentration and humidity affect the spawn running period and quality (Wuest and Bengston, 1982; Royse, 2014). The inoculated bags must be kept in the dark until the mycelia fully invades the substrate (Horn, 2004). The reproductive phase is reached when the mycelia fully colonize the substrate and perhaps when some nutrients run out. It is only the reproductive structure that comes out of the substrate and forms the fruiting body (Thakkar, 2010).

2.6.9 Cropping

Cropping is the developing of the mycelia into pinheads and then the fruit. Although spawn run needs dark room (Horn, 2004), high CO_2 and even a moderate humidity of about 65 – 70% (Thakkar, 2010), formation of pinhead and fruiting need less CO_2 of below 2% and high humidity of about 80 – 90% and low temperature of about 16 – 18^{o}C (SAMFA, 2011). In order to keep these conditions constant, there is the need of constant supply of ventilation to reduce the CO_2 content in the cropping room (SAMFA, 2011). The pinheads are too delicate that heavy watering should be avoided during pinning (Kang, 2004). Watering should be done for at least twice or thrice a day (Hasan *et al.*, 2010) in order to maintain a high humidity.

2.7 Why Oyster Mushroom Cultivation?

The widespread malnutrition coupled with increasing protein requirement in the country has necessitated the search for new and alternative means to meet the protein requirement of the country and one of the alternative ways is the production of mushroom which is noted for its high protein content (Hasan *et al.*, 2010). Shah *et al*. (2004) and Tewari (1986) reported that mushrooms contain about 85 – 95% water, 3% protein, 4% carbohydrate, 0.1% fats, 1% minerals and vitamins. Mushrooms also have very strong medicinal properties (Shah et al., 2004). On dry weight basis, mushrooms contain 20 - 40% protein which consists of all the essential amino acids required in human diet (Horn, 2004).

Since mushrooms can grow on a wide variety of agro-waste materials because of their ability to convert these less useful products into a high quality protein, it naturally opens new job opportunities especially in rural areas where the standard of living is very low (Amuneke *et al*., 2011). Low income earners can start this job with very little capital since all these agro wastes can be taken free of charge from the farms.

Shen (2002) reported that the commercial production of oyster mushroom has increased 25-fold worldwide since 1981 and is responsible for the peak production. Constant demand of the mushroom has led to increase in the price (Roach, 2006). This indicates how the usage of this mushroom increases day by day for its significant role in human health, nutrition and disease; making it the second most cultivated mushroom worldwide (Uddin *et al.*, 2011). Increase in consumer demand for oyster mushroom has continued its rapid space over the last six years and the world demand of the mushroom has remained steady, with about 900,000t annually (Royse *et al.*, 2004). Forty-five percent of the world's supply is consumed un-processed and the rest dried (Roach, 2006). Europe remains the most major consumer of mushrooms (Roach, 2006).

The whole production process of oyster mushroom takes about 42 days and this is less than the life cycle of most arable crops (Horn, 2004). It is therefore an early income generating business that can give rapid income to the family.

The mycelia of oyster mushroom has the unique ability to split the chemical structure of highly toxic PCB's (Polychlorinated biphenyls) into non-toxic substances without enclosing toxics in their fruits (Dietsler, 1997). With this ability, oyster mushroom can be used for bioremediation when cultivated on industrial wastes.

The list can continue exponentially when the importance of the cultivation of the oyster mushroom worldwide is considered. In a developing country like Ghana where the unemployment rate is very high, mushroom cultivation can be recommended to the youth in order to make several unemployed Ghanaians earn a living.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site for the Research

The research was conducted at Department of Biochemistry and Biotechnology Annex of the Kwame Nkrumah University of Science and Technology, Kumasi. The site is located under the tropical zone of the forest belt of Ghana. The area enjoys a bimodal rainfall pattern with minor and major seasons respectively. The minor season ranges between October and March while the major season ranges between April and September (Appendix 14). The average monthly rainfall ranges between 165 mm and 215 mm. Average monthly temperature of the area is between 21. 5 ^{o}C and 30.70 ^{o}C with humidity ranging from 60 % to 85% within the year (World Weather and Climate, 2014; AccuWeather, 2014; Open Weather, 2014; Jensen and Eriksen, 2014)

3.2 Materials

The materials and tools for the research work included mushroom spawns, coconut coir, bean straws, metal barrel for sterilization, fire wood, 1kg capacity poly-propylene bags, black poly ethylene sheets, rubber bands, 2 cm thick polyvinyl chloride (PVC) pipes as bottle necks, paper sheets, cropping house and weighing balance.

The coconut coir was collected from the coconut farms at Half Assin in the Western Region. The coconut coir was sent to the yard of Bas Van Buureu Limeted at Takoradi, a company that manufactures and processes coconut coir into fibre. The coir was sun dried for one month and then milled into particle sizes of about 0.5 mm thick and 1 – 2 cm long. The fibers of the coir were transported to the research site in jute bags. In all, half ton of the coconut coir was

gathered for the research. The bean straws were gathered from the bean farms around Ejura in the Ashanti region. In total, half ton of bean straw was used for the research work.

Mushroom spawns and 1kg capacity poly-propylene bags were bought from Robart Enterprise; a commercial producer of oyster mushrooms at Kenyasi, Kumasi in the Ashanti Region. The other tools and materials were bought from the central market of Kumasi.

3.3 Methodology

3.3.1 Composting of Substrates

The coconut coir and the bean straws were cleaned and air dried. The bean straws were beaten and chopped into pieces of about 1 – 1.5 cm by width and 2 – 5 cm by length according to the suggestions made by Kimenju *et al.* (2009). The coconut coir was composted in three groups with composting time of 1 day, 14 days, and 21 days. The bean straw was also treated the same with composting time of 1 day, 7 days and 14 days according to literature (Upland Holistic Development Project (UHDP), 2012; Siqueira *et al.,* 2012; Musieba *et al.,* 2012). The combination of the bean straw and the coconut coir with a ratio of 20 kg of bean straw: 30 kg of coconut coir were also composted for 1 day, 7 days and 14 days. The table below indicates the various composting times.

Table 3.1 Composting Periods for the Substrates

Treatments	Composting Period		
Bean straw	1 day	7 days	14 days
Coconut coir + Bean Straw (Mixed Substrates)	1 day	7 days	14 days
Coconut coir	1 day	14 days	21 days

The skipping of the 7 days composting time of the coconut coir substrate was due to the high C:N ratio of the coconut coir and therefore would need more composting time than the beans straw for an appreciable amount of substrate fermentation and lignin degradation.

Fifty Kilogram (50 kg) of each substrate was weighed and mixed thoroughly. The amount of water in the compost was checked with the squeezing test in the process of mixing the substrates with water. Water was added gradually to the pile and a handful of the pile was squeezed to check if water would come out of the pile. The recommended water content was that water would not ooze out when the pile was squeezed between the fingers. This was done till all the pile was mixed with water. The substrates were then heaped to about a height of 50 cm and covered with poly-ethylene sheets (Plate 3.1 A). The heaped substrates were turned at three days interval (CSIR, 2003) within the compost duration for aeration.

3.3.2 Bagging the Substrates

The partially composted substrates were packed into 1kg capacity polypropylene bags (about 33 cm by 17.8 cm and 0.08 – 0.10 mm thick). The bags were compressed and pieces of polyvinyl chloride (PVC) pipes of 3 cm long and 2 mm thick were used as bottle necks; the left over poly-propylene bags were pulled through the PVC pipes and were held in place with rubber bands (Plate 3.1B). Cotton wool and sheets of paper were used to cover the opening of the bags.

3.3.3 Bags Sterilization

The steam pasteurization method was used and the substrates were pasteurized for 6 hours (Plate 3.1 C). Three cement blocks were placed at the bottom of a metal barrel. Wooden racks, about 45 cm by 10 cm in sizes were placed on top of the cement blocks at the bottom of the metal barrel. The metal barrel was filled with water to the 25 cm level. The bags were placed onto the wooden racks and then on top of one another until the barrel was full. The top layer bags were turned up-side-down for effective sterilization. A hole of about 12 cm^2 was created in the lid of the barrel to monitor formation of steam. Substrates were heated until steam jets

out of the hole in the lid and continued to be sterilized for six hours. The bags were then cooled for four hours and then were transferred to the inoculation room.

3.3.4 Inoculation/Spawning

A disinfectant (Methylated spirit) was used to clean the hands, spawn bottle as well as the neck of the bags. Two candles were lit and the bags were placed in-between the candles to prevent pathogenic infection when the bags were opened for spawning (Plate 3.1D). The spawns (about 10 grains of the sorghum) were introduced into each bag and cotton wool was placed quickly at the open end of the bags. The bags were shaken gently for proper distribution of the spawns.

3.3.5 Spawn Running

The bags were arranged on the wooden shelves in the incubation room according to the experimental set up of Randomized Complete Block Design (RCBD). Spaces of about 10 cm were left between treatments for aeration. Formation of mycelia was monitored regularly by observing the development of white threads through the substrates (Plate 3.1E). The rate of mycelia growth was measured at five days intervals using ruler until total colonization was achieved.

3.3.6 Cropping

The bags were then transferred to the cropping house after full mycelia formation. The bags were also arranged according to the experimental design of RCDB on the shelves in the cropping room and were then slit open at the bottom of the neck to enhance flushing (Plate 3.1). The cropping room was watered regularly to provide proper humidity for the growth of the mushroom. The cropping house was covered with palm fronds to provide 50% shade for flushing.

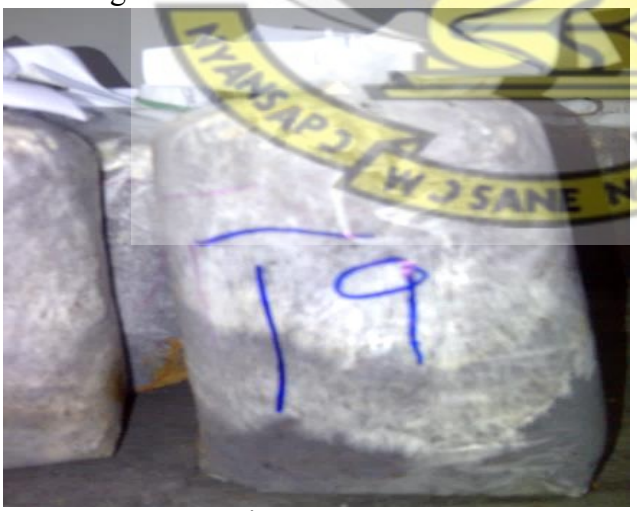
Production Process

A. Substrates Composting

B. Substrates Bagging

C. Bags Sterilization

D. Inoculation and Spawning



E. Spawn Running

F. Cropping and Mushroom Growth

Plate 3.1 Production Process of Oyster Mushroom

3.3.7 Experimental Design

The experiment was laid out in a 3 x 3 factorial randomized complete block design (RCBD) with three replications. The factors and their levels studied were

A) Types of substrates [(i) Sole Bean Straw, (ii) Coconut Coir plus Bean Straw (iii) Sole Coconut Coir]
B) Composting time [(i) 1 day (ii) 7 days and (iii)14 days for beans straw and the mixed substrates; (i) 1 day, (ii) 14 days and (iii) 21 days for coconut coir substrate]

Each treatment had ten (10) polypropylene bags with three replications each. A total of 30 bags were used for each treatment making up 270 bags in total.

