UNIVERSITY OF CAPE COAST

THE EFFECT OF COMPOSTING SUBSTRATES ON THE GROWTH, YIELD AND NUTRIENT CONTENT OF THE OYSTER MUSHROOM, *PLEUROTUS OSTREATUS* (JACQ. EX FR.) KUMMER

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BY

DZIGBODI AHUMA

THESIS SUBMITTED TO THE DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF CAPE COAST IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF MASTER OF PHILOSOPHY DEGREE IN BOTANY

JANUARY 2010

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

…… ……...

DZIGBODI AHUMA DATE

(CANDIDATE)

Supervisors Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

……..…… ……..

DR. D. H. A. K. AMEWOWOR DATE

(PRINCIPAL SUPERVISOR)

…… ……

DR. JEMMY TAKRAMA DATE

(CO-SUPERVISOR)

ABSTRACT

The effect of composting substrates prepared from four agricultural wastes: corn cob, oil palm fruit fibre, rice husk and sawdust on growth, yield and nutrient content of oyster mushroom, *Pleurotus ostreatus,* (Jacq. ex Fr.) Kumer was investigated.

One set of the substrates was decomposed and the other set was not. The mother spawn, obtained from oyster mushroom fruit body tissues and spores from two sources and a spawn from a third source, cultured on Potato Dextrose Agar medium grew best at 30 °C and in total darkness. Sorghum grains gave the best growth as a multiplication medium. Spawns grew best on decomposed substrates than on undecomposed substrates. Decomposed substrates gave higher yield than the undecomposed substrates. Oil palm fruit fibre produced the highest yield, followed by corn cob, sawdust and rice husks in that order.

The decomposed substrates contained higher levels of nutrients than the undecomposed substrates. The types of substrate also determined the level of nutrients in the mature oyster mushroom. Thus, fat and protein levels were highest in mature fruit bodies obtained from oil palm fruit fibre while total carbohydrates were highest in fruit bodies obtained from rice husk. Insoluble lignin breakdown in all four agricultural wastes by *Pleurotus ostreatus* was highest in rice husks, 67.01 – 78.78 %; followed by sawdust, 39.70 – 50.93 %; oil palm fruit fibre, 30.85 – 35.11 %; and lowest in corn cob, 11.32 – 22.32 % while

breakdown of soluble lignin remained generally the same for all the four substrates (0.0757 – 0.0780 mg/g).

Use of agricultural wastes as substrates for oyster mushroom production can solve the problem of malnutrition and pollution of the environment.

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DEDICATION

To my dad, Mr. George Ahuma.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Mushroom is a word that may mean different things to different people and countries. According to Eger, Eden and Wissig (1976), mushroom is a macroscopic and edible fungus that grows on decaying organic matter known as substrates. They have distinctive fruiting body and are usually categorized as ascomycetes or basidiomycetes. Unlike vegetables, they do not rely on sunlight for growth. They start as spawns which grow in the substrate to produce a fine white fibrous structure called mycelium. From the mycelium, the mushroom fruit is produced and this part is what is harvested. Interestingly, mushrooms offer lucrative business opportunities and they do not require arable land for production. They provide diversification and benefits such as increased income, employment, food and security.

Taxonomically, more than 140,000 different varieties of mushrooms are found in nature (Lu, 2008). Yet, less than 200 of these are widely accepted as food while only 80 are cultivated commercially (Lu, 2008). An example of such edible cultivated mushroom is *Pleurotus ostreatus. Pleurotus ostreatus* also called Oyster mushroom is the second most important edible mushroom produced commercially in the world. In Korea, 65 % of the total domestic mushroom production is made up of *Pleurotus ostreatus* while the world's production constitutes about 25 % (Chang and Miles, 1989). Oyster mushroom (*Pleurotus* sp) cultivation has increased tremendously throughout the world during the last few decades (Chang, 1999 and

Royse, 2002). Empirical evidence has shown that 14.2 % of the total world's production of edible mushrooms is made up of the Oyster mushroom (Chang, 1999).

Cultivated Oyster mushrooms consist of many other different species which include: *Pleurotus sajor-caju*, *Pleurotus cystidiosus*, *Pleurotus cornucopiae*, *Pleurotus pulmonarius*, *Pleurotus tuber-regium*, *Pleurotus citrinopileatus and Pleurotus flabellatus* (Chang and Miles, 1989). *P. ostreatus* cultivation began in the USA in the early 1900's. Later, other species such as *Pleurotus sajor-caju* were cultivated in India in the late 1940's. To date, approximately 70 species of *Pleurotus* have been recorded; and more species are yet to be discovered (Chang and Hayes, 1978).

Pleurotus in Latin refers to the sideways-growth of the stalk with respect to the cap; whilst *ostreatus* refers to the oyster shape of the cap or its flavour resemblance to the oyster (Chang and Hayes, 1978). *P. ostreatus* belongs to: Kingdom Fungi; Phylum Basidiomycota; Class Homobasidiomycota; Order Agaricales; Family Trichomataceae; Genus *Pleurotus* and Species *ostreatus* (Chang and Hayes, 1978; Stamets, 2000). The tribe within its genus is *Agaricus*. Quelet (1886) proposed and separated the three genera: *Pleurotus, Lentinus* and *Panus* based on the anatomic features of their hymenophore's sterile tissues as in homogeneous taxonomic groups. Many years later, Hilber (1982) also recommended the crossing of monospore cultures as a valuable basis for *Pleurotus* studies and since then, *P. ostreatus* has been the most cultivated species among the oyster mushroom species.

Hundred years later, Singer (1986) studied and divided the genus *Pleurotus* into six sections (Sect.) and classified them as: Sect. *Lepiotarii* (Fr.) Pilat; Sect. *Calyptrati* Sing., Sect. *Pleurotus* Sing., Sect. *Coremiopleurotus* (Hilber); Sect.

Lentodiellum (Murr.) Sing. and Sect. *Tuberegium* Sing. As a result, *Pleurotus ostreatus* was placed in its Section, based on the absence of veil and the possession of a monomitic hyphal system. Species identification within the genus *Pleurotus* became difficult due to its morphological similarities and environmental effects.

For many reasons, members of the genus *Pleurotus* have been intensively studied in many different parts of the world; they have been found to have high gastronomic values. They are able to colonize and degrade a large variety of lignocellulosic residues, they require shorter growth time when compared with other mushrooms, they demand few environmental controls and their fruiting bodies are not very often attacked by diseases and pests and can be cultivated in a simple and cheap way (Jwanny, Rashad and Abdu, 1995).

Structurally, *P. ostreatus* has macroscopic features that include: pileus, context, lamellae and stipe; while its microscopic features include: spores, basidia, hymenophoral trama, pileipellis and cystidia. The pileus is 40-250 mm broad and oyster-shaped. It is smooth, oblong and convex. However, the margin becomes somewhat undulated and striated as it grows. It is variable in colour ranging from violet-brown to blackish lilac when young; then grayish lilac and later cream-beige (Breitenbach and Kranzlin, 1991; Donk, 1962; Imazeki and Hongo, 1987; Moser, 1983; Kornerup and Wanscher, 1983).

The context is grey-white in colour but sometimes white. It is thin to thick, fleshy; radially fibrous with a fungoid odour but mild in taste. The lamella on the other hand is long crowded and decurrent. It is sometimes cream or pale grayish with smooth edge which later becomes an undulating lamellulae (1-3 tiers). The stipe is 10-20×10-25 mm. It is rudimentary and usually lateral. It has several crescents surfaces which are longitudinally striated with whitish villose-pilose which makes

the context solid. The spores are cylindrical or cylindrical-ellipsoidal and 6.5-9.0×2.8-3.5 µm in size. They are smooth and hyaline and form a white to lilac-gray print on dark background. The spore prints are normally dingy grey or pale lilac grey and basidia mostly measure 23.6-27×5.0-7.5 µm. They are slenderly clavate, 4-spored and with a basal clamp connection. Hymenophoral trama are usually regular or irregular and monolithic in nature. Often, cystidia are absent; but if present, they are cystoids and are rarely seen. The pileipellis are usually irregular, densely interwoven, flexuous and branched hyphae. They are usually 2-4 µm across with brown pigment and somewhat gelatinized septa with clamp connections (Breitenbach and Kranzlin, 1991; Donk, 1962; Imazeki and Hongo, 1987; Moser, 1983; Kornerup and Wanscher, 1983).

Nutritionally, nutrients provided in the form of simple molecules obtained from the substrates on which it grows enhance the development and growth of *P. ostreatus*. The mycelia of *P. ostreatus* are made of hyphae which produce extracellular enzymes. These enzymes break down complex carbohydrates, lipids and proteins which are absorbed by the hyphae. Thus, they are heterotrophic with microscopic hyphae that penetrate the substrate (Stahl and Esser, 1976).

The success of every growing mushroom is very dependent on the quality of the substrate as it provides all the energy and nutrients the mushrooms uses while growing. Thus, different strains of mushroom require different substrate mixtures. Research has shown that there is great potential of using some lignocellulosic materials as raw materials for the production of *Pleurotus ostreatus* (Zandril, 1987); however, not every kind of lignocellulosic substances is likely to be used as substrate for *Pleurotus* cultivation. As a result, the main and co-substrate differ among countries and even regions on the basis of availability and cost (Oei, 1991).

The source of materials normally used as substrates for the cultivation of *P. ostreatus* is agricultural wastes. Examples includes cassava stalks, cocoa pods, coffee bean husks, coffee pulp, corn cobs, corn stubble, cotton seed cake, pulse husks, rice hulls, sawdust, sugarcane bagasse, tea leaves, tobacco stalks, wheat straw and water hyacinth.

The type and nature of lignocellulosic material is a growth factor; however, other factors also affect growth of mycelia. These are: carbon, nitrogen, temperature, pH and light. According to Chang and Miles (1989), carbon may be obtained from starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose, ethanol and lignin. Citrate, oxalate and other organic acids are compounds from which carbon can also be obtained. Its tolerance for CO_2 is rather strong most especially at carbon dioxide concentration of 15-20 %. It is only when the concentration of CO_2 is raised to 30 % that the growth of mycelium decreases rapidly.

During mycelial growth, nitrogen is utilized (Kang, 2004). The sources of the nitrogen include peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine, glycine and urea, unfortunately, urea gives very poor results (Chang and Quimio, 1982).

According to Stamets (1993), not only in the temperate zones of the world do oyster mushrooms grow, they also grow in the tropical and subtropical zones. The low temperature oyster mushrooms are cultivated between 12 °C and 20 °C whereas those described as high temperature *Pleurotus* are cultivated between 15 °C to 30 °C and at pH between 5.5 and 6.5. Many commercial mushrooms only fruit at around 20 °C and are therefore not suitable for tropical regions. However, Chang and Miles (1989) noted that development of fruit bodies of *P. ostreatus* required an optimum temperature range of 10 to 18 °C. Thus, Stamets (1993) and Kang (2004) have

proposed that growers chose a suitable strain for their own natural environment considering the fact that each *Pleurotus* species need different environmental conditions for fruit body development.

Generally, the reaction of oyster mushroom to visible and ultra-violet light are of three types: inductive, inhibitive and tropic (Cochrane, 1958). Inductive effect includes both absolute light requirements for initiation or maturation of reproductive structures and quantitative response such as an increase in reproductive structures upon illumination. However, exposure to light intensities greater than 50 lux during spawn run may inhibit primordial formation (Ishikawa, 1967). Therefore, an edible mushroom like *P. ostreatus* requires light for initiation and maturation of fruit body and it is positive phototropic (Cochrane, 1958).

In fruit body formation, CO_2, light and temperature are the key environmental factors. When CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06 %), the stipe elongates and the growth of the caps is prevented. Growth of mycelium of *P. ostreatus* does not need light. They are mostly cultivated in darkness; however, primordial formation and growth of fruit bodies require light (Ishikawa, 1967). Whereas the former requires light of 200 lux intensity for over 12 hours, the growth of the fruit bodies requires light of 50 - 500 lux intensity. The intensity of colour of the caps is directly related to the intensity of light; thus when light intensity is low, the colour is pale.

In sexual reproduction, the mating system is heterothallism (self-sterile). Sexual reproduction is governed by mating type genes. Mating type genes have bifactorial tetrapolar incompatible mating systems which have two unlinked mating type factor, designated factor A and B (Eugenio and Anderson, 1968). They function by preventing the mating between genetically identical cells. Factor A, controls

nuclear pairing, clamp cell formation, coordinate cell division and clamp cell septation while factor B is responsible for the control of nuclear migration, septa dissolution and clamp cell fusion. Two monokaryotic mycelia are compatible especially when they have different alleles at both loci (Terakawa, 1957).

Typically, the major event of the life cycle of *Pleurotus* is a single basidiospore which germinates to become a mass of homokaryotic hyphae, the mycelium with each cell containing a single haploid nucleus. The homokaryotic mycelium continues to grow until the hyphae fuse with another hyphae which have a compatible mating type. After fusion between the compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed Kim (2000); (Figure 1.)

Subsequently, conjugate division occurs in the two nuclei. The distribution is regular as they pair throughout the mycelium by clamp connections. Heterokaryotized mycelia with enough mycelia mass and appropriate environmental stimuli such as low temperature ranging between 10 – 21 ^{o}C, relative humidity of 85-90 %, light intensity ranging between 1000 and 2000 lux and $CO_{2,}$ < 1000 ppm then induce the formation of fruit bodies. During fruit body formation, nuclear fusion and meiosis occur only in the specialized basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each basidium has four monokaryotic basidiospores which later germinate to produce homokaryotic hyphae Kang (2004); (Figure 1).

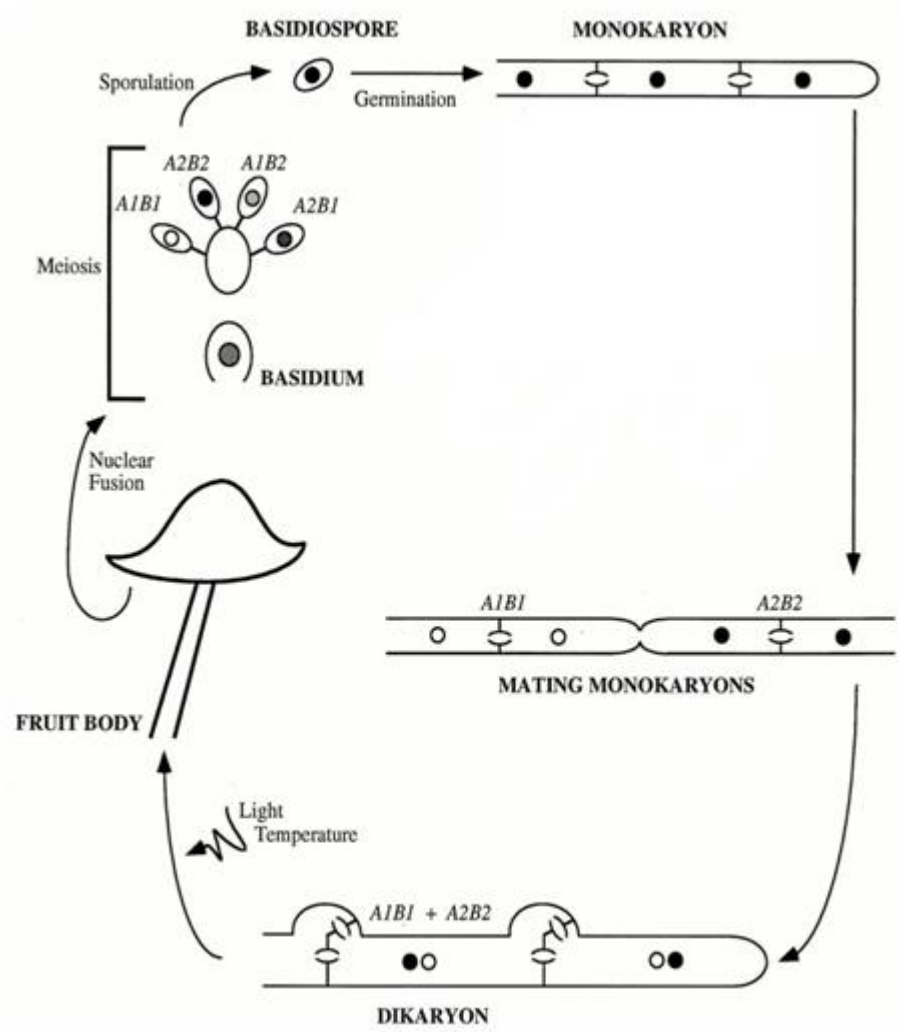


Figure 1: The life cycle of *Pleurotus ostreatus*

Conspicuously, there are three disadvantages that persist in the cultivation of *P. ostreatus*. First, it is perishable but presentable for the market for just a few days. Secondly, the spore load generated within the growing room is a potential health hazard to workers that are allergic to the spores. As a result, sporeless strains *P. ostreatus* with short gills and thick fleshy oyster mushrooms with prolonged storage are highly sought after by oyster mushroom growers. The third challenge is the constant battle growers would have to wage against the intrusion of sciurids and

phorids (mushroom flies) which to a very great extent are attracted to oyster mushroom than any other group of mushrooms because of its pleasant smell (Eger, 1978).

To a very large extent, *P. ostreatus* is attacked by various pathogens. *Chrysonilia sitophila* is the cause of red bread mould; *Cladobotryum apiculatum* is the cause of brown spot and white soft rot; *Cladobotryum dendroides* is the cause of cobweb disease, cobweb mould, mildew, soft decay, soft mildew and *Fusarium pallidoroseum* is the cause of *Pleurotus* wilt (Pacumbaba, Beyl, Pacumbaba Jr. and Wang, 2002). Some bacterial diseases that attack *P. ostreatus* include: brown blotch and mummy disease caused by *Pseudomonas aeruginosa*. Other infections show up as absent or disappearing of mycelium, deformed fruit bodies, and fruit discolouration that may appear greyish or brownish. Occasionally, it can be severe destroying the whole crop. Other diseases are: brown blotch, drippy gill and yellow blotch caused by *Pseudomonas agarici*; biovar, brown blotch caused by *Pseudomonas fluorescens* Biotype G (=Biovar V). Partitiviruses and totiviruses are also known viruses that attack oyster mushrooms; however, *Verticillium* is a known fungus that attacks *P. ostreatus* (Bessette, Kerrigan and Jordan, 1985). Other disease causing agents are rodents and nematodes. Notwithstanding, the disadvantages and the various pathogens and diseases that attack *P. ostreatus*, the advantages of cultivating oyster mushroom far outweigh the disadvantages. The advantages include: rapid mycelial growth, high ability for saprophytic colonization of substrates, simple and inexpensive cultivation techniques and several species available for cultivation under different climatic conditions.

Even though *P. ostreatus* has been reported to be inferior to animal protein (Kurtzman, 1976), the fresh weight proximate water content is between 85 and 95 %;

protein, 10.5 and 27.38 %; carbohydrate 46.6 and 81.6 % (Bano and Rajarathnam, 1982). In addition, its vitamin content is twice as high as that of vegetables with the exception of peas and other legumes (Manning, 1985). The dry weight proximate analysis however, is as follows: 3 % protein, 4 % carbohydrate, 0.1 % fat, 1 % minerals and vitamins (Tewari, 1986). It therefore serves as food low in calories, sodium, fat and cholesterol but rich in carbohydrate, fibre, riboflavin, niacin, thiamine, vitamin C, iron, calcium and phosphorous especially when dried (Anderson and Fellers, 1942). It is also comparable to a protein rich legume; hence, it serves as suitable food for diabetic patients as well as a substitute for meat for vegetarians (Manning, 1985). It was no wonder

that the Greeks, Chinese and Japanese described it as the “elixir of life.”

Not only do humans depend on mushrooms, but animals such as ruminants also depend on spent mushroom composts. Spent mushroom composts are used in improving the nutrition of ruminants by converting these materials into digestible rich protein substances; regardless of the heavy mycelial growth which consists of thick bundles of hyphae with chitinous cell wall as a major component (Flegg, Spencer and Woode, 1985). The spent compost is digested by their unique digestive system aided by the resident rumen microorganisms. These organisms decompose cellulose and hemicelluloses and so the final products are volatile acids like acetic acid, propionic acid and butyric acid.

The consumption of oyster mushrooms is known to have health benefits. They are therefore perceived by many as special substances eaten to regularize both blood cholesterol and sugar blood levels. Subsequently, the mushroom described by many as a good source of statin drugs although they are among the few types of mushrooms for example beef stick known for their carnivorous nature (Kues and Liu,

2000). They have mycelia that enable the killing and digestion of nematodes to obtain nitrogen. Normally, droplets from the mushroom paralyze and kill the nematode leaving the paralyzed victims located in the specialized directional hyphae, while, the cuticle is penetrated and the contents digested. Therefore, they can be used as nematicides or used as other forms of biological control for other pests and plant diseases.

Other uses of the oyster mushrooms include its use as a research tool in specific areas in biochemistry, molecular biology, pharmacology, paper pulp bleaching and cosmetology industries (Arias, Ramirez and Leal, 2000). An example is the use of cellulases of oyster mushroom for the production of single cell proteins in biotechnology.

Even though, cultivating mushroom is said to be labour intensive; it provides opportunities for landless people, collectors and sellers of mushrooms who are usually small and marginal farmers to improve their livelihood and sustainable income.

Asian countries have technologically developed the use of cellulose or lignocellulosic wastes by using rice straw as a substrate for oyster mushroom cultivation. Through this, they have demonstrated a practical way of transforming lignocellulosic wastes directly into a highly acceptable food for human consumption. Another specific example is an estimated 30,000 tons of cotton waste produced in Hong Kong per year serving as substrate in producing approximately an equal weight of fresh mushrooms (Chang and Miles, 1989).

In most African countries including Ghana, about 60 % of the population live in villages and mainly engage in agricultural and allied activities. Thus in practical terms, oyster mushroom cultivation has the advantages of rural employment

generation and gainful utilization of natural and farm resources together with large quantities of renewable lignocellulosic residues generated every year as a result of extensive agriculture practices. To solve the nuisance created by these piles of agricultural wastes, oyster mushroom farming is the solution. The reason is that *P. ostreatus* like other mushrooms are gregarious in nature. They thrive mostly on wood and wood by-products including sawdust, corn cobs, banana fronds, sugar cane bagasse, palm fruit fibres, rice husk and straws, water hyacinth leaves among others (Quimio, 1986). They are found in clusters on dead broad-leaf of hardwoods (oaks, alders, maples, aspens, ash, beech, birch, elm, willows and poplars) but not on conifers. They are also found in parks and alongside walks and so are said to exhibit primary saprophytism. Sometimes, they may behave as a facultative parasite at the least opportunity (Singer, 1986).

Mushrooms and for that matter *P. ostreatus*, are known to have the ability of transforming agricultural wastes into highly acceptable nutritious food for animals. This should be explored to cut down on the burning of agricultural wastes that pollute the environment.

Empirical evidence has it that *P. ostreatus* has the ability to biodegrade lignin; a complex polymer of phenylpropane units which are cross-linked to each other with a variety of different chemical bonds. The complexity associated with lignin has been far proven as resistant to detailed biochemical characterization which has by large extent, greatly obstructed the understanding of the effects of microbes. Nonetheless, some organisms, particularly fungi, have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Kirk and Farrell, 1987). Similarly, Actinomycetes have also been proven to decompose lignin;

but typically degrade less than 20 percent of the total lignin present (Crawford, 1986; Basaglia, Concheri, Cardinalis, Pasti-Grig and Nuti, 1992). Lignin degradation is primarily an aerobic process and in an anaerobic environment, it can persist for very long periods (Van Soest, 1994).

Lignin is usually described as the most resistant portion of the cell wall. Higher proportions of lignin correspond with lower bioavailability of the substrate. Hence, the effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available for enzymatic penetration and activity (Haug, 1993). Despite its resistant nature, oyster mushroom has the ability to degrade ligninocellulosic materials. Thus, oyster mushroom has the ability to eliminate xenobiotic pollutants such as pentachlorophenol (PCP), dioxin, polycyclic aromatic hydrocarbons (PAHs). They may as well be used as an environmental bioremediation, bioconversion and industrial mycoremediation agent (Kubatova *et al*., 2001) and (Hirano, Honda, Watanabe and Kuwahara, 2000).

Today, much attention is being given to the possible use of micro-organisms to convert relatively high-quality biomass from the abundant agricultural wastes to a more valuable use. For instance, the use of white rot fungi and mushrooms to break down the lignocellulosic content in plant wastes despite the variation in the structure and the nature of the by-products that may be available for utilization by micro-organisms. For that reason, a number of factors are taken into consideration before embarking on the use of organic residue or high-quality biomass material for microbial conversion to other substances. For example: (i) Is there a ready and continuous supply of the raw product to be converted? (ii) If the material is removed from cropland or forests, will this contribute to soil erosion and depletion of plant

nutrients? (iii) Are expensive equipment and large amounts of capital necessary for the processing? (iv) Are external energy supply and large amounts of water necessary? and (v) Which microorganism possess potentials for the bioconversion of the organic material under consideration?

Thus, subsequent consideration should be given to natural conditions of the indigenous microbial flora as well as the dynamics of biomass undergoing interaction with reference to the transformation of organic matter. Only in a few cases can any one species or genus be given a sole credit for natural bioconversions. For instance, mixed cultures of micro-organisms are usually involved in the transformation of organic residues; there are also cases where pure cultures of bacteria, yeasts, moulds, or enzyme preparations may be used for processing such materials.

Many studies have shown that biodegradability of lignocellulosic materials can be enhanced by pre-treating with acid (Grethelin, 1985) or alkali (Jackson, 1977; Van Soest, 1994) ammonia or urea (Basaglia, Concheri, Cardinalis, Pasti-Grigsby and Nuti, 1992; Van Soest, 1994). Other treatments such as physical grinding and milling (Ladisch, Lin, Voloch and Tsao, 1983; Fahey, Bourguis, Titgimeyer and Atwell, 1992) for fungal degradation and steam explosion had been observed (Sawada, Nakamura and Kobayashi, 1995) in addition to combining alkali and heat treatment (Gossett *et al*., 1976; Gharpuray, Lee and Fan, 1983). In the end it was found that those treatments which exposed specific surface areas were most effective in increasing enzymatic hydrolysis.

Though pretreatment is possible, it is on the contrary uneconomical, more especially when considered as a separate process in compost feedstock preparation. However, in some cases it may be incorporated in other pre-processing operations at little additional cost. Many lignocellulose ingredients in composting serve dual roles

as energy sources as well as enhancing porosity of the compost (Latham, 1979). Thus, they may reduce the effect of treatments that are percolative and may also result in pore size distribution that may be counterproductive to maintaining an aerobic process.

While delignification appears to be significantly possible during aerobic composting, decomposition however is likely to be affected by a number of factors especially when using white-rot fungi. The factors responsible include adequate nitrogen, moisture, and temperature. All these factors are important in encouraging lignin decomposition as does the composition of the lignocellulosic substrate itself. As an assimilator of lignin, oyster mushroom, is normally the last member of the fungal succession to develop on decomposing plant remains. oyster mushroom decomposes organic matter as compared to other microorganisms in the ecosystem, thus, oyster mushroom farmers have evolved a process to prepare its food carefully. This process is accomplished so that growth is undisturbed by competitors that grow faster and which may result in the crowding out of the oyster mushroom.

Significantly so is the variability in the rate of lignin decomposition in an aerobic system (Lynch and Wood, 1985). They stated that little lignin degradation occurred during composting; (Iiyama, Lam, Stone, Perrin and Macauley, 1995) and used the constant breakdown of lignin by mushroom as the basis for their calculations of polysaccharide degradation.

To confirm this, Hammouda and Adams (1989) measured lignin degradation ranging from 17 to 53 % in grass, hay and straw during 100 days of composting, while Tomati, Galli, Pasetti and Volterra, (1995) measured a 70 % reduction in the lignin content of olive waste compost after 23 days under high moisture (65-83 %) and thermophilic conditions. After the initial high decomposition rate under

thermophilic conditions, Tomati, Galli, Pasetti and Volterra, (1995) observed there was no further reduction in lignin content during the subsequent 67 days under mesophilic conditions. Later, in a laboratory incubation study, Horwath, Elliot and Churchill, (1995) recorded 25 % lignin degradation during mesophilic composting and 39 % during thermophilic composting of grass straw during a 45 day experiment.

The ratio of carbon to nitrogen is also an important factor to consider in the propagation of mushrooms. The approximate requirement at the time of filling the bags or trays is 20:1. Component of carbon are obtained from straw while that of nitrogen from manure. Cotton seed meal and castor bean meal contain both elements. Gypsum on the other hand is a useful ingredient as it provides calcium to the growing mushrooms and regulates the acidity level of the substrate and counters potassium, magnesium and phosphorus concentration and increases water holding capacity thus decreasing the risk of over wetting. It also improves the physical structure of the substrate.

In Ghana and other countries, the main common substrate for the production of mushroom is sawdust. Sawdust is a mixture of shavings from trees. The type of tree and the amount of lignin present determines the growth of the spawn. Other farm by-products that are also available for mushroom production are corn cob and stalks and palm cones and fibres. During the main harvesting period, these farm by-products are in abundance and farmers dispose of them by burning (Mondal, Rehana, Noman and Adhikary, 2010). These farm by-products in the grounded or chopped form can support the growth of oyster mushroom hence serve as a cheap source of substrate for mushroom growers. The grounded form of corn cob is very firm and retains good amount of moisture to make it a plausible alternative to sawdust (Buah, Vander Puije, Bediako, Abole and Showemimo, 2010).

An experiment conducted on palm cones and corn cobs in various proportions with a view to determine the cheapest substrate with best yield performance showed that mycelium running rate (MRR) in spawn bags ranged from 0.6132 to 0.8604 cm/day. The highest mycelium running rate was observed on corn cobs and palm cones (1:1) which was statistically similar to corn cobs and palm cones (1:3). The presence of the right proportion of alphacellulose, hemicellulose and lignin was the probable cause of higher rate of mycelium running in corn cobs and palm cones. Also suitable is C: N ratio which may be responsible for the higher mycelia growth in corn cobs and palm cones. The appreciable days to complete mycelium running of oyster mushroom on different substrates might be due to variation in their chemical composition and C: N ratio (Bhatti, Mir and Saddiq, 1987).

Poppe (1973) reported that Indole Acetic Acid (IAA) increased the number of fruiting bodies of oyster mushroom. Among three flushes of *P. ostreatus*, the highest stalk length (3.90 cm) was found in sawdust of first flush and the lowest (1.91 cm) was recorded in palm cones of third flush. Oyster mushroom *(Pleurotus ostreatus)* quality he concluded depended on the length of stalk, thus, the higher the stalk length, the poorer the quality of the mushroom (Zadrazil, 1987). Other researchers (Laborde, Lanzi, Francescutti and Giordani, 1993; Sangwan and Saini, 1995; Ragunathan and Swaminathan, 2003) held the view that using varied substrate media for the cultivation of mushrooms results in different yields because of the biological and chemical differences between the substrate medium and genotype of the cultured mushroom. Comparatively, Soyabean stalk (SS) substrate medium was found to be the most convenient culture medium for productivity of *P. ostreatus* (Poppe, 1973). The protein content of mushrooms also depended on the substrate medium due to biological, chemical differences and the C/N ratio of growth media. Again, *Pleurotus*

ostreatus was cultivated on different agro wastes like soybean straw, paddy straw, wheat straw alone and in combination of 1:1 ratio by (Akindahunsi and Oyetayo (2006) as Sangwan and Saini, (1995); Ragunathan and Swaminathan, (2003) experimented.

Maximum yield of *P. ostreatus* was recorded on soybean straw. Maximum protein, fat, ash, P, K and Na content was recorded when *P. ostreatus* was cultivated on soybean straw alone whereas maximum Ca and Fe content was recorded when *P. ostreatus* was cultivated on combination of soybean and paddy straw. The amino acid profile showed *P. ostreatus* proteins are rich in glutamic acid, aspartic acid and lysine content whereas vitamin C and folic acid were also recorded. Biochemical changes take place in the substrates because of the mushroom growth. A decrease in cellulose, hemicellulose, crude fibre, carbohydrate lignin and tannin content was observed, while an increase in protein, ash and mineral content in spent straw was recorded.

The practice of mushroom cultivation not only produces a nutritious food but also improves the straw quality. The reduction in the lignin, cellulose, hemi cellulose, tannin and crude fibre content of straw makes it ideal as animal feed (Ortega, Martinez, Betancourt, Gonzalez and Otero, 1992). The spent straw contains a large quantity of N, P, K and can be used as manure (Maher, 1991). Although *Pleurotus* species can easily be cultivated on different agro wastes, they yield potential varieties with the substrate used and a large quantity of biomass is produced after cultivation of soybean. Use of protein rich soybean agro-waste in combination with cereal agro-waste can help to increase the yield of *P. ostreatus.* Thus, it has been reported that not only the protein content of the substrate is the only factor but

also the nature of protein in the substrate influences the protein content of the fruiting bodies (Wang, Sakoda and Suzuki, 2001).

The fat content on dry wt basis ranged between 2.56 % to 2.82 %. This range of fats is lower than that of earlier report (Wang, Sakoda and Suzuki, 2001) instead; much depends on the nature of substrate. The moisture content of *P. ostreatus* ranged from 88.51 % to 89.88 % confirming high moisture content of the fruiting bodies (Manzi, Gambelli, Marconi, Vivanti and Pizzoferrato, 1999). The moisture content of *P. ostreatus* grown on different substrates was found to be significant which shows the moisture content is independent of the type of substrate and is associated with mushroom species. The mixture of wheat and paddy straw produced the maximum moisture (89.88 %) and total carbohydrate (56.20 %) in three different *Pleurotus* sp (Akindahunsi and Oyetayo (2006). The report also had it that the range of soluble carbohydrates (44.44 ±0.02% and 51.71 ±0.03%). Maximum ash content of *P. ostreatus* was recorded when grown on soybean straw (6.70 %) and was followed by soybean and paddy straw (6.42 %). The crude fibre (%) was maximum when *P. ostreatus* was grown on paddy straw (7.70 %) followed by soybean and paddy straw (7.68 %) and minimum was recorded on soybean straw alone (7.15 %). Bonatti, Karnopp, Soares and Furlan, (2004) reported similar values for ash and moisture content.

The mineral content of *P. ostreatus* harvested varied with different substrates and their combinations. The highest Ca content was recorded when mushroom was cultivated on combination of soybean and paddy straw (330 mg/100 g). The Ca content reported on combination of wheat and paddy straw (240 mg/100 g) was least. Akindahunsi and Oyetayo (2006) reported that the calcium content of *P. tuber-regium* as 2.9 mg/g in pileus and 1.2 ± 0.2 mg/g in stipe. Syed, Kadam, Mane, Patil and Baig (2009) reported similar results in *P. florida*. The nutrient content of

mushroom varies according to the substrate composition (Patrabansh and Madan, 1997). Maximum phosphorus content of 1000 mg/100g was recorded on soybeanstraw. *P. ostreatus* is high in P content, therefore can contribute to human nutrition as good source of phosphorus (Çağlarırmak, 2007). Sodium concentration of *P. ostreatus* varied significantly with different substrates. The highestsodium concentration was recorded on soybeanstraw (310 mg/100 g) and minimum was obtained on combination of Soybean and wheat straw (260 mg/100 g). The reported value agreed with earlier reports (Mattila, Kanko, Earola, Pihlava, Astola and Vahterist, 2001). Potassium (K) content was higher compared to other minerals in *P. ostreatus*.

Ghana has a significant percentage of farmers who do not have the requisite skill to go into mushroom cultivation; more especially as the spawns are quite difficult to produce. For this reason, Ghana Export Promotion Council in collaboration with the Food Research Institute, Accra introduced mushroom production on a commercial scale. The National Mushroom Development Project (NMDP) was established to make mushroom spawns readily available to farmers and run training courses for commercial growers. Unfortunately, their efforts have not yielded the anticipated results and thus needs to be complemented.

There is wide spread of protein malnutrition in sub-Saharan Africa including Ghana. Among these are the inadequate inclusion of fish, meat and meat supplements in the diet of most individuals and families. Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran and Ramabadran, 2000).

Currently, high prices of fuel have caused an increase in food prices and food scarcity in many countries (World Bank, 2008). To alleviate hunger and malnutrition in a world of rising food prices, cultivation of *P. ostreatus* is a very reliable and profitable option as a substitute to prevent low resistance to infectious diseases.

It is therefore necessary to support services and training that leads to making the commercial production mushrooms possible and easier. It is only when the cultivation of oyster mushroom and other local mushrooms are promoted would the problems of low income, environmental pollution and protein malnutrition be solved.

General objective

The general objective is:

- To explore and promote the maximum use of agricultural wastes through *Pleurotus ostreatus* cultivation to reduce environmental pollution and malnutrition.

Specific objectives

The specific objectives of this study include; to determine the:

1. Optimum environmental conditions appropriate for the production of *Pleurotus ostreatus* spawn.
2. Genetic differences in *Pleurotus ostreatus* collected from different sources.
3. Type of substrate appropriate for *Pleurotus ostreatus* cultivation.
4. Percentage of nutrients in different types of substrate.
5. Effect of the various agricultural wastes on the growth, yield and nutrient content of *Pleurotus ostreatus*.

6. Extent of lignin breakdown in various agricultural wastes by *Pleurotus ostreatus.*

CHAPTER TWO

MATERIALS AND GENERAL METHODS

Materials

Fruit bodies of *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer (Oyster mushrooms)

Fresh Oyster mushroom fruit bodies were obtained from the Technology Village of the School of Agriculture Farm, University of Cape Coast (UCC), Cape Coast and from Madam Christiana Boahene, a commercial mushroom farmer at Dzorwulu, Accra.

Oyster mushroom spawn

Spawn of Oyster mushroom was obtained from the Food Research Institute of the Council for Scientific and Industrial Research (CSIR), Accra.

Compost bags

Autoclavable transparent polyethylene compost bags, aluminium foil and PVC pipes (2.5 cm diameter x 2.5 cm length) used as necks were obtained from the Technology Village of the School of Agriculture, University of Cape Coast, Cape Coast.

Bottles for multiplying spawns

Heinz salad cream bottles of 250 ml capacity used for multiplying the mother spawns were obtained from the Bottle Market at Kotokuraba, Cape Coast.

Grains of sorghum

Grains of sorghum were obtained from the Nima market, Accra.