The experiment was conducted in two trials. Trial one (T1) took place between February and April while trial two (T2) took place between April and June. Analysis of variance (ANOVA) was used for the experimental design. GenStat software was used for the ANOVA. The means were separated using least significant differences at $p \leq 0.05$

3.4 Data Collection

3.4.1 Chemical Analysis of the Substrates (% Moisture, C, N and pH)

Fifty gram (50g) samples of each of the substrates were taken during the day of bagging. The percentage moisture, C: N ratios as well as the pH of the various samples were analyzed according to the procedure below;

3.4.1.1 Moisture Content of Substrates

The moisture contents of the substrates used were determined according to AOAC (2000) method. The empty weight of Petri-dish with their covers was weighed using the electronic balance and the data recorded. Five grams each of the samples were weighed using the

electronic balance and were transferred into the Petri dish. The total weight of the Petri-dish and the samples were also taken. The Petri dish with the samples was then transferred into the oven and the temperature set at 105^{o}C for 24 hours. The samples were then transferred into desiccators for 30 minutes. The weight of the samples and the Petri-dish was taken again. Percentage moisture was then calculated according to the formula below;

$$\text{Percentage moisture} = \frac{(Weight\ of\ dry\ sample + Petri\ dish) - (Weight\ of\ empty\ Petri\ dish)}{(Weight\ of\ fresh\ sample + Petri\ dish) - (Weight\ of\ empty\ Petri\ dish)}\ x\ 100\%$$

3.4.1.2 Percentage Carbon

The carbon content was determined according to literature reports (Nelson and Sommers, 1982; Heanes, 1984). The substrates were sun dried and grounded using mortar and pestle into very minute particles in order to pass through a 0.5 mm sieve. The samples were thoroughly mixed. Two grams (2 g) of the substrates were weighed into a 500 ml Erlenmeyer flask. From a burette, exactly 10 ml of 1.0 N $K_2Cr_2O_7$ solution was added followed by 20 ml of conc. H_2SO_4. The mixture was swirled ensuring that the solution was in contact with all the particles of the substrates. The flask and the content were allowed to cool on an asbestos sheet for 30 minutes. Two hundred millilitres (200 ml) of distilled water was added followed by 10 ml of orthorphosphoric acid. Two millilitres (2.0 ml) of diphenylamine indicator was then added. The mixture was titrated with 0.5 N ferrous sulphate solution until the colour changed to dark blue and then to a green end-point. The titre value was recorded and was corrected for blank solution ($\geq$ 10.5)

The percentage organic carbon content was then calculated according to the formula below;

$$\%C = \frac{M \times \langle V_{bl} - V_s \rangle \times 0 \cdot 003 \times 1 \cdot 33 \times 100}{g}$$

where

M =Molarity of $FeSO_4$

V_{bl} = ml $FeSO_4$ of blank titration

V_s = ml $FeSO_4$ of substrates titration

g= mass of substrates taken in gram

0.003= milli-equivalent weight of C in grams (12/4000)

1.33 = correction factor used to convert the Wet combustion C value to the true C value since the Wet combustion method is about 75 % efficient in estimating C value , (i.e. 100/75 = 1.33)

3.4.1.3 Nitrogen

The nitrogen content of the samples was determined according the procedure described by Bremner and Mulvaney (1982). The samples were sun dried for 30 minutes and then uniformly mixed. Ten grams (10g) of air dried samples were weighed into a 500 ml long – necked Kjeldahl flask. Ten milliliters (10 ml) of distilled water was added to the sample and allowed to stand for 10 minutes to moisten. One spatula full of Kjeldahl catalyst [mixture of l part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4] was added to the sample. This was followed by the addition of 30 ml conc. H_2SO_4. The samples were then digested until clear and colorless or light greenish (1-11/2 hrs). The digests were then decanted into a 100 ml volumetric flask and made up to the mark with distilled water with rinsing from the digestion

flask. Ten millilitres (10 ml) aliquot of the digest was transferred by means of pipette into the Kjeldahl distillation apparatus. Twenty millilitres (20 ml) of 40% NaOH were added. . Two hundred millilitres (200 ml) of distillate over 10 ml of 4% Boric acid and three (3) drops of mixed indicator in a 500 ml conical flask for 5 minutes was collected. Light blue coloration was observed. The distillate was then titrated with 0.l N HCl till blue color changed to grey and then suddenly flashed to pink. A blank determination was also carried out without the substrate samples.

The nitrogen content of the samples was then calculated according to the formula below;

14 g of N contained in one equivalent weight of NH_3

$$\text{Weight of N in the substrates} = \frac{14 \times (A - B) \times N}{1000}$$

Where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

Weight of substrate sample used, considering the dilution and the aliquot taken for distillation

$$= \frac{10 \text{ g} \times 10 \text{ ml}}{} = 1.0 \text{ g or } 100 \text{ ml}$$

Thus, the percentage of Nitrogen in the substrate sample was,

$$\% \text{ N} = \frac{14 \times (A - B) \times N \times 100}{1000 \times 1}$$

When N = 0. 1 and B = 0

% Nitrogen = A x 0.14

3.4.1.4 pH

The pH of the samples was determined using the Electrometric method with 1: 2.5 sample solutions (Page *et al.,* 1982). Five grams (5g) of the air dried substrate was weighed into a 50 ml beaker. Distilled water (12.5ml) was added. The suspension was stirred vigorously for 20 minutes. The suspension was allowed to stand for 30 minutes by which time most of the suspended particles had settled out of the suspension. The pH meter was then calibrated with blank pH of 7. The pH meter electrodes were then inserted into the partly settled suspension. The pH values were read from the pH meter and the results recorded.

3.4.2 Rate of Mycelia Growth

After spawning, a line was drawn across the bags using a permanent marker at where the spawns had settled to serve as a reference point for the measurement of the rate of mycelia formation. A measuring rule was used to measure the distance travelled by the mycelia in the transparent bags at 5-day intervals. The rate of mycelia formation was then calculated by subtracting the new measurements from the previous measurements at each 5-day intervals.

3.4.3 Time for Total Mycelia Formation

The colonization of the substrate by the mycelia within the bags was monitored by measurement at five days intervals. The number of days the mycelia fully colonized the substrate after the day of spawning was then recorded. The colonization was seen by the formation of white mycelia throughout the substrates within the bags.

3.4.4 Time for Primordia Formation

After the bags were slit open, the formation of primordia was observed every two days intervals and the number of days it took for first primordia formation was observed and recorded.

3.4.5 Weight of Harvested Mushroom

The total weight of mushrooms harvested from the various treatments was measured using the electronic balance. The weight of the harvested mushrooms at two days intervals were weighed and recorded. The total weight of the harvested mushrooms 30 days after cropping was then calculated by simple addition.

3.4.6 Length of Stalk

The length of the stalk was measured using the ruler. Five fruits were randomly selected using simple random technique and the lengths of the stalks were measured from the tip of the stalk to the base of the caps. This was done for each harvest within 20 days and the average calculated.

3.4.7 Perimeter of the Cap

The perimeter of the caps was measured using a thread and the measuring rule. The thread was used to trace the perimeter of the caps of the five randomly selected fruits. The length of the thread that covered the perimeter of the caps was then measured on the tape rule and the value recorded. This was done for each harvest within 20 days and the average calculated

3.4.8 Moisture Content of Harvested Mushroom

Five samples of the fruit body of the mushrooms were randomly selected and the moisture content was determined. After the weight of the empty Petri-dish and their covers were recorded, the samples were placed into the Petri-dish and weighed again. The weight of the Petri-dish plus the samples was then recorded. The Petri-dish plus the samples were then placed into the oven and the temperature set at 105^{o}C for 24 hours. After this period, the Petri-dish plus the samples were removed and placed in a desiccator for 30 minutes. The weight of

the dry sample plus the Petri-dish was then measured and recorded. The percentage moisture of the substrates was calculated according to the formula:

Percentage moisture

$$= \frac{(Weight\ of\ dry\ sample + Petri\ dish) - (Weight\ of\ empty\ Petri\ dish)}{(Weight\ of\ fresh\ sample + Petri\ dish) - (Weight\ of\ empty\ Petri\ dish)}\ x\ 100\%$$

3.4.9 Biological Efficiency

Total weight of the fruiting bodies harvested from the substrates within 30 days of fruiting was measured as total yield of the mushroom. The biological efficiency (yield of mushroom per kg substrate on dry weight basis) was calculated by the formula proposed by Chang *et al.* (1981).

$$\%\ \text{Biological Efficiency (B. E \%)} = \frac{Fresh\ Weight\ of\ Mushrooms}{Dry\ Weight\ of\ Substrates} x100$$

3.4.10 Yield

The yield per kilogram of substrate was calculated by dividing the total yield in grams taken from each treatment by the number of cropping bags fully colonized within each treatment.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical Changes in Substrates as Influenced by Composting Time and Substrate Types

The C:N ratio of the sole bean straw substrates after the composting periods ranged between 33 and 42, the mixed substrates (the coir plus the bean straw substrates) recorded C:N ratio between 33 and 53 while the sole coir substrates gave the highest C:N ratio ranging from 93 to 107 (Table 4.1).

Table 4.1 Chemical Changes in Substrates as Influenced by Substrate Types and Composting Time

Substrate Types and Composting Time	% Organic Carbon	% Total Nitrogen	C:N	pH
Bean Straw				
1 day	31.52	0.75	42.03	7.95
7 days	45.89	1.34	34.25	6.60
14 days	34.71	1.03	33.70	6.12
Average	**37.37**	**1.04**	**36.66**	**6.89**
Mixed Substrates				
1 day	6.78	0.20	33.90	7.73
7 days	10.57	0.22	48.04	7.70
14 days	17.09	0.25	53.48	7.58
Average	**11.48**	**0.22**	**45.14**	**7.67**
Coconut Coir				
1 day	14.96	0.14	106.85	6.53
14 days	17.09	0.17	104.47	6.46
21 days	18.66	0.20	93.75	6.42
Average	**16.90**	**0.17**	**101.69**	**6.47**
S.e (ST X CT)	1.73	0.02	1.52	0.07
$Lsd_{(0.05)}$ (ST X CT)	3.66	0.04	3.22	0.15
S.e (ST)	1.00	0.01	0.88	0.04
$Lsd_{(0.05)}$ (ST)	2.11	0.02	1.86	0.09
S.e(CT)	1.00	0.01	0.88	0.04
$Lsd_{(0.05)}$ (CT)	2.11	0.02	1.86	0.09

Where ST – Substrates Types, CT – Composting Time, C:N - Carbon : Nitrogen., S.E – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at $p \leq 0.05$

Generally the availability of percentage organic carbon and percentage total nitrogen in the substrates increased with time in the mixed substrates and the coconut coir. This pattern was generally observed in all the substrates (Table 4.1). The amount of percentage organic carbon and percentage total nitrogen in the bean straw substrates were significantly higher ($P \leq 0.05$) than those of the mixed and the sole coconut coir substrates (Table 4.1).

The analysis of variance showed significant differences ($P \leq 0.05$) in the effect of composting time on the availability of nutrients in the substrates. The increase of carbon and nitrogen content of the compost with time could be due to the microorganisms that got the required environmental conditions such as favourable C:N ratio and required pH range to break the giant structure of the lignin, cellulose and hemicelluloses into their simpler forms and made them available. TNAU (2008) gave similar report and stated that increasing composting time increases the amount of available nutrients and cation exchange capacities in the compost. Taylor and Francis (2013) noted that as organic matter is decomposed; nutrients such as nitrogen, phosphorus and potassium are released and recycled in various chemical forms. Proteins decompose into amino acids such as glycine or cysteine and these nitrogen compounds then further decompose to yield simple inorganic ions such as ammonium (NH_4^+) and nitrate (NO_3^-) that become available for uptake (Taylor and Francis, 2013). Epstein (1997) further explained that Carbon : Nitrogen (C:N) ratio of the substrates reduces with time because each time organic compounds are consumed by microorganisms, two thirds of the carbon is lost into the atmosphere as CO_2, while most of the nitrogen is recycled into new microorganisms. Nitrate nitrogen and ammonium nitrogen are involved in the composting process (Wu *et al*., 2010). The nitrate nitrogen increases throughout the composting process

while the ammonium nitrogen increases at the start, but reduces with time as composting process continues (Wu *et al*., 2010).

The amount of hydrogen ion concentration had an opposite pattern to the availability of carbon and nitrogen in the mixed and coconut coir substrates. The pH values decreased as time increased, ranging from a high of 7.95 to a low of 6.12 for all the substrates (Table 4.1). The mixed substrates were slightly basic with average pH of 7.67 while the pH of the sole coir substrate was in the acidic range. Aside the one day composted sole bean straw substrate which showed slightly basic pH of 7.95, the 7 and the 14 days composted sole bean straw substrates were also slightly acidic.

Generally the pH of the substrates attained acidic medium as the nutrients (carbon and nitrogen) increased with increasing composting time (Table 4.1). Hermandez *et al.* (2003) observed the same phenomenon of a declining pH as composting time increased when they worked on procedures for preparing substrates for *Pleurotus ostreatus* cultivation. Ogunwande *et al.* (2008) reported that the decrease of the pH (the substrate becoming more acidic) may be due to the decomposition of the organic matter in the piles and therefore the production of short chain organic acids. As noted by Sundberg (2005), the final pH in the compost is reduced as a result of the pH being influenced by three acid-base systems: the carbonic system which is formed during the decomposition that dissolves in the liquid forming carbonic acid (H_2CO_3), bicarbonate (HCO_3) or carbonate (CO_3^{2-}); ammonium (NH_4^+) or ammonia (NH_3) which is formed when protein is decomposed; and the third system comprising several organic acids, of which acetic and lactic acids dominate.

4.2 Moisture Content of Substrates during the First Day of Composting

The mixed substrates had 76.53% moisture during the first trial and 76.81% during the second trial (Table 4.2) and these were significantly higher ($P \leq 0.05$) than the moisture contents of the sole coir and the sole bean straw substrates. Similarly, the moisture contents of the sole coir substrates, during the two trials, were significantly higher ($P \leq 0.05$) than the moisture contents of the sole bean straw substrates (Table 4.2).

Table 4.2 Moisture Content of Substrates at first Day of Composting

Substrate	Moisture Content (%)	
	First Trial	Second Trial
Sole Bean Straw	62.50	60.61
Mixed Substrates	76.53	76.81
Sole Coir	73.90	72.65
S.e	1.098	0.829
$Lsd_{(0.05)}$	2.328	1.758

First Trial was performed from February to April, 2014 while the second trial took place from April to July, 2014.

The particle size of the substrates was a contributing factor to the differences in the water holding capacity (Saunders *et al.,* 2006). The particle sizes of coir substrates were 0.5 mm thick and 1.0 to 2.0 cm long as against 1.0 to 1.5 cm thick and 2.0 to 5.0 cm long for the bean straw. Those are the natural particles sizes of the substrates within the reach of farmers and therefore were not changed during the experimental design. The fine particle sizes of the coir substrates might hold higher amount of water as against the porous particles sizes of the bean straw. When the two substrates were mixed together to formulate the mixed substrates, the density of the mixture contributed to the ability of the mixture to maintain higher water content. Media components that differ significantly in particle sizes have higher bulk density as a mixture (Raviv *et al.,* 2002; Pokorny *et al.,* 1986). Wightman (1999) stated that a substrate quality is determined by both the physical characteristics such as good drainage and

chemical characteristics such as high nutrient content. The physical qualities may include water holding capacity, available air spaces, texture, weight per container, etc. while the chemical properties may include the available nutrients, how easily they are available to the mycelia and the rate at which they are released. Saunders *et al.* (2006) noted that a decrease in substrate particle sizes usually results in an increase in water holding capacity and a decrease in aeration.

4.3 Rate of Mycelia Formation

During the first trial, the formation of mycelia was slow among all the substrates, taking between 5 and 10 days after spawning. Rate of mycelia growth for the mixed substrates and the sole coconut coir substrate attained their peak of 1.4 cm and 1.9 cm per day respectively on the 20th day after spawning (Figure 4.1). These were significantly higher than the bean straw which recorded a declining growth of mycelia of 0.1 cm on the 20th day after spawning during the first trial. Growth of mycelia after the 20th day of spawning of all the substrates then experienced a declining rate till the 30th day after spawning (Figure 4.1).

A similar pattern was observed during the second trial where sole coconut coir and the mixed substrates showed higher rate of mycelia growth than the sole bean straw substrates (Figure 4.2). In the second trial, the mycelia growth in the mixed substrates and the sole coconut coir rose from the 5th day after spawning until they attained their peaks of 1.2 cm and 1.0 cm of growth per day, 10 days after spawning (Figure 4.2). There was then a declining rate from the 10th day till the 15th day after spawning after which growth rate remained constant till the 20th day (Figure 4.2). The peak mycelia growth rate was attained during the 10th day after spawning which was significantly different from the records of the first trial. This might be

due to the differences in the prevailing environmental conditions at the experimental site during the two trials.

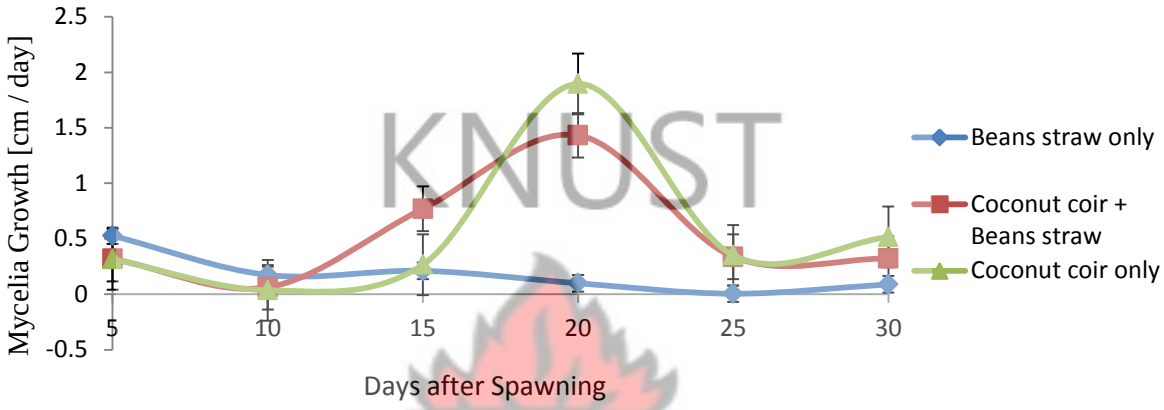


Figure 4.1 Mycelia Formation as Influenced by Substrate Types - 1st Trial (Feb - April, 2014)

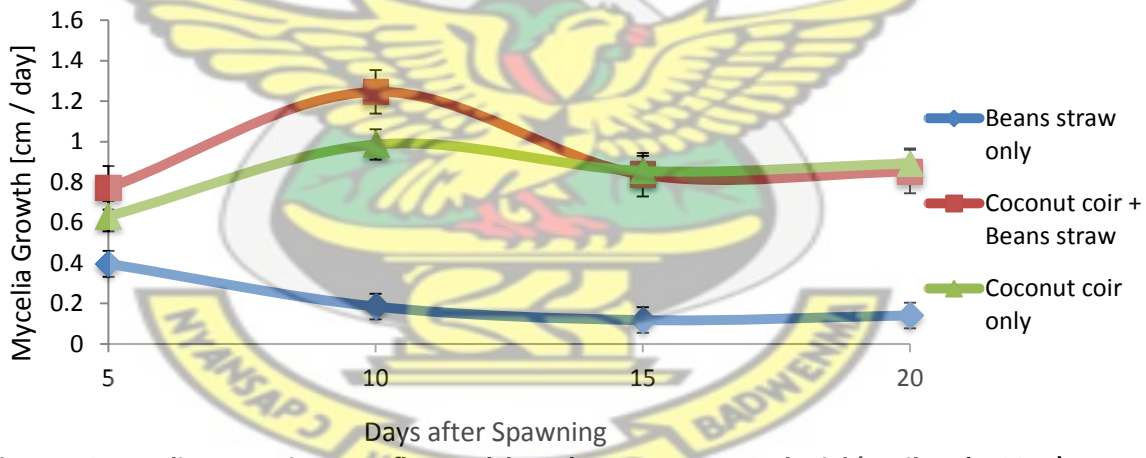


Figure 4.2 Mycelia Formation as Influenced by Substrate Types - 2nd Trial (April - July, 2014)

. The first trial took place between February and April which is the beginning of the raining season while the second trial was conducted between April and July, the raining season (Ghana Meteorological Agency, 2013). The raining season is noted for average rains of 30 to 40 hours in a month but less than 10 hours in a month during the dry season (Ghana Meteorological Agency, 2013). This rainfall distribution pattern affects temperature and

relative humidity of an area where moderate relative humidity and temperature are recorded during raining season. The experimental site recorded average temperature of 29^{o}C and precipitation of 3 mm in February when the first trial took place and then 28^{o}C, 91 mm in April when the first trial ended. Precipitation in June was 163 mm with 27^{o}C average monthly temperature when the second trial ended (AccuWeather, 2014; Appendix 14). Faster mycelia formation during the second trial might be due to moderate monthly average temperature of 27^{o}C during the second trial as against 29^{o}C during the first trial (AccuWeather 2014; Thakkar, 2010). The bean straw showed the least mycelia growth during the two trials. There was no significant difference between the mycelia growth of the sole coconut coir or the mixed substrates during the two trials. Also composting time of the substrates recorded no significant effect on the rate of mycelia formation (Table 4.3).

The particle sizes of the substrates might influence the mycelia development (Verma and Marschner, 2013). The fine particle sizes of sole coir and the well-blended particles sizes of the mixed substrates influenced the faster mycelia formation of these substrates (Figures 4.1 and 4.2). The bean straw substrates on the other hand gave poor mycelia growth and this might be due to the porous nature of the particles. Similar results were obtained by Banerjee *et al.* (1995) who concluded that both the rate and the overall conversion of cellulose to fungal protein were enhanced by reduction in sizes of the solid substrate particles. Small particles have larger surface area for microbial activity and are easier to break down for the release of the nutrients (Cogger and Sullivan, 2009). After their research on the effects on microbial biomass and soil p pools as affected by particle size and soil properties, Verma and Marschner (2013) concluded that fine compost size fractions increased microbial biomass and, that

phosphorus availability was more than the coarser fraction and these factors are due to higher surface area to volume ratio and higher decomposability compared to the coarser fraction.