Tubers of Irish potato

Irish potatoes were obtained from the Kotokuraba Market, Cape Coast.

Cotton waste

Cotton waste used for plugging compost bags were obtained from Dr. D.H.A.K. Amewowor, Department of Molecular Biology and Biotechnology, School of Biological Sciences, University of Cape Coast.

Substrates (Undecomposed)

Rice husk

Dry rice husks were obtained from the rice mill of the Kpong Irrigation Project, Asutuare in the Eastern Region of Ghana.

Sawdust

Sawdust was obtained from the Africana Sawmill, Pedu and Apewosika Sawmill, Apewosika, all in Cape Coast.

Oil palm fruit fibre and corn cob

Oil palm fruit fibre and corn cob were obtained from Domeabra near the Jukwa Market in the Central Region of Ghana.

The substrates were sun-dried (Plate 1) for five days and stored in woven sacs until they were required. The corn cobs were milled before drying.

x1/8

Plate 1: Sun-drying of compost substrates

A. Palm fruit fibre **B. Corn cob (milled)**

C. Rice husk **D. Sawdust**

Chemicals

Lime ($CaCO_3$) and gypsum salt ($CaSO_4.2H_2O$) were obtained from Messiah Enterprise, Kotokuraba in Cape Coast and Dr. D.H.A.K. Amewowor of the Molecular Biology and Biotechnology Department of the University of Cape Coast.

All chemicals used in the preparation of the media, composts and proximate analysis were either of the AnalaR or of the general purpose grade. They were obtained from the stores of the School of Biological Sciences of the University of Cape Coast, Cape Coast.

General Methods

Methods of sterilization

All glassware, forceps, scalpels, cork borers were washed with liquid soap and rinsed in several changes of tap water, dried and wrapped in baking sheets, autoclaved at 121 ^oC for 15 minutes. They were heated in an SPF- 450 electric oven at 50 ^oC for 3 hours to vaporize all condensed water and then allowed to cool before use. Inoculation needles were heated in a spirit lamp flame until red-hot, air-cooled before and after use.

The inoculation chamber including the working bench was sprayed heavily with methylated spirit one hour before use. The spraying was repeated and the working bench mopped with cotton wool soaked in a methylated spirit after use.

Preparation of Potato Dextrose Agar (PDA)

A quantity of 200 g of peeled and washed Irish potato was cut into cubes and cooked in 700 ml of distilled water until soft. The fluid was strained into a beaker and the filtrate made up to 1000 ml with distilled water. Twenty grams of glucose

and 20 g of agar were added to the filtrate and heated in a water bath to melt the agar and swirled frequently to obtain a thoroughly mixed mixture and then distributed into five 300 ml conical flasks each holding 200 ml of the mixture. The flasks were plugged with non-absorbent cotton wool covered with aluminium foil and autoclaved at 121 ^{o}C for 15 minutes and stored until ready to be used.

Field collection of fresh oyster mushroom fruits

Fresh fruit bodies of *Pleurotus ostreatus* (Plate 2) were kept in transparent plastic bags and were brought to the laboratory for storage under humid condition until used.

Spore print of *P. ostreatus*

The spore prints of fruiting bodies collected from both the Dzorwulu Farm, Accra and School of Agricultures Technology village, UCC, were obtained by placing the pileus of the basidiocarps of fruit bodies, with the gill-side facing downwards, on clean plain A4 white sheet of paper for 24 hours (Plate 3). After 24 hours, the basidiocarps were removed and the spore prints labelled and stored in a refrigerator at 4 ^{o}C until they were required (Plate 3).

x1/3

Plate 2: A basidiocarp of *P. ostreatus*

x1/3

Plate 3: Spore print of *P. ostreatus*

Production of mother spawns from spores and tissues of basidiocarps

Spore culture

Spores from the spore print were aseptically transferred onto Petri plates of PDA and incubated at 30 ± 2 ^{o}C for 4 to 7 days. Subcultures were made until pure cultures were obtained.

Tissue culture

Fresh large healthy fruiting bodies of *P. ostreatus* were divided vertically into two parts from the stalk and small pieces of tissue aseptically transferred onto PDA plates and incubated at 30 ± 2 ^{o}C for 4 to 7 days, sub- cultured until pure cultures were obtained. Plates of the pure culture, the mother spawn was stored at 4 ^{o}C in the refrigerator.

Maintenance of pure cultures of the mother mushroom spawns

Pure cultures of the mother spawn on slants of PDA medium were stored at 4 ^{o}C in the refrigerator.

Determination of the effect of temperature on the vegetative growth of *P. ostreatus*

One centimeter disc of PDA bearing mycelium obtained from spores were asceptically placed on Petri plates of PDA and incubated at 15 °C, 20 °C, 25 °C, 30 °C, 35°C and 40 °C. The diameter of the colony was measured at 2-day intervals for 14 days. There were ten replicate plates.

The procedure described for cultures obtained from spores was repeated using mycelium obtained from tissues of the basidiocarp.

Determination of the effect of light on the vegetative growth of *P. ostreatus*

One centimeter disc of mycelium obtained from spores and tissues of basidiocarps were placed on Petri plates of PDA and incubated under different light intensities ranging from total darkness of 0 lux, total light of 28.7 lux, partial darkness of 1.9 lux and 12 hours light of 40.2 lux and 12 hours darkness at room temperature of 30 ± 2 °C for 14 days. The colony radii were measured at 3-day intervals for 14 days. There were 10 replicate plates for each of the light intensities.

Hybridizing test of spores culture of *P. ostreatus*

Hybridization of cultures of *P. ostreatus* obtained from spores and tissues from different sources was done by inoculating two of any of the cultures close to each other on the same PDA Petri plate and incubated at 30 ± 2 °C for 8 days.

Preparation of sorghum grain medium

Eight hundred grams of red sorghum grains were washed thoroughly and soaked in 1000 ml of water for 20 minutes. The seeds were drained and washed and boiled in 2000 ml of distilled water for another 20 minutes until they expanded but were not quite broken. The water was drained off and the boiled seeds allowed cooling. A precipitated mixture of chalk lime ($CaCO_3$) and gypsum salt ($CaSO_4.2H_2O$) in a ratio of 1:4 respectively was mixed with the seeds. 250 ml Heinz salad cream bottles which served as culturing bottles were packed loosely to 2/3 full v/v with the prepared seeds, plugged tightly with non-absorbent cotton wool, covered with aluminium foil and then autoclaved at 121 °C for 1 hour. After autoclaving, the grains were allowed to cool and the pH as well as the moisture content of the grains determined after which the grains were inoculated.

Inoculation of the grain medium

Three of 1.0 cm discs of 14-day old culture of the mycelia obtained from each of the three sources were inoculated separately onto the grain media. The radial growth and mycelial density of the mushroom were measured and recorded. However, uninoculated sterile sorghum served as control.

Proximate Analysis of Samples Before Inoculation

Determination of water content of compost substrate samples

Two grams each of sawdust, corn cobs, rice husks and oil palm fruit fibres were separately placed in a crucible and heated at 105 ^{o}C for 24 hours in an electric oven. Intermittently, the samples were removed, cooled and weighed until a constant weight was obtained. Finally the samples were removed from the oven and cooled in a desiccator after which it was reweighed. The % moisture content was then calculated using the formula:

$$\%\ \text{moisture content} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1 = Weight of sample before drying

W_2 = Weight of sample after drying

Determination of ash content of compost substrate samples

Two grams each of sawdust, corn cobs, rice husks and oil palm fruit fibres was separately weighed into a crucible. The samples were then ignited in a muffled furnace at a temperature of 600 °C for 24 hours until a constant weight of residue was obtained. The sample was then cooled in a desiccator and reweighed. The % ash content was calculated using the formula:

$$\% \text{ ash content } = \frac{\text{Weight of ash}}{\text{Weight of compost sample}} \times 100$$

Determination of water absorption capacity of compost substrate samples

Two grams of each sample were put into a crucible and 10 ml of distilled water added. Intermittently, the crucibles were weighed until constant weights were obtained. When constant weights were finally reached, the excess water was discarded leaving each sample in the crucible. It was reweighed. There were three replicate determinations for each substrate. The % absorption capacities were calculated using the formula:

$$\% \text{ absorption capacity } = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1 = Weight of compost sample before wetting

W_2 = Weight of compost sample after wetting

Determination of the fat content of compost in substrates samples

Sample of the compost substrate, weighing 0.2 g was placed into an extraction thimble and stopper with cotton wool. The thimble was then put into an extractor with a weighed clean dry soxhlet flask attached to the bottom. One hundred millilitres of petroleum ether with boiling point of 40 °C - 60 °C was poured into the extractor bottle. The heating mantle was switched on to boil the petroleum ether. After 5 hours, the flask was removed and put in an oven at 60 °C for the ether to vaporize leaving the fat. The flask was put into a desiccator for it to cool and reweighed. There were three replicate determinations for each substrate. The % fat content was calculated using the formula:

$$\% \text{ oil } = \frac{W_3 - W_2}{W_1} \times 100$$

Where: W_1 = weight of sample

W_2 = weight of flask

W_3 = weight of flask and oil

Determination of the protein content of compost in substrate samples

Sample of the compost substrate, weighing 0.2 g was put in a 50 ml Kjeldahl flask, 4.4 ml of digestion mixture was added and gently heated till the mixture began to boil. After the reaction had subsided, the flask was reheated until the liquid cleared. It was then left to cool for 30 minutes. An aliquot of the digest was transferred into a 100 ml volumetric flask and made up to 100 ml with distilled water. 20 ml of the solution was put into a Kjeldahl flask and 12 ml of an alkali mixture added. The content of the flask was distilled and the distillate collected into a receiver. Boric acid indicator solution was added after which the solution was titrated against HCl. The addition of the Boric acid indicator changed the solution to green. At end point, the colour changed from green to pink. There were also three replicate determinations. The titre value was substituted into the equation (1) to obtain the percentage of nitrogen whose value was then used to calculate the percentage of protein. A blank determination was also carried out using cane sugar instead of the substrate samples. The procedure was repeated with each of the compost substrates and the mean value calculated using the formula:

$$\% \text{ N}_2 = \frac{\text{Titre value } \times \text{Vol of Solution}}{100 \times \text{Aliquot vol} \times \text{wt of empty sample}} \times 100 \text{ ..... (1)}$$

$$\% \text{ Protein} = \%\text{N}_2 \times 6.25 \text{ .... (2)}$$

Determination of the soluble carbohydrate content of compost in substrate samples

An amount of 0.2 g air-dry ground sample was weighed into a 100 ml conical flask. About 30 ml water was added. A glass bubble was placed in the neck and simmered gently on a hot plate for 2 hours but was periodically topped up to 30 ml. It was allowed to cool slightly and then filtered through a No. 44 Whatman filter paper into a 50 ml volumetric flask. The filter paper was washed and diluted to the 50 ml mark of the volume of the flask when cooled. The same was done for the blank.

There was colour development from yellow to green; 2 ml of each standard was pipetted into a set of boiling tubes. From that point onwards, both standard and samples were treated the same way. Ten millilitres of anthrone reagent was rapidly added and mixed with the tube immersed in an ice bath.

The tubes were then placed in a beaker of boiling water in a darkened fume cupboard and boiled for 10 minutes. The tubes were place in cold water and allowed to cool in the dark. The optical density was determined at 625 nm. A standard graph was plotted using values obtained using known concentrates of glucose (carbohydrate). From the standard graph, the soluble carbohydrate content was determined. The blank was also determined the same way

Thus, if C = mg glucose obtained from the graph then for the substrate samples,

$$\%\ \text{Soluble Carbohydrate} = \frac{\text{C(mg)} \times \text{Extract Vol (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample wt (g)}}$$

The insoluble carbohydrate content was determined by summing up the percentages of the fat, protein, soluble carbohydrate, water and ash content and subtracting it from 100 as shown below:

$$\% \text{ Carbohydrate} = 100 - x$$

$$\text{Where } x = \sum (\% \text{ Fat}) + (\% \text{ Protein}) + (\% \text{ Soluble Carbohydrate}) + (\% \text{ Water absorbed}) + (\% \text{ Ash content})$$

Proximate analysis of Oyster mushroom basidiocarp (fruit body)

The proximate analysis of percentage (water, ash, fat, protein, carbohydrate, water content) of the Oyster mushroom fruit bodies was also determined using same procedures as already described for compost substrates.

Pre-treatment of undecomposed substrate samples

Rice (*Oryza sativa L.*) husk

One thousand grams of rice husk were soaked in 1500 ml of warm distilled water in an Erlenmeyer flask at a temperature of 50 ^{o}C for 30 minutes. The water was then drained off thoroughly. Ten grams of the moist rice husk were placed into each of Petri dishes compacted and autoclaved at 121 °C for 15 minutes.

Corn (*Zea mays L.*) cob

One thousand grams of corn cob were pounded and ground to 2 mm crumbs and then stirred in 1500 ml of distilled water in an Erlenmeyer flask. The water was then drained off and 10 g of the wet ground cob were placed into each of 9 cm diameter Petri dishes, compacted and autoclaved at 121 °C for 15 minutes.

Oil palm fruit (*Elaesis guineensis L.*) fibre

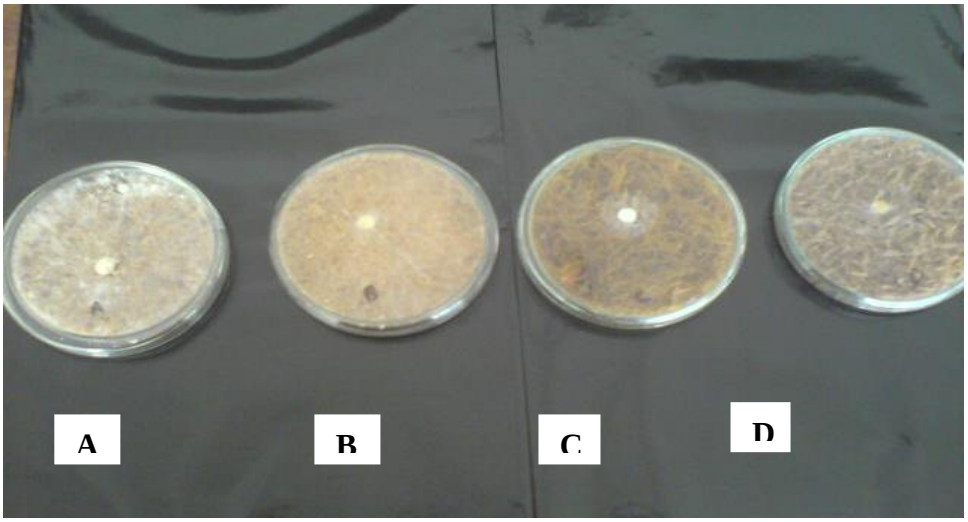
One thousand grams of oil palm fibre were shredded into 2 cm pieces and stirred into 1500 ml of distilled water in an Erlenmeyer flask. The excess water was later drained off and the fibres distributed into 9 cm diameter Petri dishes, compacted and autoclaved at 121 ^{o}C for 15 minutes.

Sawdust of wawa (*Triplochiton seleroxylon L.*)

One and half litres of distilled water were added to 1000 g of sieved wawa sawdust in Erlenmeyer flask sawdust and the excess water drained off. The wet sawdust was then distributed into 9 cm Petri dishes, compacted and autoclaved at 121 °C for 15 minutes.

Determination of vegetative growth of Oyster mushroom mycelium on undecomposed compost substrates

Petri plates of undecomposed compost substrates were each inoculated with one centimeter diameter disc of mycelium from the mother spawn obtained from tissue and spore prints of *P. ostreatus* from Dzorwulu Farm. They were then incubated at room temperature at 30 °C ± 2 °C Growth was observed for 14 days (Plate 4 a - d). There were three replicate determinations for each substrate.



x1/4

Plate 4: Colonization of compost substrate types by mycelium of *P. ostreatus*

A. Sawdust B. Corn cob C. Oil palm fruit fibre D. Rice husk

Preparation and bagging of compost

For each compost type, 3.0 kg of bran, 0.3 kg of lime and 50.0 kg of the compost substrate were put together, sprayed with water and thoroughly mixed. The mixture was left for 30 days at room temperature to decompose, turning it at 4-days intervals. The temperature within the fermenting heap (Plate 5 a - 5 d) and the pH and water content were determined before and after the fermentation process.

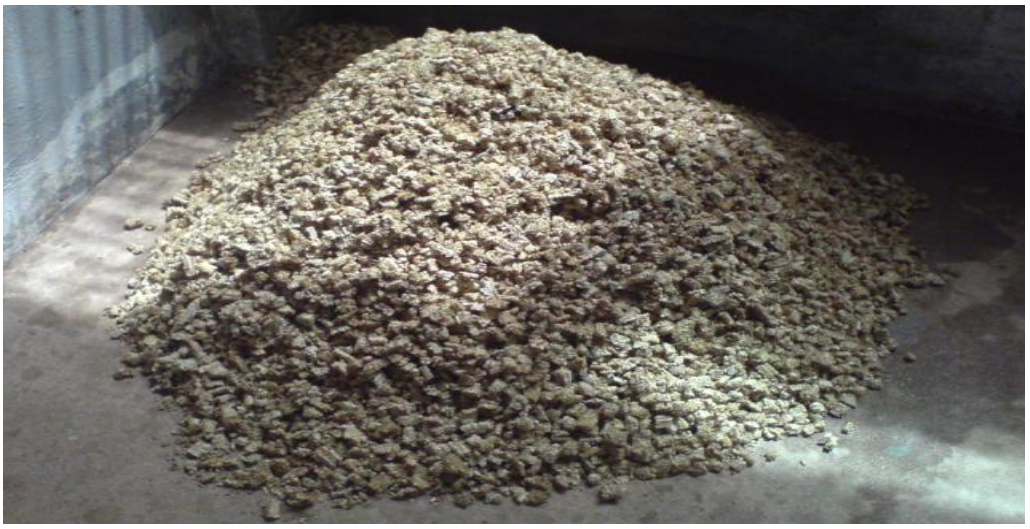
The compost mixture after 30 days was packed compactly, using a wooden pestle as a compactor, into 28 cm x 10 cm transparent polypropylene bags. A neck was created for the bags by pulling the open end through a cut PVC tube, 2.5 cm in length and 2.5 cm in dimension and folding the polypropylene back over the neck of the PVC tube to provide an opening. The polypropylene covering the PVC tube was held in place with an elastic band. The opening was plugged with cotton waste, covered with newspapers and held in place with an elastic band.

Control polyethene bags were filled with undecomposed substrate without wheat bran and lime supplement. The substrate was moistened, allowed to stand for 20 minute then to drain and then bagged. There were 15 replicate bags each for both the decomposed compost and undecomposed substrate.

A)

x1/8

B)



x1/8

Plate 5: Decomposed compost substrates

A. Sawdust B. Corn cob

C)

x1/8

D)

x1/8

Plate 5: Decomposed compost substrates

C. Oil palm fruit fibre D. Rice husk

Sterilization of bagged compost

A metal drum with a rack in it was placed on a gas burner. Water was poured into the drum until the water was just below the level of the rack. The compost bags were placed vertically on the wooden rack to form four layers, with the bags in the bottom three layer standing upright and those on the top layer turned upside down. The substrates were sterilized by steaming by steaming at 100 °C for 2-3 hours. The water was heated until a steady steam came out of a small opening punched in the lid of the drum indicating that all the air in the drum has been expelled and replaced by steam. After steaming, the sterilized bags were allowed to cool overnight and then conveyed to the inoculation room for inoculation.