The poor mycelia growth of the sole bean straw (Figures 4.1 and 4.2) might be due to the higher nitrogen content of the substrates (Table 4.1). Excess nitrogen may cause stratum degradation when nitrogen is excessively added (Rajarathan and Bano, 1989). The activities of Laccase, which is the main enzyme used by oyster mushroom to degrade the lignin content of the substrate is reduced when excess nitrogen is added to the substrate (D'Agostini *et al.*, 2011). When working on effect of organic nitrogen supplementation in *Pleurotus* species, Upadhyay *et al.* (2002) concluded that substrates supplemented with 1% de-fatted soybean meal performed better than those supplemented with 2.5%, 5%, 7.5% and 10% of the same material or cotton seed cake and that substrates with higher supplementation gave lower yields of the mushroom. Additionally, the sole bean straw substrates also recorded low water holding capacity (Table 4.2) due to their porosity. Microorganisms need moisture for their activities and the accessible moisture might be the water film around the substrates. A very porous substrate drains water easily and does not make it accessible to the decomposers. Kwon and Kim (2004) noted that substrates such as cotton seed hull alone is not desirable for mushroom production due to their low moisture retention capacity and therefore such substrates must be supplemented with other substrates to effect higher water content. The poor mycelia growth of bean straw is therefore in contrast with the observations made by Musieba (2012), Poppe (2004) and Siqueira (2012) who noted that bean straw is one of the best substrates in mushroom production. The findings of this research indicate that the straw responds well when used with other substrates.

The typical growth of the mycelia of sole coir and the mixed substrates (Figure 4.1 and Plate 4.1) might be in response to the availability of nutrients and their timely release to the developing mycelia. The sole coconut coir substrates recorded an average pH of 6.47 which is the best pH for mycelia growth as the sole coir substrate gave higher mycelia growth especially during the first trial (Figure 4.1). This confirms the work of Ibekwe *et al.* (2008) who observed that the pH range between 6.0 to 9.0 influenced mycelia growth with the highest growth at pH of 6.4.

Table 4.3 Effect of Composting Time and Substrate Types on Mycelia Formation – Trials One and Two

Mycelia Formation Rate (DAS)	Substrate Type (ST)		Compost Time (CT)		ST x CT	
	1st T	2nd T	1st T	2nd T	1st T	2nd T
5 DAS	*	*	ns	ns	ns	ns
S.e	0.0445	0.0751	0.0445	0.0751	0.0771	0.1300
$Lsd_{(0.05)}$	0.0943	0.1592	0.0943	0.1592	0.1634	0.2757
10 DAS	ns	*	ns	*	ns	*
S.e	0.0330	0.0315	0.0330	0.0315	0.0571	0.0545
$Lsd_{(0.05)}$	0.0699	0.0667	0.0699	0.0667	0.1210	0.1156
15 DAS	*	*	ns	ns	*	ns
S.e	0.1149	0.1015	0.1149	0.1015	0.1990	0.1758
$Lsd_{(0.05)}$	0.2435	0.2152	0.2435	0.2152	0.4218	0.3728
20 DAS	*	*	ns	ns	ns	ns
S.e	0.1465	0.1335	0.1465	0.1335	0.2354	0.2312
$Lsd_{(0.05)}$	0.3105	0.2830	0.3105	0.2830	0.5379	0.4902
25 DAS	ns	-	ns	-	ns	-
S.e	0.1621	-	0.1621	-	0.2808	-
$Lsd_{(0.05)}$	0.3437	-	0.3437	-	0.5953	-
30 DAS	*	-	ns	-	ns	-
S.e	0.0893	-	0.0893	-	0.1547	-
$Lsd_{(0.05)}$	0.1894	-	0.1894	-	0.3280	-

Where 1stT – First Trial, 2ndT – Second Trial, S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Different at $P \leq 0.05$, * - Significantly Different, ns – Not Significantly Different, DAS – Days after Spawning. Generally, composting time did not affect rate of mycelium growth but substrate types had effect.

When the coir was mixed with bean straw, the high content of carbon in the coir balanced the high content of nitrogen in the bean straw creating a favorable C:N ratio for microbial activity. From the results (Table 4.1), the C:N ratio of the mixed substrates ranged from 33.90 to 53.48 which were within the recommended range in literature (Rajarathnam and Bano, 1989; Quimio and Sardsud, 1981; Shroomery, 2011). As such, this resulted in well-balanced cellulose, hemicellulose and lignin content in the mixed substrate and that influenced faster mycelia formation as well as higher yield (Kang, 2004).

Plate 4.1 Mycelia Growth Indicated by White Treads within the Substrates (Fifteen Days after Spawning)

4.4 Number of Bags Fully Colonized and First Primordia Formation

The coir and the mixed substrates gave average of 90% of bags fully colonized out of the 10 bags that were monitored for mycelia growth within a period of 30 days during the first trial and 20 days during the second trial (Table 4.4).

Table 4.4 Mycelia Colonization of Bags after Spawning

Substrates/ Composting Time	Average No. of Bags Fully Colonized [%]		1st Primordia Appearance [DAC]	
	1st T	2nd T	1st T	2nd T
Bean Straw				
1 day	33.0	20.0	9.3	-
7 days	13.0	23.0	-	-
14 days	0.0	0.0	-	-
Average	**15.3**	**14.3**	**3.1**	**-**
Mixed Substrates				
1 day	73.0	97.0	2.0	6.0
7 days	60.0	100.0	2.0	6.0
14 days	100.0	100.0	2.0	6.0
Average	**77.7**	**99.0**	**2.0**	**6.0**
Coconut Coir				
1 day	97.0	100.0	4.0	6.7
14 days	100.0	87.0	8.0	6.7
21 days	100.0	87.0	4.0	6.0
Average	**99.0**	**91.0**	**5.5**	**6.5**
S.e (ST X CT)	0.86	0.65	0.63	0.46
$Lsd_{(0.05)}$(ST X CT)	1.83	1.38	1.33	0.97
S.e (ST)	0.50	0.38	0.36	0.26
$Lsd_{(0.05)}$ (ST)	1.06	0.80	0.77	0.56
S.e (CT)	0.50	0.38	0.36	0.26
$Lsd_{(0.05)}$ (CT)	1.06	0.80	0.77	0.56

Where DAS – Days after Spawning, DAC – Days after Cropping, T – Trial, Where T - Trial; ST – Substrate type; CT – Composting time, S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at $p \leq 0.05$

The bean straw gave the least number of bags fully colonized of maximum of 3 bags out of 10 bags (Table 4.4). This then affected the first primordia formation as the bean straw substrates gave no primordia with the exception of the 1 day composted bean straw in the first trial.

These effects may be due to contamination and partial colonization in the sole bean straw. Some of the bags of the sole bean straw substrates had black moulds which were evidences of contamination; other bags showed partial colonization of the mycelia (Plate 4.2).

Plate 4.2. Contamination and Partial Colonization of the Sole Straw Substrates

Contamination in the bean straw composted for 7 and 14 days were higher as compared to the same substrate composted for just a day. The contamination and the partial mycelia colonization might be due to the activities of some competitive fungi and moulds in the straw substrates. In nature, complex interactions among hundreds of other fungi, bacteria, nematodes

etc., maintain an ecological equilibrium (Shroomery, 2005) and they can compete among themselves within a substrate. The problem of partial colonization is also in line with the observations of Lieuxsinguliers (2012), who reported that some of the problems in mushroom production are partial colonization of some substrates which may be due to contamination. Fletcher and Gaze (2008) noted that moulds are associated with some special substrates and bean straw in our case could be one of these substrates. The most common moulds that contaminate compost include Trichoderma, *Pythium* and *Penicillium* species (Fletcher and Gaze, 2008) and these moulds were associated with the sole bean straw leading to contamination. The high nutrient content with the favorable pH of 6.89 on average provided the right medium for the competitor fungi to thrive well in the bean straw substrates.

4.5 Effect of Substrate Types and Composting Time on the Physical Characteristics of Mushrooms

Table 4.5 indicates length and width of stipe as well as the perimeter of the caps of the mushrooms harvested. The mixed substrates produced mushrooms with the highest stipe width and cap perimeter among the three substrates studied. The stipe width of 1.1 cm from one day composted mixed substrates in both trials was the highest and the least was 0.4 cm from one day composted sole coir substrate (Table 4.5). This was reflected in the cap perimeter where the one day composted mixed substrates produced mushrooms with the broadest cap of 17.9 cm and 14.7 cm during the two trials. On the other hand, length of stipe took a different pattern where the mixed substrates composted for 7 and 14 days gave mushrooms with higher stipe length of 4.3 and 4.8 cm while the sole coir substrates composted for one day produced mushrooms with longer stipe length of 5.2 and 2.7 cm respectively during the two trials. Oyster mushroom physical quality depends on the length of stipe (Ajonina and Tatah, 2012). Mondal *et al.* (2010) noted that the higher the stipe length, the poorer the quality of the

mushrooms. Averagely, the mixed substrates produced mushrooms with better physical parameters than the sole coir substrates.

Table 4.5 Physical Parameters of the Fruits as Influenced by Substrate Types and Composting Time

Substrates	Parameter of Mushrooms [cm] during Trials					
	Length of Stalk		Width of Stalk		Perimeter of Cap	
	1	2	1	2	1	2
Bean Straw						
1 day	4.5	-	0.9	-	9.5	-
7 days	-	-	-	-	-	-
14 days	-	-	-	-	-	-
Average	**4.5**		**0.9**		**9.5**	**-**
Mixed Substrates						
1 day	3.2	3.2	1.1	1.1	20.1	16.1
7 days	4.3	3.6	0.9	1.0	16.6	15.4
14 days	4.2	4.8	1.0	1.0	17.0	12.5
Average	**4.1**	**3.9**	**1.0**	**1.0**	**17.9**	**14.7**
Coconut Coir						
1 day	5.2	2.7	0.6	0.4	12.8	8.2
14 days	4.2	2.4	0.7	0.6	13.1	9.1
21 days	3.6	2.1	0.8	0.7	13.2	11.0
Average	**4.3**	**2.4**	**0.7**	**0.6**	**13.0**	**9.4**
S.e(ST X CT)	0.43	0.36	0.11	0.08	1.76	0.80
$Lsd_{(0.05)}$ (ST X CT)	0.91	0.76	0.24	0.16	3.73	1.69
S.e(ST)	0.25	0.21	0.06	0.04	1.01	0.46
$Lsd_{(0.05)}$ (ST)	0.53	0.44	0.14	0.09	2.15	0.98
S.e(CT)	0.25	0.21	0.06	0.04	1.01	0.46
$Lsd_{(0.05)}$ (CT)	0.53	0.44	0.14	0.09	2.15	0.98

Where T – Trial, S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at $P \leq 0.05$, ST – Substrate Type, CT – Composting Time, ' –' – No Observation