Inoculation of compost

Prepared grain spawns were used to inoculate the various substrates in the compost bags. The bottles containing the grains colonized by white mycelium were shaken to separate them into single grains after which the cotton waste plug was lifted and the mouth of the bottle flamed. A few grains (1-2 teaspoonfuls) were transferred to the sterile substrate in the bags through the neck. The plugs were put back in place and covered with a piece of newspaper and held in place with an elastic rubber band. The inoculated bags were slightly tilted to distribute the grains evenly on the surface of the substrate. The spawned compost bags were kept in a dark room, until the mycelia had grown and penetrated to the bottom of the substrate at room temperature (30 ± 3 °C) for 28 days. The growth rate of the mycelium was determined during this period by measuring the distance travelled down the compost by the mycelium at two days intervals. Inoculation was done under aseptic conditions. There were three replicate bags for each spawn-substrate combination.

Cropping and harvesting of mushrooms

After the 28 days, the inoculated bags were kept for an additional week. This was to ensure the mycelium was mature enough to fruit and pin heads started appearing. The bags were then sent to the cropping house where they were opened to initiate fruiting at a temperature of 30 ± 2 °C. The bags were opened by cutting off the mouth portion of the bag with a sterile razor to expose the substrate colonized by the mushroom mycelium and watered daily with a fine mist of water using a water hose. Three to four days after opening the bags, fruit bodies called mushroom primordia began to form and matured in 5-6 days and ready for harvesting. The number, total weight, width and lengths of the fruits were recorded at each harvest.

Determination of lignin content of substrates before and after fruiting

Each substrate sample was milled to pass through a 0.75 mm pore size screen. Milled samples were treated with 95 % ethanol for 6 hours in a soxhlet apparatus to remove extracts. The extracted wood samples were hydrolyzed with 72 % sulphuric acid (w/w) at 30 °C. The residual material was allowed to cool and then filtered through a 3 folded No. 44 Whatman filter paper. The solids were dried to constant weight at 105 °C and recorded as Klason insoluble lignin. The acid soluble lignin concentration in the aqueous fraction was determined by measuring the absorbance at 205 nm (A_{205}) and using the value of 105 l/g/cm as the absorptivity of soluble lignin, soluble lignin was then determined using the formula:

$$\mathrm{ASL} = \frac{\mathrm{A} \times \mathrm{D} \times \mathrm{V}}{\mathrm{a} \times \mathrm{b} \times \mathrm{M}} \times 1000\mathrm{mg/g}$$

Where: A = absorbance at 205 nm

D = Dilution factor

V = Volume of the filtrate in L (0.029 L)

A = Extinction coefficient of lignin in g/lcm (105 g/lcm)

b = Cuvette path length in cm (1 cm)

M = Weight (g) of sample (as 100 % dry matter before acid hydrolysis/suspension

The insoluble lignin content was determined using the formula:

$$\% \text{ Lignin} = \frac{(W_3 - W_2)}{W_1} \times 100$$

Where: W_3 = weight of sample and filter paper after hydrolysis

W_2 = weight of filter paper

W_1 = weight of sample

Statistical analysis

Data collected were analyzed statistically using:

- Analysis of variance (ANOVA)
- Graphical presentation

Experimental precautions

a) Inoculation room was heavily sprayed with methylated spirit before and after use to prevent contamination.

b) Glassware were thoroughly washed with liquid soap, rinsed with clean water and dried before sterilization to prevent contamination.

c) Inoculation loops, needles and forceps were flamed until red- hot but were allowed to cool before use and the process repeated after use to prevent contamination.

d) Only fresh, large and vigorous growing basidiocarps were used for spawn production.

e) During compost bag sterilization, it was ensured that the water level was not above rack in the drum. This was done to prevent water from entering the bags.

f) Watering of substrates for fruiting was sparingly done to prevent a waterlogged situation.

g) Harvesting was done by grasping fruit bodies by the stalk and gently twisting and pulling.

h) The turning of the compost was done every four days to ensure adequate aeration and fermentation.

i) Dustbins were almost always emptied to prevent the growth of any microorganism and the breeding of fruit flies.

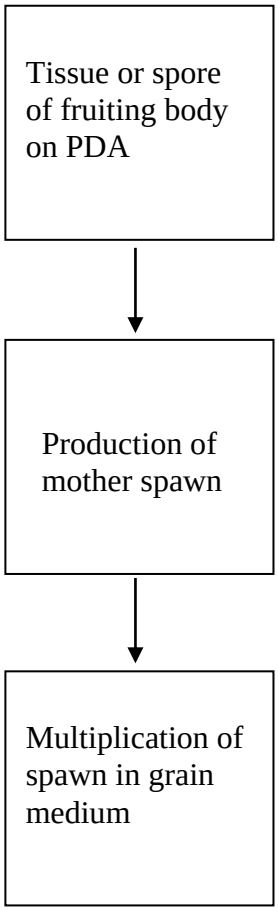


Figure 2: The basic steps in the production of *Pleurotus ostreatus* spawns in the laboratory

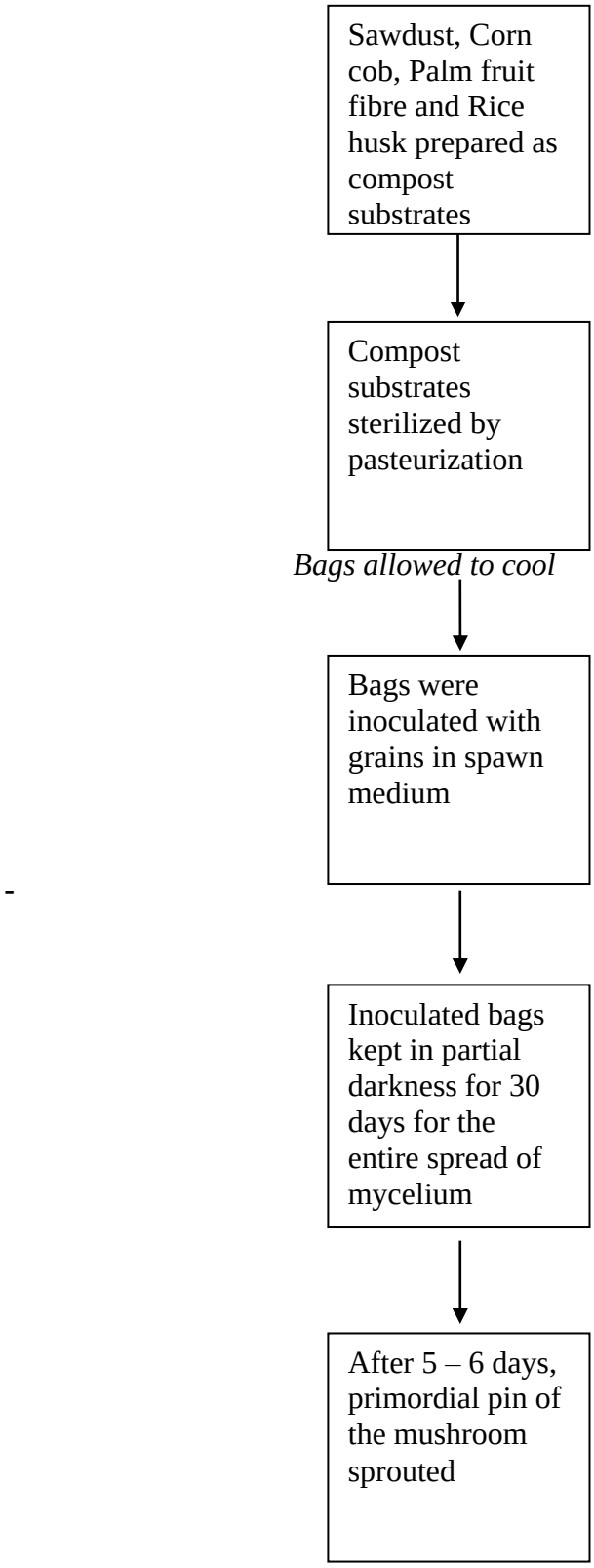


Figure 3: The basic steps in the cultivation of *P. ostreatus* in the mushroom house.

CHAPTER THREE

RESULTS

Production of Oyster mushroom mother spawn

(i) Characteristics of the Oyster mushroom fruit bodies

Fruit bodies of *P. ostreatus* collected from Dzorwulu Farm, Accra and School of Agric Farm, UCC were used for both tissue and spore culturing.

All the fruit bodies collected from these sources had varied length, width and weight. Those from Dzorwulu Farm, Accra, used for tissue culturing, ranged from 11.9 cm to 12.0 cm in length whereas, those obtained from UCC School of Agriculture Farm, ranged from 11.5 cm to 13.6 cm. Their width, ranged from 2.4 to 2.9 cm and 2.5 to 4.8 cm respectively with a corresponding weight range of 11.17 to 16.10 g and 18.06 to 23.77 g, respectively. Similar observations of varied length, width and weight were made in the other fruit bodies used for spore culturing (Table 1).

Apart from the ranges, the means of the various parameters of the individual fruit bodies were also recorded and these were as follows: 12.2 cm, 2.7 cm and 14.63 g for length, width and weight for fruits obtained from Dzorwulu Farm, Accra and used for tissue culturing; while those from the UCC School of Agric Farm recorded 12.9 cm length; 3.9 cm width and 21.57 g weight (Table 1).

Furthermore, fruit bodies originating from Accra but used for spore culturing had their means as: length 11.6 cm, width, 2.1 cm and weight 9.93 g whereas those from School Farm recorded 12.0 cm, 2.3 cm and 4.11 g (Table 1).

Indeed, all the fruit bodies collected were observed to have varied length, width and weight; but, statistical analysis showed there were no significant differences among the locality, the culture type the fruit bodies were used for, as well as the combination of the two; $p > 0.05$ (Appendix I). In terms of the weight however, only the culture type the fruit bodies were used for showed there were significant differences among them; $p = 0.002$. On the contrary, the locality and the combination of locality and culture type had $p > 0.000 – 0.050$, hence no significant differences among them (Appendix II).

Table 1: Mean length, width and weight of mushroom fruit bodies collected on different days from two different sources and used for both tissue and spore cultures.

	Tissue			Spore		
Locality	L (cm)	W_1 (cm)	Weight (g)	L (cm)	W_1 (cm)	Weight (g)
'Dzorwulu' Farm, Accra	12.2	2.7	14.63	11.6	2.1	9.93
Range	(11.9-12.0)	(2.4-2.9)	(11.17-16.10)	(10.5-11.9)	(1.8-2.3)	(8.41-10.35)
UCC School of Agric Farm	12.9	3.9	21.57	12.0	2.3	4.11
Range	(11.5-13.6)	(2.5-4.8)	(18.06-23.77)	(11.1-12.6)	(1.8-2.5)	(3.16- 6.64)

Where:

L = Length

W_1=Width

(ii) Growth of spawns produced by Oyster fruit body tissues and spores on Potato Dextrose Agar

The mean colony diameters of spawns produced by tissues and spores of Oyster mushroom fruit bodies from the Dzorwulu Farm, Accra and School of Agriculture Farm, UCC grown on PDA, are presented in Table 2 and Plate 6 a and 6 b.

All the cultures obtained from both tissues and spores grew well on PDA (Table 2, Plate 6 a and 6 b) and as expected, the colony diameter increased with increase in the period of incubation. The mean colony diameter for cultures produced by tissues and spores of Oyster mushroom from Dzorwulu Farm, Accra, was 2.8 and 1.3 cm, respectively after two days but increased to 9.0 and 8.5 cm, respectively, by the 14^{th} day (Table 2).

In the same way, the mean colony diameters of mycelia produced by tissues and spores of mushroom from School of Agriculture Farm, UCC was 1.8 cm and 1.2 cm, respectively, after two days, but increased to 8.7 cm and 8.4 cm respectively by the 14^{th} day (Table 2).

Statistical analysis shows that the differences among the parameters: locality and culture type as well as their combination were not significant as $p > 0.00 – 0.050$ (Appendix III).

Table 2: Mean colony diameter (cm) of cultures produced by both tissues and spores of *Pleurotus ostreatus* collected from Dzorwulu Farm, Accra, and School of Agriculture Farm, UCC.

Period Of Growth (days)	Dzorwulu Farm, Accra		School of Agriculture Farm, UCC	
	Tissue L (cm)	Spore L (cm)	Tissue L (cm)	Spore L(cm)
2	2.8	1.3	1.8	1.2
4	5.1	3.7	2.2	2.7
6	6.3	4.6	4.0	3.9
8	6.9	5.4	5.9	4.1
10	7.2	6.3	6.6	5.8
12	8.7	7.1	7.4	6.9
14	9.0	8.5	8.7	8.4

Where L (cm) = Mean colony diameter

x 5/9

Plate 6 a: 6-day old pure culture of *P. ostreatus* produced by spores

x 2/3

Plate 6 b: 14-day old pure culture of *P. ostreatus* produced by fruit body tissue

(iii) The effect of temperature on oyster mushroom spawn growth

The effect of temperature on the vegetative growth of Oyster mushroom spawns was determined by measuring the mean colony diameter over a 14-day period. The mean colony diameter of spawns produced by tissues and spores of mushrooms from Dzorwulu Farm, Accra, and School of Agriculture Farm, UCC together and of the Oyster mushroom spawn obtained from Food Research Institute, Accra incubated at: 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C are presented in Table 3a and 3b.

The growth rate of each culture differed with the temperature of incubation. All the factors under consideration (locality, culture type and temperature) in Tables 3a and 3b statistically showed; $p \leq 0.05$ (Appendix IV and Appendix V).

Table 3a shows that all the cultures grew poorly at 15 °C, 20 °C , 35 °C and 40 °C and the best growth occurred at 30 °C.

Somewhat, growth of cultures of spawns originating from FRI differed little from that of Dzorwulu Farm, Accra and UCC School Farm. Whereas the tissue and spore cultures originating from Dzorwulu Farm, Accra and UCC School of Agric farm grew best at 25 °C and 30 °C; those from FRI, Accra increased from 15 °C, reached a peak of 25 °C and then reduced from 30 °C to 40 °C (Table 3 b). At 40 °C, no growth was observed (Table: 3 a and 3 b, Figure 4, 5, 6, 7, 8 and Plate 7).

Table 3a: The mean colony diameter (cm) of cultures produced by tissues and spores of *Pleurotus* collected from different sources and incubated at temperatures ranging between 15°C and 40 °C over a period of 14 days.

Source of inoculum	Type of Inoculum	Temperature (°C)	Period of incubation (Days) 2	4	6	8	10	12	14
	Tissue	15	0.0	0.0	0.0	0.0	0.0	0.0	0.1
		20	0.0	0.0	0.0	0.4	0.4	1.1	3.4
		25	0.4	0.7	3.6	4.5	5.4	5.5	5.8
Dzorwulu		30	1.5	2.5	2.9	5.2	6.6	8.2	8.2
		35	0.0	0.0	0.0	0.0	0.0	1.3	1.7
Farm,		40	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Accra	Spore	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		20	0.0	0.0	0.0	0.1	0.6	1.0	1.1
		25	0.0	0.0	0.2	0.6	1.0	1.1	1.1
		30	0.5	0.0	2.3	2.9	5.2	6.0	6.7
		35	0.0	1.8	0.0	0.0	0.0	0.6	1.0
		40	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tissue	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		20	0.0	0.0	0.3	0.6	0.7	0.9	1.4
		25	0.0	0.3	1.3	1.3	1.3	1.3	1.3
School of		30	1.4	1.3	3.5	4.4	4.6	5.0	6.3
		35	0.0	2.0	0.0	0.0	0.0	1.2	1.2
Agric.		40	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Farm,	Spore	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		20	0.0	0.0	0.0	0.1	0.2	0.4	0.5
UCC		25	0.0	0.0	0.3	2.1	2.2	2.2	2.2
		30	0.4	0.0	1.7	3.1	4.4	5.2	5.4
		35	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		40	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3b: The mean colony of diameter (cm) of cultures produced by an already prepared *Pleurotus ostreatus* spawn obtained from FRI, incubated 15–40 °C for 14 days.

Period of growth	Temperature (°C)					
(Days)	15	20	25	30	35	40
2	0.2	0.5	1.8	0.0	0.0	0.0
4	0.3	1.2	2.6	0.4	0.0	0.0
6	0.4	1.5	5.3	0.7	0.4	0.0
8	0.5	1.7	6.1	1.1	0.4	0.0
10	0.8	1.8	7.2	1.4	0.4	0.0
12	1.1	1.9	8.6	2.5	0.4	0.0
14	1.4	2.3	9.0	3.4	0.4	0.0

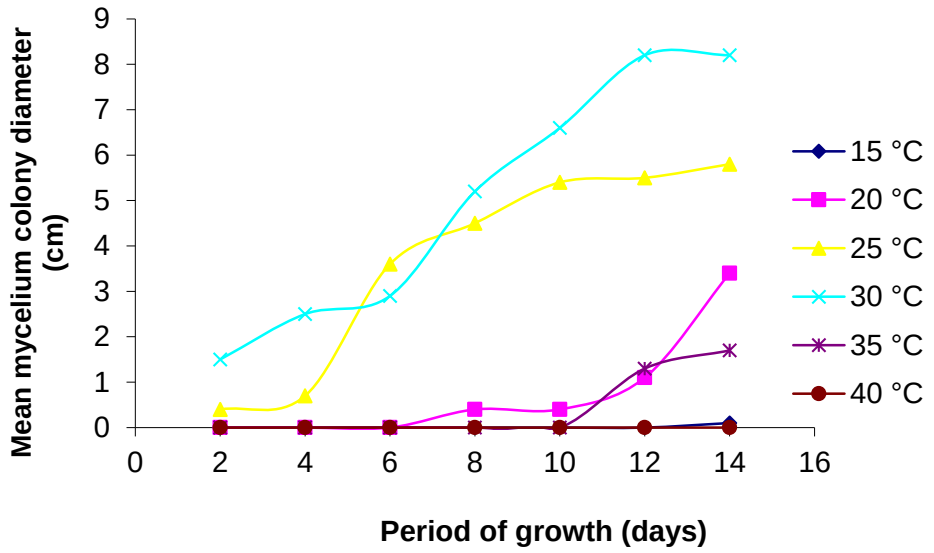


Figure 4: The effect of temperature on the growth of mycelium developed from tissues obtained from Dzorwulu Farm, Accra fruit bodies and grown on PDA over a period of two weeks.

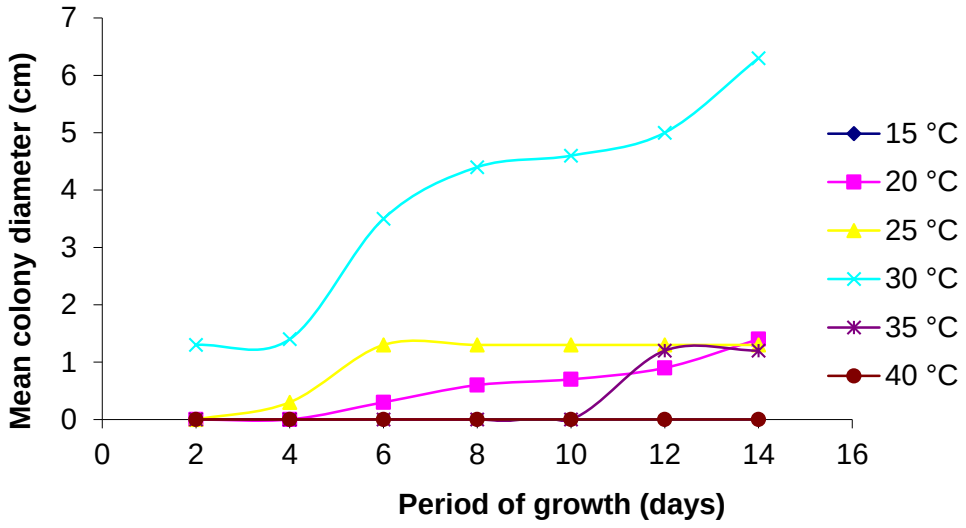


Figure 5: The effect of temperature on the growth of mycelium developed from School of Agric. Farm (UCC) tissue fruit bodies and grown on PDA over a period of two week

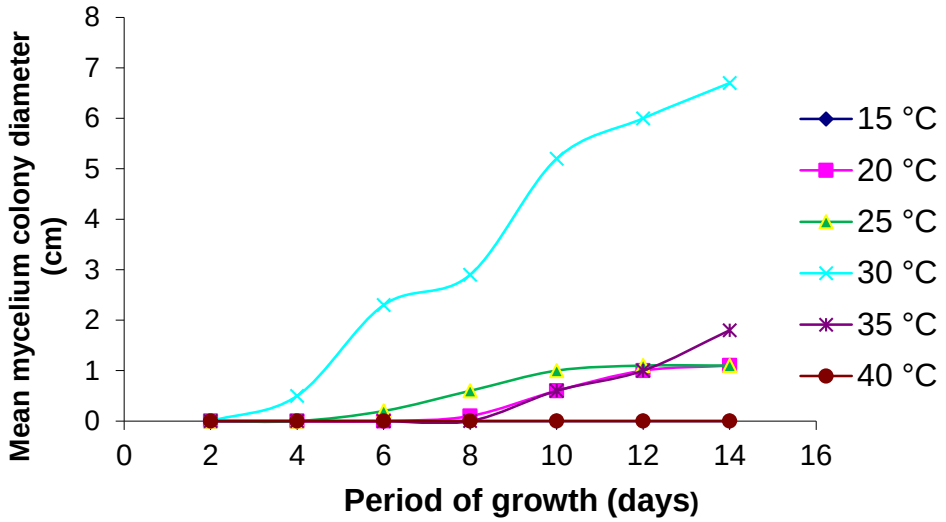


Figure 6: The effect of temperature on the growth of mycelium developed from spores produced by Dzorwulu Farm, Accra fruit bodies and grown on PDA over a period of two weeks

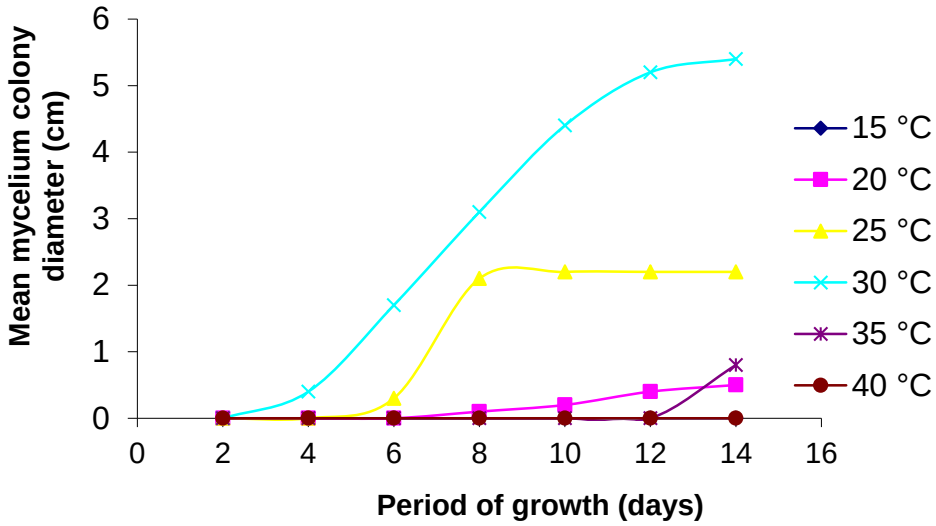


Figure 7: The effect of temperature on the growth of mycelium developed from spores produced by School of Agric. Farm (UCC) fruit bodies and grown on PDA over a period of two weeks

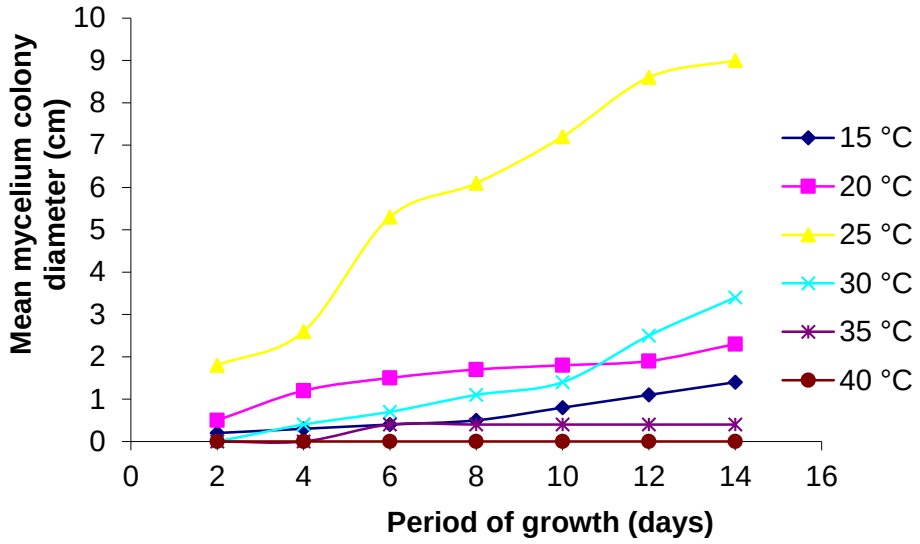
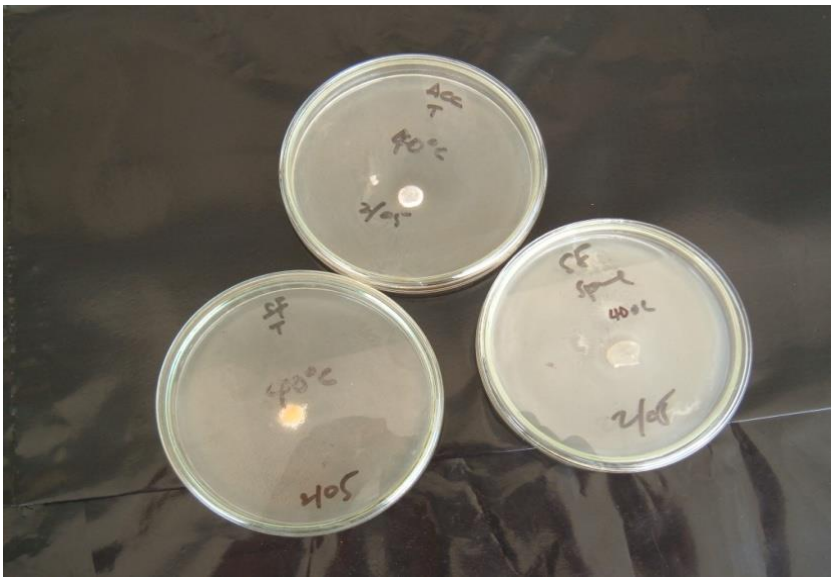


Figure 8: The effect of temperature on the growth of mycelium produced already prepared spawn obtained from FRI and grown on PDA over a period of two weeks



x1/3

Plate 7: Inoculated Petri plates showing no growths when incubated at 40 °C.

(iv) The effect of light on oyster mushroom spawn growth

Another environmental factor that affects the growth and fruiting of mushrooms is light. The effect of light on the vegetative growth of Oyster mushroom spawns was determined by measuring the mean colony diameter over a 14-day period. The mean colony diameters of cultures produced by tissues and spores of fruit bodies obtained from Dzorwulu Farm, Accra, and School of Agric. Farm, UCC and by Oyster mushroom spawn obtained from Food Research Institute, Accra incubated under four different light intensities: total darkness (0 Lux), total light (28.7 Lux), partial darkness of (1.9 Lux) and 12 hours sunlight light 12 hours darkness (40.2 Lux) are shown in Table 4 a – 4 c.

Growth of the cultures was different under the different light conditions ($p = 0.000$; Appendix VI). The best growth occurred in total darkness, followed by growth in partial darkness (1.9 lux) and then growth in total light (28.7 lux). There was no growth in 12 hours light (40.2 lux) and 12 hours darkness in all treatments (Plate 8). In total light, on the other hand, a growth lag phase, between 4 and 6 hours (Table 4 a – c) to between 10 and 12 hours (Table 4 b) was shown by the cultures (Plate 8).

Table 4 a: The mean colony diameter (cm) of cultures obtained from tissues of *Pleurotus ostreatus* fruit bodies collected from Dzorwulu Farm, Accra and School of Agriculture Farm, UCC and incubated under different light intensities.

Period of growth (Days)	'Dzorwulu' Farm, Accra				School of Agric. Farm, UCC			
	TD	TL	PD	12HRL	TD	TL	PD	12HRL
2	1.5	0.0	0.8	0.0	1.4	0.0	1.0	0.0
4	2.5	0.0	1.4	0.0	2.0	0.0	1.3	0.0
6	2.9	1.1	2.1	0.0	4.4	0.0	1.7	0.0
8	5.2	1.7	2.4	0.0	4.6	0.0	2.5	0.0
10	6.6	2.2	4.5	0.0	5.0	0.8	3.9	0.0
12	8.2	2.6	5.2	0.0	6.3	1.3	4.0	0.0
14	8.2	3.0	7.4	0.0	7.8	1.4	6.4	0.0

Where: TD = Total darkness (0 Lux)

TL = Total light (28.7 Lux)

PD = Partial darkness of (1.9 Lux)

12HRL = 12 hours sunlight (40.2 Lux)12 hours darkness

Table 4 b: The mean colony diameter (cm) of cultures produced by spores of *Pleurotus ostreatus* fruit bodies collected from 'Dzorwulu' Farm, Accra, and School of Agriculture Farm, UCC and incubated under different light intensities.

Period of growth (Days)	'Dzorwulu' Farm, Accra				School of Agric. Farm, UCC			
	TD	TL	PD	12HRL	TD	TL	PD	12HRL
2	1.0	0.0	0.6	0.0	0.7	0.0	0.4	0.0
4	1.6	0.0	1.1	0.0	0.8	0.0	0.5	0.0
6	1.9	0.3	1.3	0.0	1.0	0.0	0.7	0.0
8	2.4	0.7	1.5	0.0	1.3	0.0	1.1	0.0
10	3.2	2.0	2.1	0.0	2.1	0.0	1.6	0.0
12	4.3	2.1	2.2	0.0	2.5	0.4	2.0	0.0
14	5.6	2.5	2.7	0.0	3.1	0.9	2.1	0.0

Where: TD = Total darkness (0 Lux)

TL = Total light (28.7 Lux)

PD = Partial darkness of (1.9 Lux)

12HRL = 12 hours sunlight (40.2 Lux)12 hours darkness

Table 4 c: The mean colony diameter (cm) of a culture of an already prepared spawn obtained from Food Research Institute (FRI) and incubated under different light intensities.

Period of growth (Days)	Treatment			
	TD	TL	PD	12HRL
2	1.8	0.0	1.4	0.0
4	2.6	0.0	2.0	0.0
6	5.3	1.2	3.2	0.0
8	6.1	1.3	4.6	0.0
10	7.2	1.8	5.7	0.0
12	8.6	2.3	6.5	0.0
14	9.0	3.0	8.6	0.0

Where: TD = Total darkness (0 Lux)

TL = Total light (28.7 Lux)

PD = Partial darkness of (1.9 Lux)

12HRL = 12 hours sunlight (40.2 Lux) 12 hours darkness

x1/4

Plate 8: Inoculated Petri plates showing virtually no growth of myceliumwhen exposed to 12 hours sunlight and 12 hours darkness

(v) Hybridization test

The hybridization mycelium spore cultures from Dzorwulu Farm, Accra and School of Agriculture Farm, UCC is presented in (Plate 9).

Pure spore cultures from Dzorwulu Farm, Accra, and School of Agriculture. Farm, UCC was grown on the same plate and medium. After 8 days of growth they grew into each other, thus overlapping, however no junction was observed between them.

x7/9

Plate 9: Hybridization test of *Pleurotus ostreatus* spore cultures from Dzorwulu Farm, Accra and School of Agriculture Farm U.C.C.

(vi) Growth of Oyster mushroom mycelium on sorghum grain medium

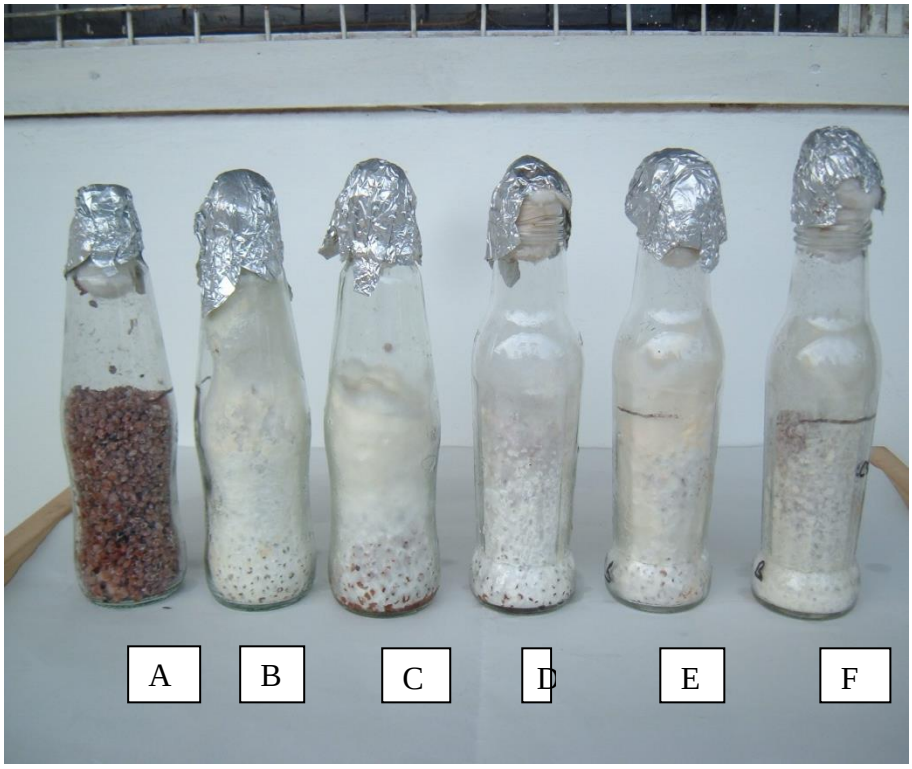
The availability of spawns is very critical to the cultivation of mushrooms like *P. ostreatus*. Therefore, the differences in the growth rate of the various cultures obtained from the three different sources and grown on sorghum grain medium are presented in Table 5 and Plate 10.

Growth was generally rapid for all the spawns. However, the pattern of growth throughout the fourteen days was more rapid in the spores than in the tissue cultures for Dzorwulu Farm, Accra and School of Agric Farm, UCC with the spawn cultures of FRI growing the slowest. For example, whereas growth in spawns produced by Dzorwulu Farm, Accra fruit bodies was 2.4 cm on day two and increased to 10.4 cm after fourteen days, those of its counterpart spore, had a growth of 1.3 cm on day two but also increased to 11.0 cm on the fourteenth day. Similarly, spawns obtained from UCC School of Agriculture Farm tissue and spore as well as those from FRI, Accra gave the same growth patterns.

Statistical analyses (two-way analysis of variance; Appendix VII and Appendix VIII) show the differences in the colony diameter obtained from tissues and spores of Dzorwulu Farm, Accra as well as those of UCC School of Agriculture Farm and those of FRI were not significant; $p = 0.000$ thus, the period of growth and the localities had no effect on the growth of the spawns on the sorghum medium.

Table 5: Growth of *Pleurotus ostreatus* mycelia on sorghum grain media in culture bottles at 30 °C.

Period of Growth (Days)	Extent of mycelial growth in culture bottle (cm)				
	Source of inoculum				
	Fruit body from Dzorwulu Farm, Accra		Fruit body from UCC School of Agric Farm		FRI Spawn
	Tissue	Spore	Tissue	Spore	
2	2.4	1.3	2.1	1.4	1.2
4	4.1	2.4	3.7	2.8	2.9
6	7.0	4.1	5.5	3.5	3.8
8	7.2	7.1	7.1	6.6	5.5
10	8.5	9.0	7.3	8.4	6.9
12	9.6	10.1	7.8	10.3	7.2
14	10.4	11.0	9.7	10.5	8.2



x1/3

10: Bottles of sorghum inoculated with *Pleurotus ostreatus*

A. **Control (not seeded with spawn).**
B. **PDA culture of spores of fruit body from Dzorwulu Farm, Accra.**
C. **PDA culture of spores of fruit body from School of Agriculture Farm, UCC.**
D. **PDA culture of spawn from FRI.**
E. **PDA culture of tissue of fruit body from Dzorwulu Farm, Accra.**
F. **PDA culture of tissue of fruit body from School of Agriculture Farm, UCC.**

(vii) Growth of Oyster mushroom mycelium on substrates

The growth of *P. ostreatus* mycelium on four types of substrates is presented in Tables 6 a, 6 b, 6 c, 6 d and 6 e. The differences in the level of performance were assessed with regard to the source of the fruit bodies as well as the type of culture and substrate used.

The trend of results showed mycelial length in all the four substrates increased with growth period. Growth was rapid in all substrates except in rice husk which was slowest. Spawns of tissue originating from Dzorwulu Farm, Accra grew 4.3 cm in length in four days but 18.9 cm in twenty-eight days on decomposed sawdust substrate; whereas growth on undecomposed sawdust was 3.5 on day four but 17.0 cm at the end of twenty eight days (Table 6 a).

Similar growth patterns were also observed in corn cob, oil palm fruit fibre and rice husk of Dzorwulu Farm, Accra spore substrates, UCC School of Agric Farm tissue and spore substrates and FRI, Accra, spawn substrates (Tables 6 a, 6 b, 6 c, 6 d, and 6 e).

Meanwhile statistical analyses of the data presented in Tables 6 a, 6 b, 6 c, 6 d, and 6 e revealed there were significant differences between the substrates, treatments and the culture used for the inoculation ($p = 0.000$) in all the parameters and hence different growth lengths (Appendix IX and X).

Table 6 a: Growth of *P. ostreatus* spawns originating from tissue of fruit body of Dzorwulu Farm, Accra on different decomposed and undecomposed composts at 30 ± 2 °C.