Stalk width and cap perimeter of mushrooms from the sole coir substrates, as compared to the mixed substrates, was however, poor (Table 4.5) and this might be due to the very high C:N ratio observed in these substrates (Table 4.1). Very high C:N ratios mean higher carbon content of the coir substrates and lesser nitrogen content as a result of their slow decomposition within the time range. The sole coir substrates recorded very high C:N ratio

ranging from 97 to 107. This high carbon to nitrogen ratio of these substrates imply that as the carbon continued to break down, microorganisms would draw on substrate nitrogen to assist in the process and this phenomena would eventually leave the substrate virtually poor in nitrogen (Aggie Horticulture, 2009). Chisholm *et al.* (2014) and Aggie Horticulture (2009) reported that using high organic matter with excess carbon can create a problem in a substrate and to complete the nitrogen cycle and continue decomposition, the microbial cells will draw any available substrate nitrogen in the proper proportion to make use of available carbon. Chisholm *et al.* (2014) and Aggie Horticulture (2009) termed this phenomenon as "robbing" the substrate of nitrogen and it delays availability of nitrogen for the growing mushroom. Thus, very high C:N ratio indicates inefficient nitrogen content in the substrate to balance the carbon content in the substrate. Cornell Composting (1996) indicated that higher C:N ratios mean that there is not sufficient nitrogen for optimal growth of the microbial population, so the compost remains relatively cool and degradation proceeds at a slower rate. When the nitrogen content is too low for the amount of carbon e.g. 80:1 of C:N ratio, organisms will have to recycle the nitrogen through many generations in order to break down the carbon containing materials (Aggie Horticulture, 2009). This situation results in slow decomposition and as such inefficiency of the substrate as a growing medium. This agrees with Thomas *et al.* (2012) who also reported that the high C:N ratio of around 100: 1, lignin content of 37% and polyphenol content of 100mg per 100g makes the coir pith sometimes not a better choice for mushroom cultivation.

4.6 Yield as Influenced by Substrate Types and Composting Time

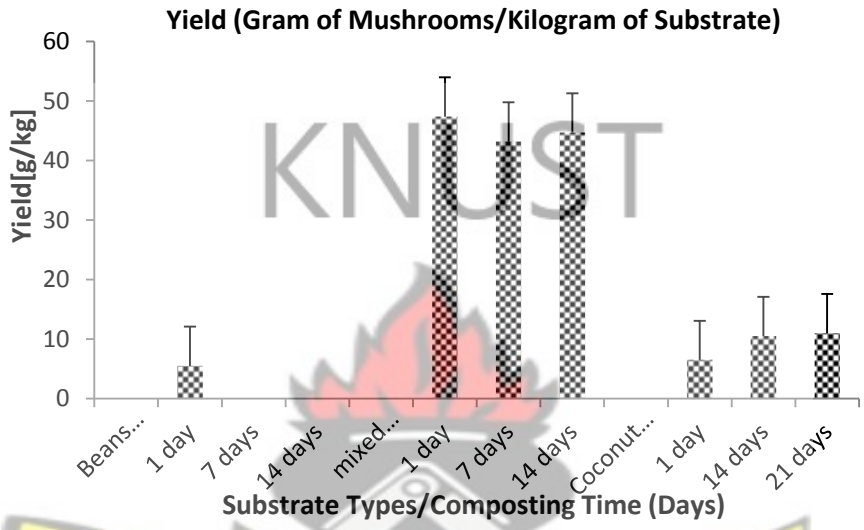
The mixed substrates gave the highest yield in grams of mushrooms per kilogram of substrates (g/kg) among the three substrate types studied, ranging from 26.5g/kg to 51.1g/kg and these were significantly higher ($P \leq 0.05$) than the 5.0g/kg to 11.0g/kg of the sole coir substrates in the two trials (Figures 4.3A and 4.3B).

The only yield recorded from the bean straw substrates was from the one day composted substrate (5.5g/kg) during the first trial and it was significantly lower ($P \leq 0.05$) than the yield obtained from the mixed substrates, but not significantly ($P \geq 0.05$) different from those of the sole coir substrate (Figure 4.3 A).

This higher yield observed in the mixed substrates may be due to good physical and chemical qualities of these substrates that ensured a smooth transition from vegetative phase to reproductive phase. This agrees with reports of Shashirekha and Rajarathnam (2007) who observed that supplementing the coir with rice straw increased the activities of cellulases, hemicellulases and protease enzymes from inoculation till the end of fructification, while laccase activity decreased during frutification in consonance with decreased lignin degradation during frutification. The observation also confirms the work of Kapoor *et al.* (2009) who noted that the supplementation of different brans (wheat or rice brans) into a substrate for mushroom growth resulted not only in improved linear growth, but also in higher activity of cellulases in supplemented straw as compared to the unsupplemented straw. Mondal *et al.* (2010) reported that a well-balanced C:N ratio influences mycelia growth. Oseni *et al.* (2012) also noted that supplementing sawdust with wheat bran increases mycelia growth and yield of mushrooms. The coir, having fine texture and the straw being porous in nature blended well with good environmental conditions for the Mushroom. These created balanced physical

conditions such as aeration and good water holding capacity to support the mycelia growth as well as yield.

A. First Trial



B. Second Trial

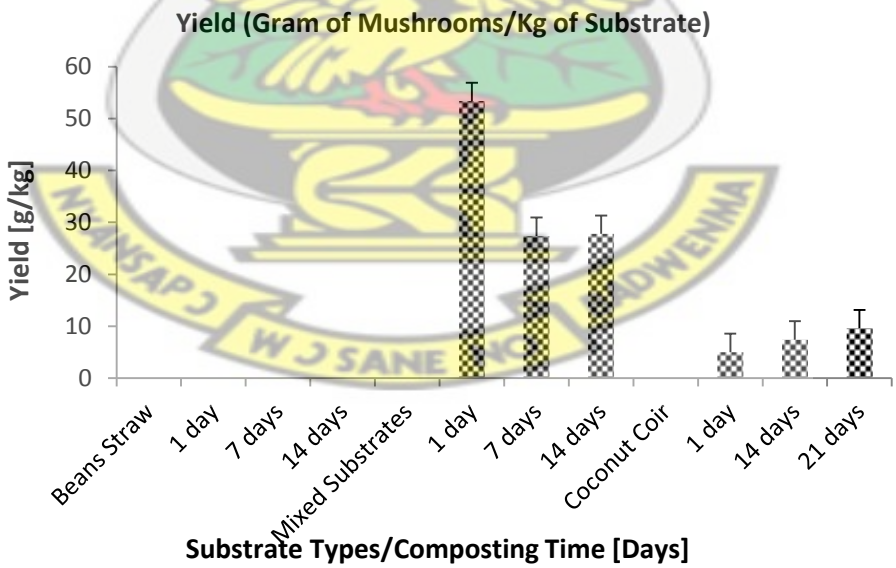


Figure 4.3 Yield of Mushrooms as Influenced by Substrate Types and Composting Time during the First and the Second Trials

The one day composted mixed substrates, comparatively, performed better during the two trials than the other two substrates. On the other hand, the 21 days composted sole coir substrate comparatively performed significantly higher ($P \leq 0.05$) than the 1 day though significantly the same as the 14 days during the two trials (Figures 4.3). It was observed that yield of the mushroom from the sole coir substrates increased with increasing composting time as against those of the mixed substrates where the one day composted substrate recorded the highest yield. These might be due to the chemical constituents of the substrates at such composting times. Oyster mushroom being lignocellulotic in nature have the required enzymes, such as laccases, to break the lignin to get access to the cellulose and the hemicelluloses when the C:N ratio is favorable (Dzomeku, 2009; Oei 1991; Kang, 2004; Shroomery, 2011). The oyster mushrooms feed by secreting a range of enzymes such as peroxidase, laccases, cellulases, hemicellulases and xylanases and the activities of these enzymes are affected by the chemical constituents of the substrates (Cohen *et al.* 2002). The oyster mushroom is well adapted on lignin and cellulose containing substrates such as sawdust, rice straw etc. as it prefers the lignin that makes up the secondary cell walls of hard woods (Woller, 2007). As oyster mushroom is saprophytic and lignocellulotic in nature, it is able to grow on lignocellulotic substrates when the C:N ratio is low (Woller, 2007).

The one day composted mixed substrate could also maintain high moisture content as well as low C:N ratio for the growth of the mushroom. Low C:N ratio, optimum moisture content, and regular turning of substrate increase microbial activity (Mlangeni *et al.*, 2013; Pan *et al.*, 2012) and therefore the release of more nutrients for the developing caps. Most researchers used their lignocellulotic substrates by just soaking them in water, but had very good results (Diego *et al.*, 2011; Kwon and Kim, 2004; Thomas *et al.*, 1998). Diego *et al.* (2011) reported

that a lignocellulotic mushroom such as *Pleurotus* spp. can be grown on raw lignocellulotic material without composting, but other mushrooms such as *Agaricus* and *Lentinula* species need composting for their production. Kwon and Kim (2004) just chopped straws into pieces and soaked for 12 hours for the growth of oyster mushroom. Thomas *et al.* (1998) soaked coconut coir for 16 hours for the growth of oyster mushroom and still had good results. These researchers and many others proved that substrate fermentation for longer periods are not all that necessary for the production of oyster mushrooms when the initial C:N ratio of the substrates are within recommended range, i.e. 30 – 60 (Jenkins and Zwieten, 2003; Shroomery, 2011) and a pH range between 5.5 and 8.0 (Chen *et al*, 2011; Ibekwe *et al.*, 2008). This gives an indication that initial chemical analysis of the carbon and the nitrogen of the substrate as well as the pH of the medium are needed in order to save time, energy and resources in the mushroom business.

4.7 Average Number of Fruit Bodies per Flush and Percentage Dry Matter

During the first trial, an average of 10.7 fruit bodies per flush was harvested on the bean straw substrates, which was significantly higher ($P \leq 0.05$) than the 6.6 and the 5.3 obtained from the mixed and the sole coir substrates respectively (Table 4.6). The growth of mushrooms from the sole straw substrate was more of a cluster type. The same clusters of mushrooms were observed on the coir and the mixed substrates during the first trial (Plate 4.3). Different pattern of appearance of mushrooms during the second trial occurred whereby mushrooms appeared in duos and in trios and sometimes in solitary. This effect might be due to changes in weather conditions within the two trials.

Composting time also influenced number of mushrooms per flush during the two trials. The substrates composted for one day among the mixed substrates significantly produced higher

number of fruits per flush while the substrates composted for 14 and 21 days in the sole coir substrates gave similar pattern (Table 4.6). This was due to the required C:N ratio within such specific periods of composting as well as other good physical conditions such as aeration, water holding capacity and particle sizes of the substrates (Chisholm *et al*., 2014; Mlangeni *et al.*, 2013; Jenkins and Zwieten, 2003; Ibekwe *et al.*, 2008).

Among the composting times investigated, the one day composted mixed substrate produced the highest number of fruit bodies per flush during the two trials (7.9 and 3.7 fruits per flush) and this was reflected in the % Dry matter of 11.7% and 13.9% respectively.

Table 4.6 Number of Fruit Bodies per Flush, Percentage Dry Matter and Biological Efficiency of Mushrooms as Influenced by Substrate Types and Composting Time

Substrate	Av. No. of Fruits per flush		Dry Matter[%]		Biological Efficiency [%]	
	1st T	2nd T	1st T	2nd T	1st T	2nd T
Bean Straw						
1 day	10.7	0.0	14.0	0.0	1.40	0.00
7 days	0.0	0.0	0.0	0.0	0.00	0.00
14 days	0.0	0.0	0.0	0.0	0.00	0.00
Average	**3.6**	**0.0**	**4.7**	**0.0**	**0.46**	**0.00**
Mixed Substrates						
1 day	7.9	3.7	11.7	13.9	20.36	22.90
7 days	5.7	3.3	11.6	13.7	17.95	11.40
14 days	6.2	3.0	9.4	10.8	17.75	11.00
Average	**6.6**	**3.3**	**10.9**	**12.8**	**18.69**	**15.12**
Coconut Coir						
1 day	4.3	2.3	8.6	13.7	2.19	1.90
14 days	6.0	3.0	8.4	13.7	4.03	2.50
21 days	5.7	2.7	8.1	11.4	3.30	2.90
Average	**5.3**	**2.7**	**8.4**	**12.9**	**3.17**	**2.40**
S.e(ST X CT)	1.06	0.32	1.78	0.45	2.66	1.40
$Lsd_{(0.05)}$ (ST X CT)	2.25	0.68	3.78	0.64	5.65	2.96
S.e(ST)	0.61	0.18	1.03	0.37	1.53	0.81
$Lsd_{(0.05)}$ (ST)	1.30	0.39	2.18	0.78	3.25	1.71
S.e(CT)	0.61	0.18	1.03	0.37	1.53	0.81
$Lsd_{(0.05)}$ (CT)	1.30	0.39	2.18	0.78	3.25	1.71

Where T - Trial; ST – Substrate type; CT – Composting time; Averages were calculated on the ten (10) bags used for the treatments.

No significant differences ($P \geq 0.05$) actually existed in the number of fruits per flush among the sole coir substrates. The particle sizes as well as the good chemical properties of the mixed substrates such low C:N ratio and a recommended pH range contributed to their higher number of fruits per flush as well as their dry matter content (Wightman 1999; Verma and Marschner, 2013; Mondal *et al.,* 2010). Yoshida *et al.,* (1993) observed that the number of fruiting bodies increased when the substrates were mixed with different supplements. The same observation was also made by Sarker (2004) who noted that number of primordia increases with levels of supplements.

Plate 4.3 Mushrooms in Flushes during First and Second Trials

Mushrooms appeared in clusters during the first trial (Plate 4.3 A and C) and in trios, duos and solitary during the second trial (Plate 4.3 B and D)

4.8 Biological Efficiency (B. E)

Biological efficiency is the yield of mushrooms per kg of substrates on dry weight basis (Chang *et al.*, 1981). Generally, the efficiency of all the substrates to produce mushrooms was low within the days data on yield was collected. However, the mixed substrates were more efficient than the sole coir and the sole straw substrates (Table 4.6). Differences in biological efficiencies of the various substrates were due to different substrate compositions (Ajonina and Tatah, 2012).

The efficiency of the one day composted mixed substrate was comparatively constant and higher among the substrates during the two trials. The one day composted coir plus straw substrate had the recommended C:N ratio as well as the right pH for mushroom growth. The C:N ratio of 33.90 and pH of 7.73 (Table 4.1) measured from the one day composted mixed substrates were appropriate chemical properties needed for oyster mycelia growth and fruiting (Jenkins and Zwieten, 2003; Shroomery, 2011; Chen *et al*, 2011; Ibekwe *et al.*, 2008).

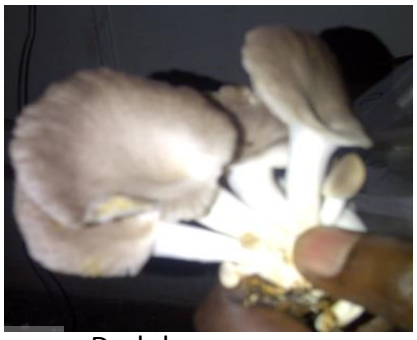
4.9 Aesthetic Features of the Harvested Fruits

Substrate type as well as composting time did not influence the colour or the shape of the mushrooms harvested. The colours of the fruits were, however, affected by the stage of the development of the mushroom and the prevailing environmental conditions (Burge, 2008). Three main colors of the mushrooms were identified from all the substrates and their composting times. The colours were cream, ash and dark brown (Plate 4.4). The shapes of the harvested mushrooms were conical, lobate and circular (Plate 4.4). Griensven (2000) noted that the genetic background of the strain, along with the environmental conditions and the cultivation techniques could affect the physical characteristics of the mushroom.

Colors Identified

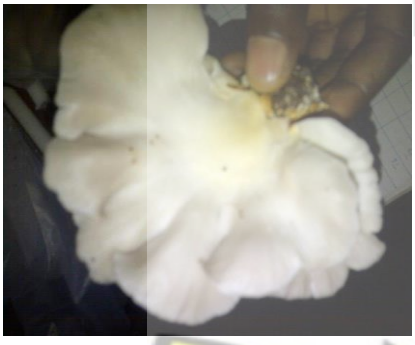
Cream

Ash

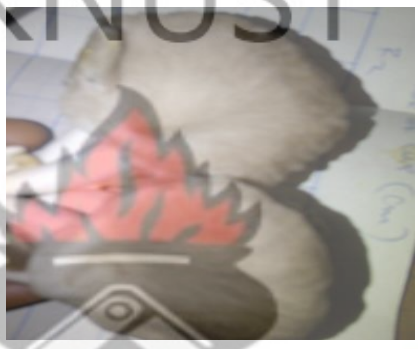


Dark brown

Shapes Identified



Lobate



Conical

Circular

Plate 4.4 Colors and Shapes of Mushrooms Identified
Color and shape of mushrooms not affected by substrate types or composting time

4.10 Cost Benefit Analyses

Tables 4.7 and 4.8 show the cost benefit analysis of the various substrates and their respective composting times. The mixed substrates gave the highest rate of return among the three substrates. This was as a result of the highest yield obtained from these substrates (Figure 4.3) due to their favorable chemical constituents such as low C:N ratio and recommended pH (Griensven 2000; Oseni *et al*., 2012; Mondal *et al.,* 2010). The one day composted mixed substrate was cost effective and profitable in the two trials than the 7 and the 14 days. High profit recorded in the one day mixed substrates may be linked to the effective activities of hydrolyzing and oxidizing enzymes which are capable of utilizing organic compounds in the substrate to convert biomass into mushrooms (Bhattachrjya *et al*., 2014).

Table 4.7 Partial Budget Analysis of the Substrates – First Trial

	Sole Bean Straw			Mixed Substrates			Sole Coconut Coir		
Composting Time	1 day	7 days	14 days	1day	7 days	14 days	1 day	14 days	21 days
Gross Benefit Yield [kg/ton]	5.5	0.0	0.0	47.4	43.2	44.7	6.5	10.5	11.0
Adjusted Yield (10%) Downward [kg/ton]	4.95	0.00	0.00	42.66	38.88	40.23	5.85	9.45	9.90
Price per 100g of Mushroom [GH₵]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Gross Benefit (GB) [GH/ton of subs.]	247.50	0.00	0.00	2133.0	1944.0	2011.5	292.5	472.5	495.0
Total Variable Cost (TVC) [GH₵/ton]	163.3	163.3	163.3	258.7	258.7	258.7	223.3	223.3	223.3
Net benefit(NB) (GB-TVC) [GH₵]	84.2	-163.3	-163.3	1874.3	1685.3	1752.8	69.2	249.2	271.7
RR=NB/TVC ×100%	51.6	-100.0	-100.0	724.5	651.4	677.5	31.0	111.6	121.7

Where RR – Rate of Return

Table 4.8 Partial Budget Analysis of the Substrates – Second Trial

	Sole Bean Straw			Mixed Substrates			Sole Coconut coir		
Composting Time	1 day	7 days	14 days	1day	7 days	14 days	1 day	14 days	21 days
Gross Benefit Yield [kg/ton]	0.0	0.0	0	53.4	27.4	27.8	5	7.4	9.6
Adjusted Yield (10%) Downward [kg/ton]	0	0	0	48.06	24.66	25.02	4.50	6.66	8.64
Price per 100g of Mushroom [GH₵]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Gross Benefit (GB) [GH₵//ton of subs.]	0.0	0.0	0.0	2403.0	1233.0	1251.0	225.0	333.0	432.0
Total Variable Cost (TVC) [GH₵/ton]	163.3	163.3	163.3	258.7	258.7	258.7	223.3	223.3	223.3
Net benefit(NB) (GB-TVC) [GH₵]	-163.3	-163.3	-163.3	2144.3	974.3	992.3	1.7	109.7	208.7
RR=NB/TVC ×100%	-100	-100	-100	828.9	376.6	383.6	0.7	49.1	93.5

Labour cost was not factored into this analysis. The total variable cost (TVC) was the cost incurred for using such specific substrates which include transportation, buying the spawns, and the composting and bagging materials.

Comparatively, high profit recorded from the substrates during the first trial might be due to the effect of the differences in environmental conditions between the two trials. The first trial took place at the beginning of the raining season while the second trial took place during the raining season (Appendix 14). The raining season is characterized by reduced temperature and moderate to high relative humidity while the beginning of the raining season is characterized by high temperature with reduced relative humidity (Ghana Meteorological Agency, 2013; AccuWeather, 2014). Although reduced temperature and high relative humidity is a good condition for mushroom growth, it also favors the growth of insects and parasites that affect the mushrooms (Petzoldt and Seaman, 2012; Shroomery, 2005). Less pathogenic effect and low pest infestation due to low atmospheric moisture might have resulted higher profit during the first than the second trial of almost all the substrates. Mushroom farming during such season is more profitable but it goes with heavy labour of watering the bags every day

Generally the profit level of using the sole substrates (sole coir and sole straw) was very low (Table 4.7; 4.8) and that might be due to the poor chemical and physical properties of the substrates. Selecting the mixed substrates (coir plus straw substrate) would mean higher profit in the mushroom business. Frempong (2000) stated that estimates of benefit-cost ratios, net profit values and internal rates of return suggest that mushroom cultivation is generally profitable. The results of the cost benefit analysis allow mushroom growers to know how substrate selection affects the profitability of the mushroom business (Roth and Hyde, 2002). Using the one day composted mixed straw is very profitable in both trials.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Cost management is very crucial in the quest for gaining profit in a mushroom business. Cost minimization and profit maximization is the main goal of every mushroom farmer. Substrates selected must be biologically efficient, time effective, high yielding and profitable. Base on this research, the mixed substrates (coconut coir plus bean straw substrate) could be one of the potential substrates for mushroom production. The biological efficiency, although low, was the highest compared to the sole straw and the sole coir substrates.

Composting time of substrates has an effect on pH and the C:N ratio of the substrates and hence the availability of the nutrients for the developing mycelia and mushroom growth. Coconut coir composted for 21 days recorded the least C:N ratio with the highest yield among the same substrate while the mixed substrate composted for one day recorded the best physical and chemical properties with the highest yield. The one day composting time of the mixed substrates recorded the highest yield among all the substrates used during the two trials.

Higher C:N ratio reduces the biological efficiency of the substrates and therefore reduces the profit margin of the mushroom business. The C:N ratio between 30 and 60 is ideal for oyster mushroom.