Period Of Growth (Days)	Distance of advancing culture front from inoculum (cm) on Sawdust		Corn cob		Oil palm fruit fibre		Rice husk	
	D	U	D	U	D	U	D	U
4	4.3	3.5	4.3	2.2	5.4	4.7	2.3	2.2
8	6.8	4.8	6.5	5.3	6.8	6.2	3.2	2.8
12	10.3	7.9	11.0	8.5	10.1	10.2	3.7	3.6
16	13.7	12.1	13.0	12.2	13.0	12.6	5.9	5.1
20	16.2	16.0	15.6	16.3	16.4	13.9	9.8	6.8
24	16.7	18.0	19.2	18.8	19.2	15.5	12.5	7.8
28	18.9	17.0	20.0	19.7	20.0	17.0	15.6	8.5

Where: D = Length of growth of *P. ostreatus* in decomposed substrate

U = Length of growth of *P. ostreatus* in undecomposed substrate

Table 6 b: Growth of *P. ostreatus* spawns originating from spores of fruit body of Dzorwulu Farm, Accra, on different decomposed and undecomposed composts at 30 ± 2 °C.

Period of Growth (Days)	Distance of advancing culture front from inoculum (cm) on							
	Sawdust		Corn cob		Oil palm fruit fibre		Rice husk	
	D	U	D	U	D	U	D	U
4	2.8	2.6	4.1	2.4	4.2	2.1	2.1	1.9
8	5.1	4.5	5.4	4.9	5.6	4.8	3.0	2.6
12	9.0	6.5	6.9	6.5	8.0	7.9	3.8	3.6
16	11.3	7.2	10.5	10.2	10.7	10.1	4.4	4.1
20	13.1	10.2	13.4	11.5	13.8	11.8	6.9	4.6
24	15.1	11.4	15.7	12.0	16.1	15.4	8.3	4.8
28	15.1	13.5	19.4	14.4	20.0	17.0	10.8	4.9

Where: D = Length of growth of *P. ostreatus* in decomposed substrate

U = Length of growth of *P. ostreatus* in undecomposed substrate

Table 6 c: Growth of *P. ostreatus* spawns originating from UCC School of Agriculture Farm fruit body tissue on different decomposed and undecomposed composts at 30 ± 2 °C.

Period of Growth (Days)	Distance of advancing culture front from inoculum (cm) on							
	Sawdust		Corn cob		Oil palm fruit fibre		Rice husk	
	D	U	D	U	D	U	D	U
4	4.4	3.3	3.4	2.7	5.0	2.8	2.4	2.4
8	4.8	4.6	4.7	4.0	7.5	4.4	3.4	3.2
12	7.4	6.5	7.2	6.8	10.2	6.0	5.0	4.2
16	14.3	7.7	9.7	7.6	12.2	9.2	6.3	6.0
20	16.4	10.8	11.9	9.0	14.2	12.9	7.8	8.8
24	18.4	12.8	15.5	11.8	15.9	14.3	11.5	10.3
28	19.6	13.3	18.8	14.6	19.2	18.5	13.3	12.7

Where: D = Length of growth of *P. ostreatus* in decomposed substrate

U = Length of growth of *P. ostreatus* in undecomposed substrate

Table 6 d: Growth of *P. ostreatus* spawns originating from UCC School of Agriculture Farm fruit body spores on different decomposed and undecomposed composts at 30 ± 2 °C.

Period of Growth (Days)	Distance of advancing culture front from inoculum (cm) on							
	Sawdust		Corn cob		Oil palm fruit fibre		Rice husk	
	D	U	D	U	D	U	D	U
4	3.0	2.4	4.2	2.8	4.6	3.6	2.5	1.7
8	5.9	4.9	5.7	5.4	6.6	5.1	5.4	3.9
12	9.2	6.0	7.2	6.8	9.0	8.9	5.5	5.0
16	11.3	6.5	9.8	9.3	13.3	12.3	7.7	5.6
20	12.6	7.1	13.7	11.0	15.9	15.3	10.7	7.0
24	14.8	8.3	16.1	13.9	18.1	17.3	11.4	8.0
28	17.3	11.7	19.3	15.7	20.0	19.9	13.5	9.9

Where: D = Length of growth of *P. ostreatus* in decomposed substrate

U = Length of growth of *P. ostreatus* in undecomposed substrate

Table 6 e: Growth of *P. ostreatus* spawns obtained from FRI, Accra, on different decomposed and undecomposed composts at 30 ± 2°C.

Period Of Growth (Days)	Distance of advancing culture front from inoculum (cm) on							
	Sawdust		Corn cob		Oil palm fruit fibre		Rice husk	
	D	U	D	U	D	U	D	U
4	4.7	4.0	3.3	3.2	3.7	4.9	3.2	1.6
8	5.5	4.9	4.6	4.2	8.0	7.3	3.6	3.5
12	7.1	5.5	7.4	6.7	11.7	8.8	4.4	3.7
16	13.6	6.7	10.8	8.7	14.3	13.5	7.4	5.5
20	17.1	7.4	16.1	11.8	17.5	16.7	10.7	6.1
24	18.0	7.9	17.3	15.3	19.7	19.1	13.9	7.7
28	20.0	12.3	19.4	18.1	20.0	19.4	16.4	10.1

Where: D = Length of growth of *P. ostreatus* in decomposed substrate

U = Length of growth of *P. ostreatus* in undecomposed substrate

(viii) Characteristics and yield of Oyster mushroom grown on decomposed and undecomposed composts

Yield is a factor that mainly depends on the texture and the type of substrate used. For this reason, the number, length, width and weight of *P. ostreatus* fruit bodies collected over a period of five weeks, in relation to the source of the fruit bodies, culture type and type of treatment of substrate were determined. These are presented in Table 7 a and 7 b and Plate 11 a – 11 d,

The number, length, width and weight of the harvested fruit bodies generally differed with one substrate and treatment. Decomposed substrates supported higher development than undecomposed substrates. For example, while the number, length, width and weight of Dzorwulu Farm, Accra tissue spawn on decomposed sawdust were 2.2, 14.2 cm, 3.9 cm and 36.70 g, respectively, those of the undecomposed were 1.0, 5.3 cm, 1.9 cm and 3.88 g, respectively. Comparatively, use of decomposed sawdust must be preferred to undecomposed substrate. This trend was also seen on corn cob, oil palm fruit fibre and rice husk Table (7 a). A similar pattern was also observed in Table 7 b.

Among the substrates, productivity on oil palm fruit fibre was incredibly highest regardless of the location and the type of culture used, followed by sawdust, corn cob and then rice husk.

Despite the differences observed, the statistical tests showed that there were no significant differences among the parameters, whether on decomposed or undecomposed media ($p>0.05$) (Appendix XI, XII and XIII).

Table 7 a: Mean yield, length width and fresh weight of harvested *P. ostreatus* over a period of five weeks in relation to source of inoculum, culture type, type and treatment of substrate.

Source of fruit body	Source of spawn	Substrate	Treatment of substrate: Decomposed Y	L (cm)	W^1 (cm)	W (g)	Undecomposed Y	L (cm)	W^1 (cm)	W (g)
Dzor-Wulu Farm, Accra	Tissue	Sawdust	2.2	14.2	3.9	36.70	1.0	5.3	1.9	3.88
		Corn cob	1.4	8.8	3.1	7.50	0.8	4.3	0.7	4.10
		Oil palm fruit fibre	2.6	9.0	3.6	16.03	0.2	8.6	3.4	10.61
		Rice husk	1.4	7.2	3.3	0.57	0.2	3.9	2.2	0.53
	Spore	Sawdust	1.4	2.9	0.5	4.72	0.4	4.0	1.1	3.41
		Corn cob	1.2	8.0	2.7	4.46	0.6	4.6	0.9	3.86
		Oil palm Fruit fibre	2.8	5.2	2.1	9.74	0.6	4.7	1.1	4.46
		Rice husk	0.8	1.5	0.4	2.56	0.4	3.0	0.7	0.69
UCC School Of Agric. Farm	Tissue	Sawdust	3.2	6.4	3.6	6.65	1.0	5.0	3.1	3.58
		Corn cob	2.2	7.5	4.3	4.76	0.8	2.7	0.8	0.88
		Oil palm fruit fibre	3.2	7.9	2.2	7.02	2.8	5.9	4.3	6.59
		Rice husk	1.4	1.3	0.5	2.78	0.2	0.4	0.8	0.96
	Spore	Sawdust	1.0	5.9	3.2	5.59	0.6	6.5	2.7	5.86
		Corn cob	0.8	6.2	1.3	3.47	0.6	3.3	1.4	2.48
		Oil palm fruit fibre	3.0	5.9	2.5	6.34	1.0	1.9	0.4	1.06
		Rice husk	0.4	2.0	0.5	1.74	1.6	4.6	2.9	2.70

Where: Y = Number of harvested fruit bodies

L = Length of harvested fruit bodies

W^1 = Width of harvested fruit bodies

W = Weight of harvested fruit bodies

Table 7 b: Mean number, length, width and fresh weight of harvested *P. ostreatus* by spawn from FRI, Accra grown over a period of 5 weeks in relation to the type and treatment of substrate.

Substrate	Treatment of substrate							
	Decomposed				Undecomposed			
	Y	L (cm)	W^1 (cm)	W (g)	Y	L (cm)	W^1 (cm)	W (g)
Sawdust	3.8	8.0	2.7	6.43	0.8	3.6	1.4	2.09
Corn cob	3.0	4.4	1.9	3.19	0.6	4.0	1.3	2.18
Oil palm fruit fibre	4.8	7.3	2.7	7.21	3.2	6.1	2.2	5.86
Rice Husk	1.2	7.5	1.6	2.99	1.0	4.3	1.1	1.98

Where:

Y = Number of harvested fruit bodies

L = Length of harvested fruit bodies

W^1 = Width of harvested fruit bodies

W = Weight of harvested fruit bodies

(iv) Characteristics of composting substrates before inoculation

The extent of the effect of an inoculum on a particular substrate type is dependent on the characteristics of the substrate type. Some of these characteristics include: pH, dry matter, water absorption capacity and ash content. Therefore, the level of these characteristics in each substrate were determined before inoculation and are presented in Table 8 a below.

Clearly, the characteristics varied from one substrate type to the other. These differences were statistically confirmed. Appendix XIV, XV, XVI, and XVII show p = 0.000 for each of the parameters under observation, a highly significant differences among the substrates with respect to the characteristics.

The pH readings among the substrates ranged from 6.46 to 6.95 (slightly acidic); while dry matter readings ranged from 10.69 to 81.81 %. The percentage of water absorbed was lowest in rice husk (18.19 %) and highest in sawdust (79.32 %); while ash content in rice husk and in oil palm fruit fibre was lowest (0.50 %) followed by cob (1.50 %) and highest in sawdust (3.00 %) (Table 8 a).

Table 8 a: Mean pH, dry matter, water absorption and ash content of the different types of substrates before treatment and inoculation.

Substrate	pH	Dry Matter (%)	Water absorbed (%)	Ash content (%)
Sawdust	6.90	10.69	79.32	3.00
Corn cob	6.52	43.15	56.85	1.50
Palm Fibre	6.46	64.44	35.56	0.50
Rice Husk	6.95	81.81	18.19	0.50

(x) Chemical composition of substrate composts before inoculation

The chemical composition of both decomposed and undecomposed substrates was determined before being inoculated with the grain spawns of *P. ostreatus* collected from the three different sources. The data on nutritional content of the different substrate types are presented in Table 8b.

The experiments showed that there were differences in the level of the various nutrients in each substrate. Statistically, the differences among the substrate and the treatment types were significant p = 0.000 for all the nutrient types (fat, carbohydrate and protein) (Appendices XVIII, XIX, XX).

The experiments also revealed there were more nutrients in decomposed substrates than in undecomposed substrates. Decomposed sawdust, for example, contained 3.37 % fats, 35.80 % total carbohydrate and 5.01 % proteins while the undecomposed sawdust substrate contained 0.40 % fats, 9.47 % total carbohydrate and 1.36 % protein. Similar trends were exhibited in corn cob, oil palm fruit fibre and rice husk, thus making decomposed substrate being preferred to its undecomposed counterpart (Table 8 b).

Some substrates recorded more nutrient contents than others. Decomposed corn cob for example, recorded the least fat content of 2.23 % while rice husk recorded the least protein content of 2.19 % but the highest carbohydrate content of 64.04 %. Oil palm fruit fibre, on the other hand, recorded the highest fat and protein contents of 43.30 % and 5.21 %, respectively, but recorded the least soluble carbohydrate content of 0.56 %
(Table 8 b).

Table 8 b: The initial mean values of nutrients of the substrates before inoculation.

		Percentage composition of substrate			
Treatment	Nutrient	Sawdust	Corn cob	Palm Fibre	Rice husk
Decom-posed	Fat	3.37	2.23	43.30	4.50
	Soluble carbo–hydrates	5.42	9.05	0.56	10.58
	Total carbo–hydrates	35.80	31.36	14.89	64.04
	Protein	5.01	2.41	5.21	2.19
Undecom-Pose	Fat	0.40	3.81	3.30	3.09
	Soluble carbo–hydrates	0.58	6.52	25.50	2.35
	Total carbo–hydrates	9.47	0.39	13.16	4.59
	Protein	1.36	10.41	48.39	2.46

(xi) Lignin composition of substrates

The lignolytic content of the various substrates: sawdust, corn cob, palm fibre and rice husk were determined before inoculation and after harvesting and are presented in Table 9, 10 a and 10 b.

Lignin composition in the substrate differed. Insoluble lignin in decomposed substrates ranged from 7.43 to 79.19 % while that of undecomposed substrates ranged from 24.49 to 81.68 %. Meanwhile, soluble lignin ranged from 0.0762 to 0.0777 mols in decomposed substrates but 0.0763 to 0.0780 mols in undecomposed substrates. Thus, undecomposed substrates had higher insoluble lignin content than decomposed ones. The highest, therefore, was recorded in undecomposed rice, 81.68 % as compared to 79.19 % in decomposed rice. The least was recorded in decomposed corn cob; 7.43 % compared to 24.49 % in undecomposed corn cob. The same trend was also observed in soluble lignin content of the various substrates (Table 9).

Results after harvesting (Tables 10 a and 10 b) showed a reduction in both insoluble and soluble lignin content of each substrate; with undecomposed rice substrates still recording the highest lignin content among both undecomposed and decomposed substrates.

Apparently, Dzorwulu Farm, Accra culture spawns had less effect as its substrate still had higher insoluble lignin content, followed by those of UCC School of Agric Farm and then FRI (Table 10 a). The same trend was also observed in soluble lignin (Table 10 b).

Statistically, there were significant differences between the individual parameters: locality, culture and treatment type as well as lignin contents (insoluble and soluble), $p = 0.000$. Nonetheless, there was no significant

difference between the combination of the performance of both substrates and treatment before inoculation, as well as locality and treatment after harvesting as $p > 0.05$ (Appendix XXI and XXII).

Table 9: The initial mean lignin content of the various substrates before inoculation with *P. ostreatus* spawns.

	Undecomposed		Decomposed	
Substrate Type	Insoluble lignin (%)	Soluble lignin (mols)	Insoluble lignin (%)	Soluble lignin (mols)
Sawdust	51.07	0.08	29.01	0.08
Corn cob	24.49	0.08	7.43	0.08
Oil palm fruit fibre	43.19	0.08	35.31	0.08
Rice husk	81.68	0.08	79.19	0.08

Table 10 a: Percentage of insoluble lignolytic compounds of the various substrates after harvesting the *P. ostreatus* fruit bodies

Substrate Type	Source of spawn					
	Dzorwulu Farm Accra		UCC School of Agric Farm		FRI	
	D	U	D	U	D	U
Sawdust	48.02	50.93	41.74	44.96	39.70	46.54
Corn cob	18.12	22.32	15.77	11.32	14.58	16.89
Oil palm fruit fibre	34.47	37.93	31.67	35.11	30.85	33.81
Rice husk	76.39	78.78	71.92	72.40	67.01	70.39

Where: D = Decomposed substrate

U = Undecomposed substrate

Table 10 b: The soluble lignolytic content (mols) of the various substrates after harvesting the *P. ostreatus* fruit bodies

Substrate Type	Source of spawn					
	Dzorwulu Farm Accra		UCC School of Agric Farm		FRI	
	D	U	D	U	D	U
Sawdust	0.0773	0.0771	0.0776	0.0774	0.0771	0.0771
Corn cob	0.0778	0.0777	0.0780	0.0778	0.0771	0.0777
Oil palm fruit fibre	0.0767	0.0762	0.0766	0.0763	0.0757	0.0776
Rice husk	0.0780	0.0777	0.0775	0.0773	0.0771	0.0770

(xii) Chemical composition of harvested fruit bodies of *P. ostreatus*

The chemical composition of harvested fruit bodies of *P. ostreatus* mushrooms with reference to dry matter content, ash content, water absorption capacity, pH, fats, soluble carbohydrates, total carbohydrate and protein were determined and are presented in Table 11 a, 11 b and 11 c.

Table 11 a represents harvested fruit bodies of Dzorwulu Farm, Accra tissue spawns grown separately in both decomposed and undecomposed substrates of sawdust, corn cob, palm fibre and rice husk, Table 11 b represents harvested fruit bodies of the UCC School of Agric Farm tissue spawn grown separately in both decomposed and undecomposed substrates while Table 11 c, represents harvested fruit bodies of FRI spawns grown separately in both decomposed and undecomposed substrates.

The chemical composition of each harvested fruit body under observation differed from one another with respect to the type of substrate treatment used. When fruit bodies of the same substrate treatment type under observation (Table 11 a) were compared, results indicated there were differences in their nutritional content. The dry matter content of Oyster mushroom fruit bodies originating from Dzorwulu Farm, Accra and grown in decomposed sawdust was 55.91 %, 74.00 % in corn cob, 62.07 % in oil palm fruit fibre and 83.19 % in rice husk. Corn cob had the highest ash content of 3.00 % followed by sawdust; 2.00 %, then palm fibre; 0.65 and 0.05 % in rice husk. Sawdust and corn cob had acidic pH while oil palm fruit fibre and rice husk had alkaline pH.

Fats levels were 1.27 % in sawdust, 1.66 % in corn cob, 3.05 % in palm fibre and 1.93 % in rice husk in decomposed Dzorwulu Farm, Accra tissue substrates. Other nutrients such as carbohydrate and protein had varied nutritional

contents and did not follow any specific pattern. Rather, the percentage of the nutritional content depended on the type of nutrient being determined. The pattern described was also observed in the undecomposed substrate of fruit bodies obtained from the same location (Table 11 a). Furthermore, a similar pattern was observed in Table 11 b and 11 c.

All the results of the three Table 11 a, 11 b, and 11 c) revealed that the nutritional contents of the harvested fruit bodies from decomposed substrates were higher than those from undecomposed substrates; except those of oil palm fruit fibre which were unique and were almost the same in nutritional value in both decomposed and undecomposed substrates.

Statistical analysis shows $p = 0.000$ for the parameters: fats, carbohydrate and protein, except for the source and the combination of both source and treatment for fats and protein ($p > 0.05$); hence, they were not significant and so did not have differences among them (Appendix XXVII, XXVIII and XXIX).

In the case of dry matter, the substrate, treatment, combination of source and substrate and substrate and treatment were significant ($p \leq 0.05$). The same pattern was observed in ash content and water absorption capacity. Despite the source and the treatment type, pH readings were not significantly different; $P > 0.05$ (Appendix XXIII, XXIV, XXV and XXVI).