5.2 Recommendations

- The mixed substrate with one day composted time is more recommended for oyster mushroom production since it is time saving and more economical

- Sole beans straw should be used as supplement to other substrates for the growth of oyster mushroom but not solely use as oyster mushroom substrate
- Optimum supplementation ratio of coir plus beans straw substrate was not established, further research is therefore needed.
- Mushroom growers are also advised to do initial C:N ratio analysis of substrates to determine substrates that need further composting.

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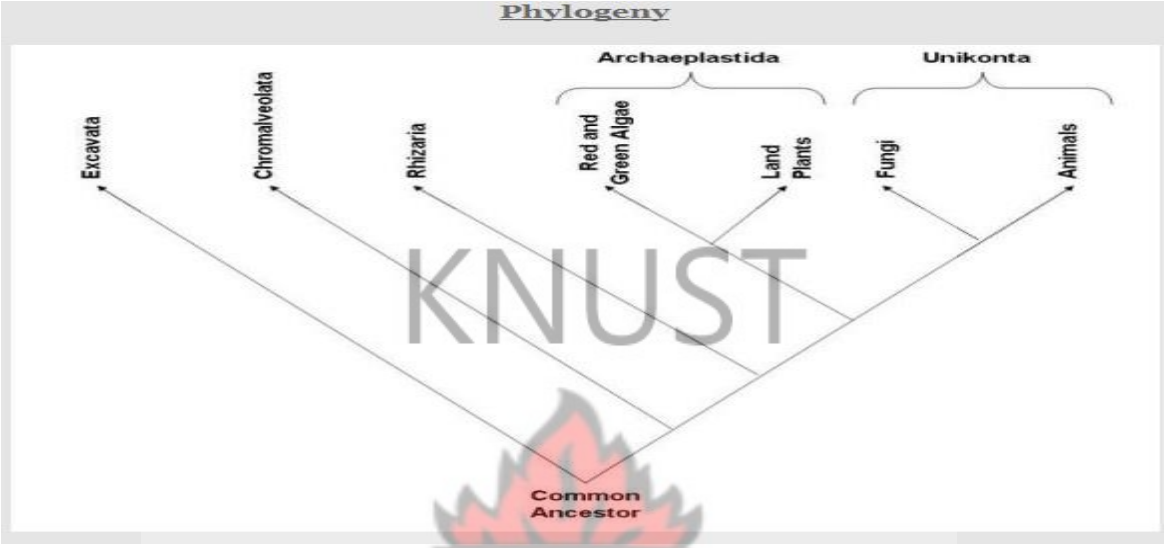
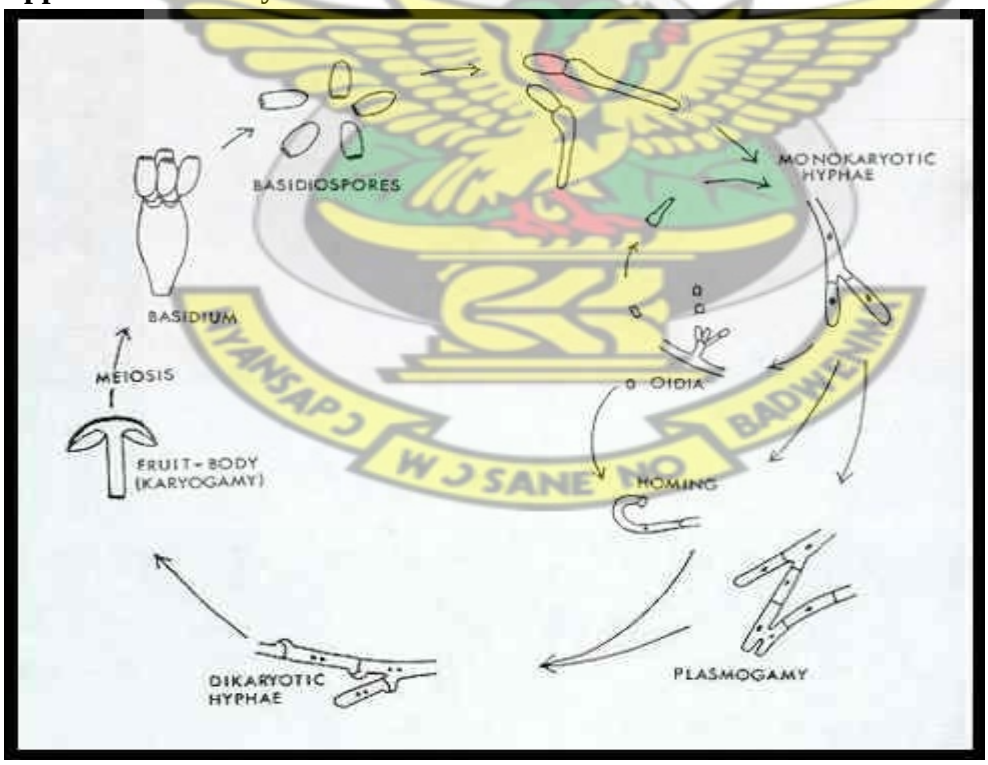
APPENDICES

Appendix 1. Phylogenic Tree of the Organisms on Earth that Show that Fungi are More Related to Animals than to Plants



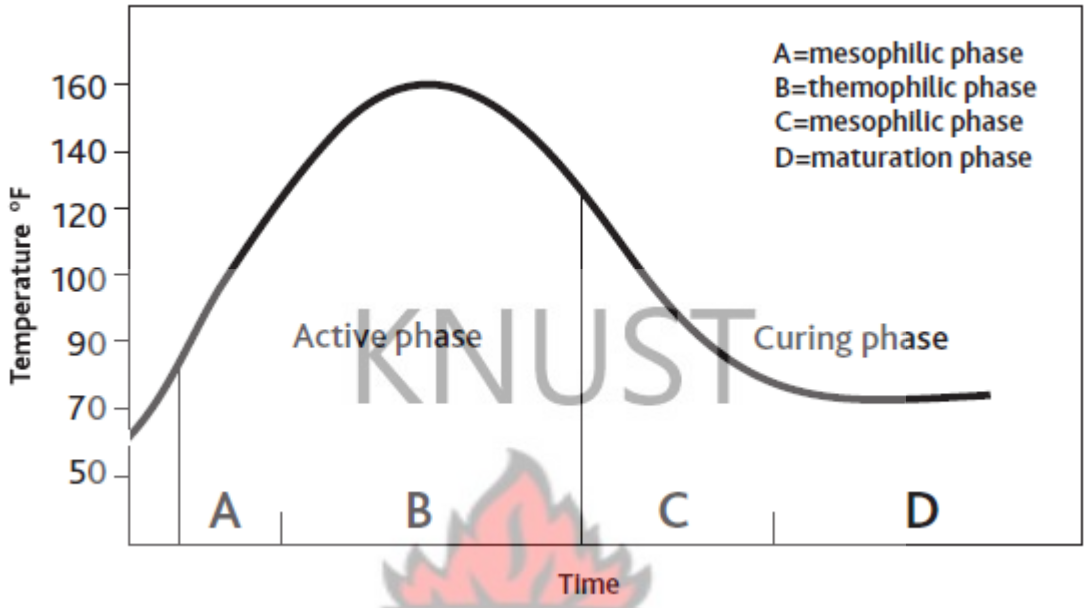
Source Christianson, E. (2007). Classification of *Amanita muscaria.*

Appendix 2. Life Cycle of Mushroom.



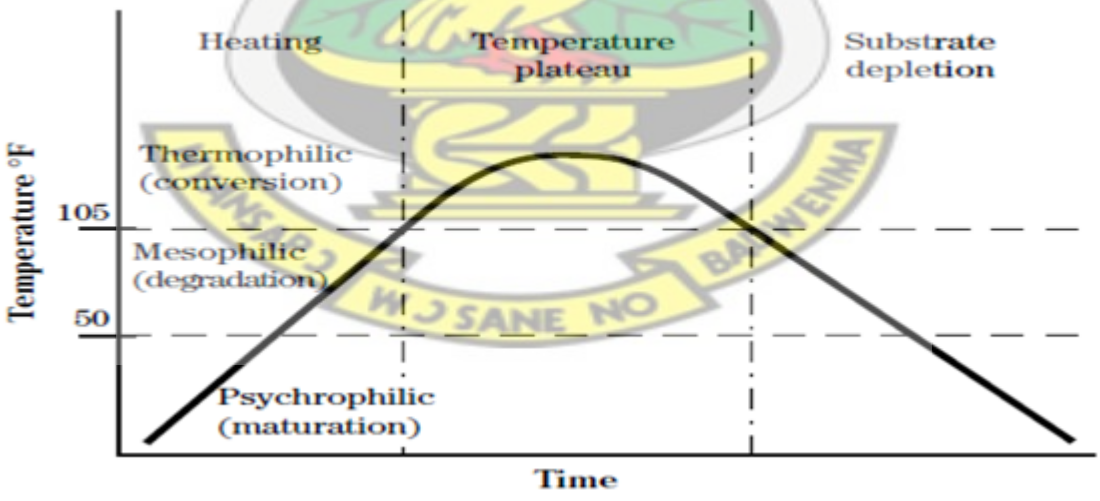
Source: Deacon, J. b. (2003). The microbial word; microorganisms and microbial activities, Institute of Cell and Molecular Biology, The University of Edinburgh.