Table 11 a: Nutrient content of fruit bodies of *P. ostreatus* originated from Dzorwulu Farm, Accra and grown on different substrates.

Treatment	Nutrient	Percentage nutrient content of fruit bodies on			
		Sawdust	Corn cob	Oil palm fruit fibre	Rice husk
Decomposed substrate	Dry matter	55.91	74.00	62.07	83.19
	Ash content	2.00	3.00	0.65	0.05
	Water Absorbed	60.04	58.79	56.36	32.14
	pH	6.59	6.83	7.14	7.47
	Fat	1.27	1.66	3.05	1.93
	Soluble Carbohydrate	12.93	15.59	16.17	11.97
	Total Carbohydrate	23.91	24.36	25.40	25.72
	Protein	9.86	4.89	10.97	3.84
Undecomposed substrate	Dry matter	48.65	66.25	61.48	42.25
	Ash content	1.08	2.33	0.30	0.50
	Water Absorbed	58.82	50.28	41.03	30.18
	pH	6.83	6.89	4.63	7.79
	Fat	1.89	0.36	2.55	2.80
	Soluble Carbohydrate	12.62	11.08	4.77	17.58
	Total Carbohydrate	21.32	23.16	22.20	38.59
	Protein	2.13	4.31	9.74	3.70

Table 11 b: Nutrient content of fruit bodies of *P. ostreatus* originated from UCC School of Agric Farm and grown on different substrates.

Treatment	Nutrient	Percentage nutrient content of fruit body on			
		Sawdust	Corn cob	Oil palm fruit Fibre	Rice husk
Decom-Posed substrate	Dry matter	95.40	90.55	86.13	92.28
	Ash content	0.10	0.60	0.30	0.10
	Water Absorbed	51.09	53.30	40.15	34.01
	pH	6.32	7.34	6.69	6.10
	Fat	1.67	2.65	1.33	0.93
	Soluble Carbohydrate	10.11	9.70	25.43	14.91
	Total Carbohydrate	13.27	21.14	10.68	27.18
	Protein	3.57	3.25	3.80	2.84
Undecom-posed substrate	Dry matter	95.15	94.00	89.05	92.58
	Ash content	0.55	1.00	0.55	0.50
	Water Absorbed	50.83	47.97	39.68	26.68
	pH	7.20	6.31	6.75	7.94
	Fat	1.60	2.81	5.48	3.46
	Soluble Carbohydrate	9.43	8.31	10.77	14.60
	Total Carbohydrate	25.12	29.63	20.24	28.89
	Protein	2.89	3.18	3.74	2.48

Table 11 c: Nutrient content of fruit bodies of *P. ostreatus* originated from FRI, Accra and grown on different substrates.

Treatment	Nutrient	Percentage nutrient content of fruit bodies on			
		Sawdust	Corn cob	Oil palm fruit fibre	Rice husk
Decomposed substrate	Dry matter	60.20	57.97	66.66	64.80
	Ash content	0.54	0.50	0.30	0.15
	Water Absorbed	60.09	53.30	60.15	42.01
	pH	6.62	7.04	6.97	6.50
	Fat	3.67	3.65	8.33	1.93
	Soluble Carbohydrate	1.75	5.35	5.33	17.39
	Total Carbohydrate	10.65	23.74	8.68	24.13
	Protein	5.57	8.57	4.32	1.80
Undecomposed substrate	Dry matter	50.11	53.00	65.05	62.58
	Ash content	0.50	0.50	0.32	0.10
	Water Absorbed	60.83	51.97	59.68	46.60
	pH	6.42	6.22	6.64	7.83
	Fat	2.60	1.81	6.48	0.46
	Soluble Carbohydrate	1.16	7.24	4.26	18.01
	Total Carbohydrate	0.12	22.23	2.26	26.11
	Protein	4.80	7.09	3.71	1.46

x1/3

Plate 11 a: Mature fruit bodies of *P. ostreatus* growing on sawdust substrate.

x1/3

Plate 11 b: Mature fruit bodies of *P. ostreatus* growing on corn cob substrate.

 **x1/3**

Plate 11 c: Mature fruit bodies of *P. ostreatus* growing on oil palm fruit fibre substrate.

 x**1/3**

Plate 11 d: Mature fruit bodies of *P. ostreatus* growing on rice husk substrate.

(xiii) The state of some harvested fruit bodies of *P. ostreatus*

The state of some harvested fruit bodies of *P. ostreatus* is presented in Plate 12 a, 12 b, 12 c and 12 d.

Plate 12 a presents some fresh and healthy matured fruit bodies of *P. ostreatus* harvested from decomposed substrate bags.

Plate 12 b and Plate 12 c show a diseased and matured fruit bodies of *P. ostreatus* harvested from an undecomposed and decomposed substrate bags respectively. While the basidiocarp in Plate 12 b appears rotten and discoloured, that in Plate 12 b appears to have been eaten by rodents.

Plate 12 d on the other hand, shows harvested fruit bodies allowed to mature and overgrow. In their overgrown state, they appear very soft and exuding water. They also appear to have some organisms growing on it.

x1/8

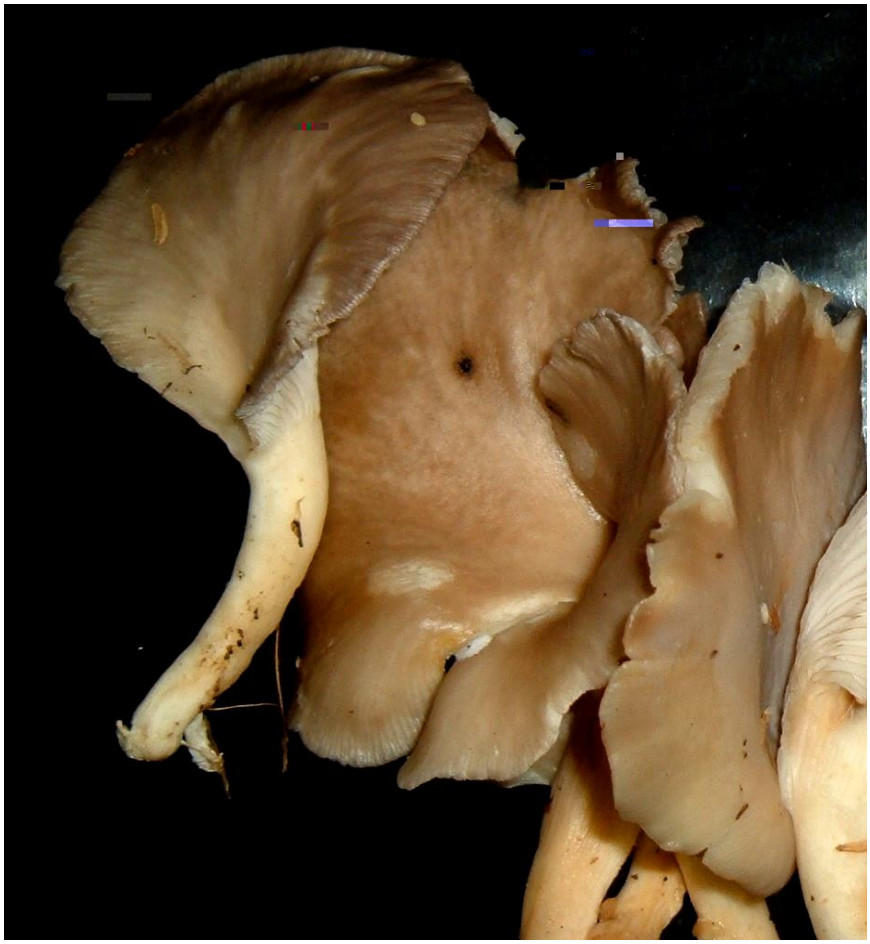
Plate 12: Some harvested *P. ostreatus* fruit bodies.
12 a) Healthy fresh *P. ostreatus* fruit bodies.

x1/2

Plate 12 b) A diseased *P. ostreatus* fruit body.

x1/3

Plate 12 c) Remains of *P. ostreatus* fruit bodies eaten by rodents.



x1/4

Plate 12 d) Overgrown harvested *P. ostreatus* fruit bodies

(xiv) Chemical composition of substrate composts after cropping

Substrate composts after cropping are called spent compost. The chemical composition of these substrate composts was also determined and the results obtained are presented in Table: 12 a, 12, b and 12 c.

Table 12 a indicates the chemical composition of substrates, decomposed and undecomposed, on which spawn of tissue of *P. ostreatus* was grown. Table 12 b indicates results of tests with substrate inoculated with tissue of *P. ostreatus* from the UCC School of Agric Farm whilst Table 12 c, indicates those of substrates, inoculated with spawn from FRI.

Once more, the chemical compositions of the substrates differed. The trend followed the same pattern as those of Table 11 a, 11 b and 11 c. There was no specific pattern as the percentage of the nutrient content depended on the type of nutrient.

However, two unique trends emerged. The nutrient contents of decomposed substrates were always higher than those of their undecomposed counterparts. The other observation was that substrates after harvesting recorded higher nutrient values than before they were inoculated with grain spawns. Sequentially, palm fibre showed highest: dry matter (76.66 %), fats (9.55 %) and protein content (10.78 %). Rice husk and corn cob on the other hand, recorded total carbohydrate contents of 66.92 % and 20.36 % respectively, and total carbohydrate content was least in Sawdust (7.75 %). The same trends were seen in its undecomposed substrate (Tables 12 a, Table 12 b and Table 12 c).

Statistically, the differences among the pH values were not significant ($p > 0.05$; Appendix XXX). There were however significant differences in relation to all the parameters in fats ($p = 0.000$), The only exception was the sources which did not show any differences ($p = 0.712$).There were also significant differences in the protein and carbohydrate contents with the exception of the source, combination of source and treatment and then substrate and treatment (Appendix XXXI, XXXII and XXXIII).

Table 12 a: Level of nutrients of spent compost after growth and fruiting of spawn of tissues of *P. ostreatus* from Dzorwulu Farm, Accra.

		Percentage of substrate			
Treatment	Nutrient	Sawdust	Corn cob	Oil palm fruit Fibre	Rice husk
Decom-posed substrate	Dry matter	57.20	51.97	76.66	64. 80
	Ash content	0.24	0.50	0.30	0.15
	Water Absorbed	78.09	70.30	54.15	52.10
	pH	7.07	7.16	7.12	7.88
	Fat	3.22	5.56	9.55	2.42
	Soluble Carbohydrate	6.75	7.04	6.09	7.27
	Total Carbohydrate	7.75	20.36	19.12	66.92
	Protein	6.06	5.18	10.78	2.98
Undecom-posed substrate	Dry matter	50.09	51.13	66.48	62.33
	Ash content	0.24	0.10	0.30	0.10
	Water Absorbed	80.83	71.97	59.68	49.60
	pH	7.14	7.34	0.04	6.92
	Fat	2.24	0.83	5.91	1.45
	Soluble Carbohydrate	6.43	6.31	5.77	6.91
	Total Carbohydrate	7.31	18.41	13.27	49.29
	Protein	3.24	2.92	4.81	2.66

Table 12b: Level of nutrients of spent compost after growth and fruiting of spawn of tissue of *P. ostreatus* from UCC School of Agric Farm.

		Percentage of substrate			
Treatment	Nutrient	Sawdust	Corn cob	Oil palm fruit fibre	Rice husk
Decom-posed substarte	Dry matter	60.00	57.53	62.66	65.60
	Ash content	0.60	0.20	0.32	0.11
	Water Absorbed	74.00	73.28	59.68	46.49
	pH	6.73	7.13	7.03	7.04
	Fat	3.37	2.50	43.30	2.30
	Soluble Carbohydrate	5.60	15.26	1.87	14.37
	Total Carbohydrate	14.77	40.10	15.00	66.56
	Protein	5.54	3.25	7.71	2.84
Undecom-posed substarte	Dry matter	60.11	59.09	67.05	67.58
	Ash content	0.50	0.50	0.30	0.10
	Water Absorbed	67.24	51.68	46.62	41.01
	pH	6.94	7.05	7.02	6.76
	Fat	2.29	2.23	25.48	2.13
	Soluble Carbohydrate	5.11	8.36	1.71	12.60
	Total Carbohydrate	14.12	33.03	13.61	42.73
	Protein	3.61	3.20	6.19	2.81

Table 12c: Level of nutrients of spent compost after growth and fruiting of spawn of *P. ostreatus* from FRI, Accra.

Treatment	Nutrient	Percentage of substrate			
		Sawdust	Corn cob	Oil palm fruit fibre	Rice husk
Decom-posed substarte	Dry matter	57.20	51.97	76.66	64.80
	Ash content	0.24	0.50	0.30	0.15
	Water Absorbed	78.09	70.30	54.15	52.10
	pH	7.07	7.16	7.12	7.88
	Fat	5.42	4.19	9.13	4.55
	Soluble Carbohydrate	9.70	10.11	5.43	14.91
	Total Carbohydrate	13.27	21.14	10.68	27.18
	Protein	3.57	3.25	5.80	2.84
Undecom-posed substarte	Dry matter	50.09	51.13	66.48	62.33
	Ash content	0.24	0.10	0.30	0.10
	Water Absorbed	80.83	71.97	59.68	49.60
	pH	7.14	7.34	7 0.04	6.92
	Fat	2.88	4.12	8.33	2.24
	Soluble Carbohydrate	8.20	9.69	5.24	14.16
	Total Carbohydrate	13.02	19.63	6.24	21.89
	Protein	3.51	2.68	4.95	2.83

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Cultivation of *Pleurotus ostreatus* is a profitable agribusiness. Thus, its cultivation as a nonconventional crop in an already existing agricultural system has led to both social and economic improvement in the status of some farmers.

In the recent past, Ghana harvested mushrooms only during the rainy seasons until the advent of artificial inception and innovation, control and improvement of the Oyster mushroom made it generally acceptable as a flavourishing and a tasty product (Shah, Ashraf, Ishtiag, 2004).

As a result of the much heightened interest in mushroom research, studies were carried out, taking into consideration a number of factors which were thought to have helped upgrade and increase the income levels of the mushroom farmer. For instance, the availability of spawns is a limiting factor to the production of mushrooms in many countries including Ghana (Auetragul, 2001); thus spawning processes are seen as critical and are always handled with much care to attain better results.

In view of the above assertions, relevant studies were carried out. The results of one of the tests (Table 2) showed that spawn developed from tissues of the fruit body of *P. ostreatus* grow better on Potato Dextrose Agar than spawn developed from spores. Thus, tissue culture, a means of cloning a living specimen while preserving its genetic constitution was the better material to use as starting

material for obtaining pure cultures than the spore. Mother spawn from spores was always overgrown with fungal contaminants such as *Penicillium sp* and *Aspergillus sp*. The difficulty in obtaining pure cultures from spores was probably due to fungal spores in the air depositing on the spore prints (Bansah, 2005). For this reason, live tissues of Oyster mushrooms are recommended for Oyster mushroom spawn making and should be sampled from fresh, healthy and vigorous growing fruit bodies.

Meanwhile, temperature, light, humidity, physical shock, nutrients and other factors are said to affect the growth of spawns and fruiting in mushrooms (Oei, 1996). According to Auetragul (2001), countries with climatic conditions like that of Ghana could grow a range of tropical mushrooms like *Volvariella volvocea, Auricularia polytricha* and *Pleurotus sp*. The present study showed that *Pleurotus ostreatus* can grow over a wide range of temperatures between 15 °C and 30 °C and best at 25 °C when the spawn originated from basidiocarp tissue (Table 3 a) and at 30 °C when the spawn originated from spores (Table 3 b) and Figs (4-8). Unfortunately, that excludes the northern regions of Ghana as they sometimes have temperatures way over 30 ^{o}C, unless temperature controlled growth rooms.

P. ostreatus grew best in total darkness (Table 4 a - 4 c) followed by partial darkness (1.9 Lux). Results of experiments by Ishikawa (1967) and Eger (1978), which studied the effect of light exposure during a spawn run, support findings obtained in this study. They stated the quality and quantity of light required during the formation and maturation process of mushrooms. They therefore stated: “lines of *P. ostreatus* have been shown to differ in their minimal and optimal requirements.” Light is essential for the formation and maturation of reproductive

structures of many species of wood-rotting basidiomycetes (Kitamoto, Suzuki and Furukawa, 1972, Perkins and Gordon, 1969).

The study also attempted to hybridize spore cultures of different locations. The extent of growth of two different spores **(**from different locations) grown on the same Petri-plates was not the same (Plate 8). So far, only a limited number of genetic studies of *Pleurotus ostreatus* have been conducted. This is as a result of the difficulty in performing direct crosses among strains. Moreover, there are quite a lot of contradictory data about the size and organization of the genetic material leading to lack of genetic linkage map. Hence, breeding of new *P. ostreatus* strains with a higher agricultural or industrial value has been traditionally carried out by trial and error (Smith, 1993).

There have been several attempts made in studying the genome organization in *P. ostreatus* which have always been hampered by small size of fungal chromosomes. Consequently, different authors had reported different chromosome numbers and different genome sizes for this particular species (Sagawa and Nagata, 1992, Peberdy, Hanifa and Jia, 1993, Chiu, 1996). Later, when Pulse Field Gel electrophoresis and linkage mapping was discovered and used, at least eleven chromosomes per haploid genome were detected; adding up to a total genomic size of 35 Mb in average with each chromosome having a size between 1.4 Mb to 4.7 Mb. The breakthrough by molecular biologists also showed that chromosome-specific single-copy probes were used to resolve to the barest minimum the ambiguities caused by chromosome co-migration (Larraya *et al*., 2000).

Beyond hybridization, multiplication of mother spawns can be used for large scale production of *Pleurotus ostreatus*. Bansah (2005) found that

colonization of millet grain medium was fastest, followed by sorghum and wheat. According to that study, there was no growth on paddy rice medium which was one of the substrates tested. Thus, sorghum grains were used due to its availability on the local markets. Amazingly, spore cultures here grew a little more appreciably than tissue cultures. The addition of precipitated mixture of chalk lime ($CaCO_3$) and gypsum salt ($CaSO_4.2H_2O$) must have enhanced the medium-containing reproductive structures thus, resulting in better growth of spore culture than tissue culture (Plate 9).

Indisputably, Ghana abounds in a lot of food crops and for that matter a lot of agricultural wastes. Popularization of cultivated *Pleurotus ostreatus* among rural folks in Ghana may therefore be quite an easy task. The collection of edible mushrooms in the rural areas and their subsequent sale at the urban centres is an old tradition and generally regarded as gender related, i.e. work for women.

Over the years, mushrooms have gained popularity in tropical and sub-tropical regions. Organic waste can play an important role of managing organic wastes whose disposal has become problematic (Das and Mukherjee, 2007). Thus, Oyster mushroom can be cultivated in any type of lingocellulose material like straw, sawdust, rice hull etc. Hiami (1990) studied the oyster mushroom cultivation on different woods and found that *P. ostreatus* gave maximum yield. Presently, sawdust is commonly used and it is the preferred medium at commercial scale. Hiami (1990) reported that *P. ostreatus* gave maximum biological efficiency on sawdust. Of the sawdust types, softwood like mango and cashew are known to be more suitable than hardwood sawdust. They normally grow on piles of decaying rice straw, sawdust, corn cob, oil palm fruit fibre, coffee pulp, sugar-cane bagasse, oil palm extraction wastes etc (Quimio, 1986).