Appendix 3. Temperature Phase of Composting



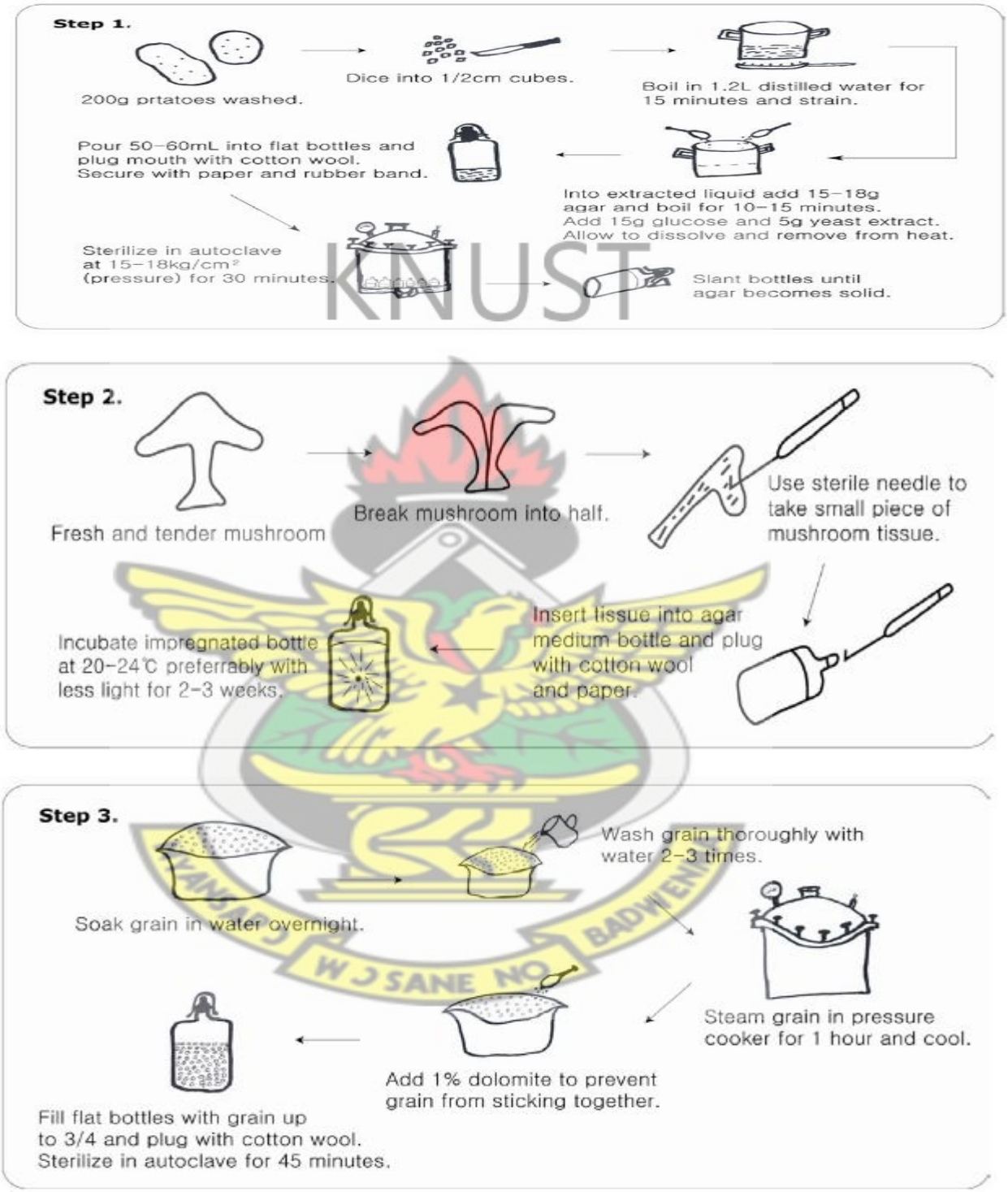
(With Reference from Chen, *et al* (2011), who quoted from Cooperband, L. (2002),The Art and Science of Composting, Center for Integrated Systems, University of Wisconsin. http://www.cias.wisc.edu/wpcontent/uploads/2008/07/artcompost.pdf.

Appendix 4. Composting Process and Compost Temperature Ranges



(Source: Graves, E. R. and Hattemer, G. M. (2000). Composting**,** Part 637 Environmental Engineering National Engineering Handbook, Natural Resources Conservation Service, United States Department of Agriculture, (210-VI-NEH)

Appendix 5. Spawn Production Process



(Source: Ogden, A., and Prowse, K. (2004). How to make oyster mushroom grain spawns in a simple way, Gourmet Woodland Mushrooms Ltd., U.K. retrieved from http://www.alohamedicinals.com/book1/chapter-4-1.pdf on 27/01/2014

Appendix 6. Rate of Mycelia Formation as Influenced by Substrate Types and Composting Time Interactions – First Trial

	Days after Spawning					
Substrates	5	10	15	20	25	30
Bean Straw only						
1 day	0.718	0.382	0.311	0.187	0.129	0.333
7 days	0.518	0.155	0.324	0.123	-0.007	-0.067
14 days	0.346	-0.015	-0.002	-0.011	-0.107	0.000
Coir + Bean straw						
1 day	0.307	0.066	1.394	1.007	0.173	0.433
7 days	0.215	0.089	0.643	1.119	0.675	0.231
14 days	0.431	0.033	0.273	2.169	0.167	0.300
Coconut Coir only						
1 day	0.322	0.031	0.153	2.073	0.253	0.533
7 days	0.271	0.045	0.115	1.676	0.593	0.627
14 days	0.355	0.031	0.560	1.940	0.207	0.387
S.e	0.077	0.057	0.199	0.254	0.281	0.155
$Lsd_{(0.05)}$	0.163	0.121	0.422	0.538	0.595	0.328
%CV	21.50	27.700	43.700	6.800	25.100	8.400

Where S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05, % CV – Percentage Co-efficient of Variation

Appendix 7. Rate of Mycelia Formation as Influenced by Substrate Types only – First Trial

	Days after Spawning					
Substrates	5	10	15	20	25	30
Bean straw only	0.527	0.174	0.211	0.099	0.005	0.089
Coconut coir + Bean straw	0.318	0.063	0.770	1.432	0.338	0.322
Coconut coir only	0.316	0.036	0.267	1.896	0.351	0.516
$S.e_{(0.05)}$	0.045	0.033	0.115	0.147	0.162	0.089
$Lsd_{(0.05)}$	0.094	0.070	0.244	0.310	0.344	0.190
%CV	21.500	27.700	43.700	6.800	25.100	8.400

Where S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05, % CV – Percentage Co-efficient of Variation

Appendix 8. Rate of Mycelia Formation as Influenced by Substrate Types and Composting Time Interactions – Second Trial

	Days after Spawning			
Substrates	5	10	15	20
Bean Straw only				
1 day	0.726	0.213	0.063	0.265
7 days	0.462	0.342	0.293	0.159
14 days	0.000	0.000	0.000	0.000
Coir + Bean straw				
1 day	0.564	1.113	1.089	0.867
7 days	0.904	1.367	0.641	0.831
14 days	0.848	1.258	0.781	0.858
Coconut Coir only				
1 day	0.662	0.989	0.893	0.983
7 days	0.726	0.975	0.932	0.783
14 days	0.509	0.993	0.74	0.911
S.e	0.130	0.055	0.176	0.231
Lsd	0.276	0.116	0.373	0.490
%CV	1.900	5.900	11.100	15.100

Where S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05, % CV – Percentage Co-efficient of Variation

Appendix 9. Rate of Mycelia Formation as Influenced by Substrate Types only – Second Trial

	Days after Spawning			
Substrates	5	10	15	20
Bean straw only	0.396	0.185	0.118	0.141
Coconut coir + Bean straw	0.772	1.246	0.837	0.852
Coconut coir only	0.632	0.986	0.855	0.892
S.e	0.075	0.032	0.102	0.134
$Lsd_{(0.05)}$	0.159	0.067	0.215	0.283
%CV	1.900	5.900	11.100	15.100

Where S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05; % CV – Percentage Co-efficient of Variation

Appendix 10. F Table for Analysis of Variance of Chemical Analysis as Influenced by Substrate Types and Composting Time

Variance	Carbon	Nitrogen	C:N	pH
Substrate type (ST)	**	**	**	**
Composting time (CT)	*	**	**	**
ST x CT	**	**	**	**
$S.e_{(0.05)}$ ST	0.997	0.011	0.878	0.040
$S.e_{(0.05)}$ CT	0.997	0.011	0.878	0.040
$S.e_{(0.05)}$ ST x CT	1.726	0.019	1.521	0.070
$LSD_{(0.05)}$ ST	2.113	0.023	1.861	0.086
$LSD_{(0.05)}$ CT	2.113	0.023	1.861	0.086
$LSD_{(0.05)}$ ST x CT	3.660	0.040	3.224	0.148
%CV	4.200	5.800	0.100	0.800

Where * - Significantly Different; ** - Highly Significantly Different; S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05; % CV –Percentage Co-efficient of Variation

Appendix 11. Yield of Mushrooms as Influenced by Substrate Types and Composting Time

Substrates	**Yield (g/kg)**	
	1st T	**2nd T**
Bean Straw		
1 day	5.5	0.0
7 days	0.0	0.0
14 days	0.0	0.0
Coir + Bean straw		
1 day	47.4	53.4
7 days	43.2	27.4
14 days	44.7	27.8
Coconut Coir		
1 day	6.5	5.0
14 days	10.5	7.4
21 days	11.0	9.6
S.e	6.58	3.54
Lsd	13.95	7.50
%CV	9.90	14.40

Appendix 12. F - Table for the Physical Parameters as Influenced by Substrate Types and Composting Time

Variance	Length of Stipe		Width of Stipe		Perimeter of Cap		Fruits Per Flush		Percentage Dry Matter	
	T 1	T2	T 1	T2	T 1	T2	T 1	T2	T 1	T2
Substrate type (ST)	*	*	*	*	*	*	*	*	*	*
Composting time (CT)	*	*	*	ns	*	ns	*	ns	*	*
ST x CT	*	ns	*	ns	*	*	*	ns	*	*
$S.e_{(0.05)}$ ST	0.25	0.21	0.06	0.04	1.01	0.46	0.59	0.23	1.03	0.37
$S.e_{(0.05)}$ CT	0.25	0.21	0.06	0.04	1.01	0.46	0.59	0.23	1.03	0.37
$S.e_{(0.05)}$ ST x CT	0.43	0.36	0.11	0.08	1.76	0.80	1.02	0.40	1.78	0.64
$LSD_{(0.05)}$ ST	0.53	0.44	0.14	0.09	2.15	0.98	1.25	0.50	2.18	0.78
$LSD_{(0.05)}$ CT	0.53	0.44	0.14	0.09	2.15	0.98	1.25	0.50	2.18	0.78
$LSD_{(0.05)}$ ST x CT	0.91	0.76	0.24	0.16	3.73	1.69	2.17	0.86	3.78	1.35
%CV	2.00	11.5	7.60	5.20	8.30	3.20	5.60	3.70	9.50	0.20

Where * - Significantly Different; ns- No Significantly Different; S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05; % CV –Percentage Co-efficient of Variation; T -Trials

Appendix 13. F – Table for Substrates Performance as Influenced by Substrate Types and Composting Time

Variance	% Moisture Content of Substrates		Number of Bags Fully Colonized (DAS)		1st primordia (DAC)	
	T 1	T2	T 1	T2	T 1	T2
Substrate type (ST)	*	*	*	*	*	*
Composting time (CT)	ns	ns	ns	ns	*	ns
ST x CT	ns	ns	*	ns	*	ns
$S.e_{(0.05)}$ ST	1.10	0.83	0.50	0.38	0.36	0.26
$S.e_{(0.05)}$ CT	1.10	0.83	0.50	0.38	0.36	0.26
$S.e_{(0.05)}$ ST x CT	1.90	1.44	0.86	0.65	0.63	0.46
$LSD_{(0.05)}$ ST	2.33	1.76	1.03	0.80	0.77	0.56
$LSD_{(0.05)}$ CT	2.33	1.76	1.03	0.80	0.77	0.56
$LSD_{(0.05)}$ ST x CT	4.03	3.05	1.83	1.38	1.33	0.97
%CV	1.50		6.10	2.60	7.4	3.10

Where * - Significantly Different; ns- No Significantly Different; S.e – Standard Error, $Lsd_{(0.05)}$ – Least Significant Difference at P = 0.05; % CV –Percentage Co-efficient of Variation; T – Trial

Appendix 14. Average Weather Conditions of the Experimental Site during the Research, 2014

Month	Temperature (^{o}C)	Precipitation (mm)
January	28	0
February	29	3
March	29	127
April	28	91
May	28	113
June	27	163
July	26	131
August	25	83
September	25	231
October	27	85
November	27	61
December	27	20

Source: AccuWeather (2014). Local Weather, Kumasi, Ghana. Accessed on 19/10/2015 from http://www.accuweather.com/en/gh/kumasi/176776/june-weather/176776?monyr=6/1/2014&view=table