When they grow on these piles (Plates 4 a – d), they play an important role in recycling the organic matter by secreting hydrolytic enzymes into the local environment. These enzymes are therefore broken down into various polymers to produce soluble products which the mushroom absorbs (Heritage, *et al*., 1996). When it is in an artificial culture such as the one prepared in compost bags (Plates 10 a – d) for cultivation, it contains mineral salts and nitrogen salts. They also contain glucose which serves as a carbohydrate source. This source then manufactures all the complex organic molecules that they require for growth from the metabolism of glucose. Nevertheless, glycogen is the principal storage polymer used by fungi of which mushroom is an example. Here, oil droplets are used as nutrient storage, thereby allowing fungi that are obligate aerobes to obtain energy from the aerobic respiration of the glucose (Heritage, Evans and Killington, 1996).

According to Rajarathnam and Bano (1989), preparation of a substratum for cultivation of *Pleurotus ostreatus* mushrooms includes wetting, cooking and spawning of mycelia. Steaming eliminates moulds and spores which negatively affect production, weakens lignocelluloses' connections and increases the efficiency of mushroom enzyme systems activity. Without the application of heat treatments to eliminate the destructive enzymes (Sivan and Chet 1989; Geremia *et al.*, 1993; Ait-Lahssen *et al.*, 2001). and organisms, there could be extensive losses in mushroom production (Badham, 1991; Jandaik and Guleria, 1999). Well–prepared and well-kept substrata do not contain mycotoxins such as aflatoxin, ochratoxin, zearalenon, patulin, sterigmatocistine, penicillin acid, citrinine, *Trichoderma* sp and soil filamentous fungi (Rajarathnam and Bano, 1989).

Basically, spent substrata consist of lignocellulose complex remains which are undecomposed or unused decomposed parts of mushroom mycelia. By the

activity of laccase, cellulase, celobiase, hemicellulase, ligninase enzymes and others, the lignocellulose complex is decomposed into simpler organic compounds which mushrooms use as nutritious substances. For that matter, the chemical composition of the substrata changes in comparison with the initial material (Flegg, Spencer and Woode, 1985).

As a result, the lignolytic content of the various substrates was studied before inoculation and after harvesting. They were studied because lignocellulosic waste products were used as culture substrates (Yildiz, Yildiz, Gezer and Temez, 2002). Study of the lignin content before inoculation showed higher lignin content (Table 9) than after harvesting (Tables 10 a and 10 b) when the analyses of the two situations were compared; with rice having the highest in both cases. Lignin, a three-dimensional network polymer in wood inhibits its use as feed for ruminant. It is feebly digestible and so proportion of lignin in cell wall increases as plant ripens. In the process, their molecules connect with the molecules of carbohydrates to decrease their digestibility to about 30 – 50% of cellulose and hemicelluloses decomposed in the tripe (Kirk and Chang, 1975; Rajarathnam and Bano, 1989). In order to use wood waste as a source material for ruminants, the lignin has to be modified or degraded by fungi such as Oyster mushroom (Apetorgbor, Darkwa and Ofosu-Asiedu, 1998). It was therefore not surprising to have poor mycelial growth in rice but best growth in oil palm fruit fibre.

Mushroom substrate study by Owusu Boateng and Dzogbefia (2005), indicated that decompose substrates are highly selective and nutritionally rich for growth of mushrooms which is in line with the amounts of nutrients in the prepared decomposed compost being noticeably higher than those in undecomposed

substrate. In addition, fruit bodies on decomposed substrates grew considerably faster and healthier than those on undecomposed substrates.

The observation of good yield (g) indicates that there was enough amounts of key nutrients present in the substrate (Chang and Quimio, 1982); (Tables 7 a - 7 b).

Just as healthy and fresh mature fruit bodies of *Pleurotus ostreatus* were obtained from mostly decomposed substrates so also were diseased fruit bodies obtained from mostly undecomposed substrates. The probable disease that attacked some of the basidiocarp of the Oyster mushroom (Plate 12 b) was yellow blotch disease by *Pseudomonas agarici*. It is characterized by primordia, with yellow droplets on their surface, which become stunted, yellow to orange, and deformed as they mature. When primordia develop on a vertical or nearly vertical surface, the resultant sporocarps grow semi laterally, forming tight clusters (Bessette, Kerrigan and Jordan, 1985). There were also those (Plate 12 c) that were suspected to have been eaten by rodents or springtail (*Lepidiocrytus sp*). Any fruit body of Oyster (Plate 12 d) that is allowed to overgrow and exude water was found to be habited by some black organisms as well as white organisms (*Cladobotryum apiculatum*). The responsible organism has been identified as *Pseudomonas tolaasi.* Excessive moisture and the presence of water on fruiting body promote its development (Chang and Miles, 2004).

Further, the analysis of substrates after harvesting suggested the pH of all the substrate composts changed. Most often, spent *Pleurotus ostreatus* substrata is said to have specific taste and smell and so are usually thought to have 20% of dry matter of a meal for cattle consumption. Subsequently, cattle consume less of it

when served alone but have better consumption when mixed with silage (Platt, Hadar and Chet, 1984).

Silaging gives the substrata some better organoleptic qualities and increases the consumption of substrata. Spent substrata have sufficient humidity to be silage. But, exploited substrata do not contain enough soluble carbohydrates, so the silaging must include supplement which has enough carbohydrates (plant or grits). That way, microorganisms are supplied with enough sugar, which when decomposed gives lactic acid, the main preserving agent and imparting also taste and aroma (Heritage *et al.*, 1996).

Proximate analysis of fruit bodies and substrate after harvesting showed an appreciable fat, protein and carbohydrate contents. They also had enough percentages of dry matter, ash content, water content and a reasonable pH level. The changes observed in the chemical composition of both fruit bodies and spent compost have been caused by the conditions of cultivation including: the number of fruit body flushed and gathered as well as the level of compost exploitation (Chang and Miles, 1989). The higher nutritional content of spent compost than substrate before inoculation might have been due to the activities of the mycelia in the substratum. Thus, after the finalization of the mushroom flushing and gathering, spent substrata could be used in the feeding of ruminants because their guts contain resident micro-organisms that digest cellulose and hemicelluloses.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

In conclusion:

Culturing of tissue and spore of mushrooms grew best at 30 °C and in total darkness, while already prepared spawn culture also grew best in total darkness but at temperature of 25 °C.

The fruit bodies, from which the spores used for hybridization, were derived from the same source population.

Decomposed and undecomposed substrates supported the growth of mushroom; however, decomposed substrates produced a better yield.

Although all the four kinds of agriculture wastes were good for mushroom cultivation, the palm fibre was the best producing the highest mean number and weight of fruit bodies in five weeks followed by corn cob, sawdust and rice husk in that order.

Proximate analysis showed that the Oyster mushrooms contained fats; carbohydrates, and proteins.

The results of the proximate analyses showed an increase in the substrates had a corresponding increase in the nutrient content of the fruit body.

There was a considerable reduction in the lignin content of the substrates after their use in raising oyster mushroom.

The fat, total carbohydrates and protein levels in the mature fruit bodies depended on the type of substrate used. Fat and protein levels were highest in

mature fruit bodies formed on oil palm fruit fibre and the total carbohydrate level, highest in mature fruit bodies on rice husk.

RECOMMENDATIONS

It is recommended that:

1. The cultures from live and fresh healthy fruits of tissues grew faster and easier than cultures from the spore of mushroom fruit bodies; therefore, mushroom spawns should be prepared from vigour and large fruit body tissues rather than spores for commercial purposes.
2. Investigations into the nature of tissues and spore colonies of fruit bodies as well as colonies of already prepared spawns should be conducted because they seem to give different and interesting growth patterns.
3. Farmers should take advantage of the temperature range of *Pleurotus ostreatus* and grow them during their lean season for extra income.
4. For further studies, investigations should be conducted into using the four substrates in various combinations as composite composts for mushroom production.
5. Growers, Environmentalists, Governments and Communities should start considering composting agricultural wastes and utilizing them as substrates for mushroom farming as a way of reducing environmental pollution.

SUMMARY

1) The effect of composting substrates on the nutritional content of the Oyster mushroom, *Pleurotus ostreatus* was studied.
2) Fruit bodies of *P. ostreatus* were obtained from two different sources ('Dzorwulu' Farm, Accra and School of Agric Farm of the University of Cape Coast).
3) A bottle of spawn was obtained from FRI, Accra.
4) These were grown in Petri-plates containing potato dextrose agar (PDA).
5) The mycelia colonizing the PDA were observed within two weeks.
6) They were sub cultured where necessary but grown under different range of temperatures and light intensities.
7) In each case, the diameters of the colonies were measured and recorded for fourteen days as: 9.0 cm and 8.5 cm for tissues and spores cultures produced by Dzorwulu Farm, Accra fruit bodies and 8.7 cm and 8.4 cm for tissues and spores cultures produced by School of Agriculture Farm, UCC respectively.
8) Grains of sorghum were obtained; grain medium was prepared and used as substrate for spawn multiplication. This was also observed for two weeks.
9) Four types of agricultural wastes (sawdust, corn cob, palm fibre and rice husk) were collected from different sources.
10) One set was decomposed for thirty days while the other set was wetted and bagged immediately without allowing it to undergo decomposition.
11) They were later sterilized and inoculated with prepared grain medium.
12) It took twenty–eight days for the mycelium to grow and ramify though the various sterilized bagged compost.

13) After one month of colonization, bags were transferred to a cropping house and opened.
14) Three to five days later, fruit bodies started appearing.
15) Fruit bodies were harvested when mature and parameters like yield, length, width, weight and proximate readings were taken. as:2.2, 14.2 cm, 3.9 cm, 36.70 g for decomposed sawdust whilst undecomposed sawdust recorded 1.0, 5.3 cm, 1.9 cm and 3.88 g in that order. In addition to sawdust, others such as: corn cob, oil palm fruit fibre and rice husk had similar pattern when inoculated with P. *ostreatus*.
16) Proximate analysis of substrates was determined before inoculation and after harvesting.

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APPENDICES

Appendix 1

Two-way ANOVA for Table 1: Length

Source	DF	SS	MS	F	P
locality	1	1.568	1.568	0.39	0.541
culture type	1	3.042	3.042	0.76	0.397
locality*culture type	1	0.128	0.128	0.03	0.860
Error	16	64.144	4.009		
Total	19	68.882			

S = 2.00225 R-Sq = 6.88% R-Sq(adj) = 0.00%

Appendix I1

Two-way ANOVA for Table 1: Weight

Source	DF	SS	MS	F	P
locality_1	1	1.56	1.56	0.04	0.853
culture type_1	1	614.29	614.29	14.01	0.002
locality_1*culture type_1	1	203.60	203.60	4.64	0.047
Error	16	701.56	43.85		
Total	19	1521.01			

S = 6.62176 R-Sq = 53.88% R-Sq(adj) = 45.23%

Appendix I1I

Two-way ANOVA for Table 2: Colony diameter in terms of spore and tissue cultures

Source	DF	SS	MS	F	P
locality_2	1	0.008	0.008	0.00	0.975
culture type_2	1	10.982	10.982	1.37	0.259
locality_2*culture type_2	1	0.008	0.008	0.00	0.975
Error	16	128.354	8.022		
Total	19	139.352			

S = 2.83234 R-Sq = 7.89% R-Sq(adj) = 0.00%

Appendix IV

Two-way ANOVA for Table 3 a: Colony diameter of Accra and School farm but grown at different temperatures

TABLE 5

Analysis of Variance for RES

Source	DF	SS	MS	F	P
LOCA	1	19.388	19.388	15.15	0.000
temp.	5	932.987	186.597	145.82	0.000
CULTURE	1	36.725	36.725	28.70	0.000
LOCA*temp.	5	23.274	4.655	3.64	0.003
LOCA*CULTURE	1	10.939	10.939	8.55	0.004
temp.*CULTURE	5	37.985	7.597	5.94	0.000
Error	485	620.609	1.280		
Total	503	1681.906			

S = 1.13120 R-Sq = 63.10% R-Sq(adj) = 61.73%

Appendix V

Two-way ANOVA for Table 3 b: Spawn colony of diameter of CSIR grown under different temperatures

Factor	Type	Levels	Values
day	fixed	7	1, 2, 3, 4, 5, 6, 7
temp._1	fixed	6	1, 2, 3, 4, 5, 6

Analysis of Variance for RES_1

Source	DF	SS	MS	F	P
day	6	77.247	12.874	322.50	0.000
temp._1	5	481.381	96.276	2411.69	0.000
day*temp._1	30	99.406	3.314	83.00	0.000
Error	84	3.353	0.040		
Total	125	661.387			

S = 0.199801 R-Sq = 99.49% R-Sq(adj) = 99.25%

Appendix VI

Two-way ANOVA for Table 4: Colony diameter in terms of tissue culture (Accra, School farm) and spawn colony radius of (CSIR) grown under different light intensities

Factor	Type	Levels	Values
locality	fixed	3	1, 2, 3
days	fixed	7	1, 2, 3, 4, 5, 6, 7
light inten	fixed	4	1, 2, 3, 4

Analysis of Variance for res

Source	DF	SS	MS	F	P
locality	2	51.386	25.693	139.09	0.000
days	6	419.991	69.998	378.95	0.000
light inten	3	952.000	317.333	1717.94	0.000
locality*days	12	19.038	1.587	8.59	0.000
locality*light inten	6	29.991	4.998	27.06	0.000
days*light inten	18	219.800	12.211	66.11	0.000
Error	204	37.682	0.185		
Total	251	1729.889			

S = 0.429788 R-Sq = 97.82% R-Sq(adj) = 97.32%

Appendix VII

Two-way ANOVA for Table 5 a: Tissue length versus locality, days on sorghum grain

Factor	Type	Levels	Values
locality	fixed	3	1, 2, 3
days	fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance for tissue length

Source	DF	SS	MS	F	P
locality	2	41.603	20.801	1221.78	0.000
days	6	383.074	63.846	3750.02	0.000
locality*days	12	7.576	0.631	37.08	0.000
Error	42	0.715	0.017		
Total	62	432.967			

S = 0.130481 R-Sq = 99.83% R-Sq(adj) = 99.76%

Appendix VIII

Two-way ANOVA for Table 5 b: Spore length versus locality, days on sorghum grain

Factor	Type	Levels	Values
locality	fixed	3	1, 2, 3
days	fixed	7	1, 2, 3, 4, 5, 6, 7

Analysis of Variance for spore length

Source	DF	SS	MS	F	P
locality	2	20.408	10.204	603.90	0.000
days	6	538.502	89.750	5311.66	0.000
locality*days	12	20.795	1.733	102.56	0.000
Error	42	0.710	0.017		
Total	62	580.414			

S = 0.129988 R-Sq = 99.88% R-Sq(adj) = 99.82%

Appendix IX

General Linear Model for Table 6 a, 6 b 6 c, 6 d: Length versus locality, days, of Accra and School Farm spawns on substrate composts

Factor	Type	Levels	Values
locality	fixed	2	1, 2
days	fixed	7	1, 2, 3, 4, 5, 6, 7
culture	fixed	2	1, 2
substrate	fixed	4	1, 2, 3, 4
treatment	fixed	2	1, 2

Analysis of Variance for Length, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
locality	1	6.9	7.3	7.3	1.09	0.297
days	6	12497.1	2819.4	469.9	70.26	0.000
culture	1	164.3	167.8	167.8	25.09	0.000
substrate	3	1096.6	1084.9	361.6	54.07	0.000
treatment	1	36.8	36.8	36.8	5.50	0.019
Error	656	4387.2	4387.2	6.7		
Total	668	18188.8				

S = 2.58608 R-Sq = 75.88% R-Sq(adj) = 75.44%

Appendix X

Two-way ANOVA for Table 6 e: Length versus locality, days of CSIR spawns on substrate composts

Factor	Type	Levels	Values
day	fixed	7	1, 2, 3, 4, 5, 6, 7
sub	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for csir

Source	DF	SS	MS	F	P
day	6	3641.56	606.93	227.08	0.000
sub	3	695.37	231.79	86.72	0.000
treat	1	87.00	87.00	32.55	0.000
day*sub	18	146.08	8.12	3.04	0.000
day*treat	6	37.70	6.28	2.35	0.035
sub*treat	3	311.41	103.80	38.84	0.000
Error	130	347.46	2.67		
Total	167	5266.59			

S = 1.63486 R-Sq = 93.40% R-Sq(adj) = 91.52%

Appendix XI

Two-way ANOVA for Table 7 a and 7 b: Number versus substrate_1, treatment_1

Factor	Type	Levels	Values
substrate_1	fixed	4	1, 2, 3, 4
treatment_1	fixed	2	1, 2

Analysis of Variance for YIELD

Source	DF	SS	MS	F	P
substrate_1	3	33.80	11.27	0.83	0.488
treatment_1	1	22.50	22.50	1.65	0.208
substrate_1*treatment_1	3	32.90	10.97	0.81	0.500
Error	32	435.20	13.60		
Total	39	524.40			

S = 3.68782 R-Sq = 17.01% R-Sq(adj) = 0.00%

Appendix XII

Two-way ANOVA for Table 7 a and 7 b: Length versus substrate_1, treatment_1

Factor	Type	Levels	Values
substrate_1	fixed	4	1, 2, 3, 4
treatment_1	fixed	2	1, 2

Analysis of Variance for LENGTH

Source	DF	SS	MS	F	P
substrate_1	3	32.93	10.98	0.49	0.691
treatment_1	1	4.42	4.42	0.20	0.660
substrate_1*treatment_1	3	75.08	25.03	1.12	0.356
Error	32	715.90	22.37		
Total	39	828.33			

S = 4.72990 R-Sq = 13.57% R-Sq(adj) = 0.000

Appendix XIII

Two-way ANOVA for Table 7 a and 7 b: Yield versus substrate_1, treatment_1

Factor	Type	Levels	Values
substrate_1	fixed	4	1, 2, 3, 4
treatment_1	fixed	2	1, 2

Analysis of Variance for WGHT

Source	DF	SS	MS	F	P
substrate_1	3	28.66	9.55	0.83	0.490
treatment_1	1	8.46	8.46	0.73	0.399
substrate_1*treatment_1	3	128.39	42.80	3.70	0.022
Error	32	370.46	11.58		
Total	39	535.98			

S = 3.40250 R-Sq = 30.88% R-Sq(adj) = 15.76%

Appendix XIV

Two-way ANOVA for Table 8 a: Dry matter of substrate before inoculation

Factor	Type	Levels	Values
d m	fixed	4	1, 2, 3, 4

Analysis of Variance for dry M

Source	DF	SS	MS	F	P
d m	3	260.358	86.786	1140.04	0.000
Error	4	0.304	0.076		
Total	7	260.662			

S = 0.275908 R-Sq = 99.88% R-Sq(adj) = 99.80%

Appendix XV

Two-way ANOVA for Table 8 a: Water content of substrate before inoculation

Factor	Type	Levels	Values
wat	fixed	4	1, 2, 3, 4

Analysis of Variance for water

Source	DF	SS	MS	F	P
wat	3	224656	74885	71413.80	0.000
Error	4	4	1		
Total	7	224660			

S = 1.02402 R-Sq = 100.00% R-Sq(adj) = 100.00%

Appendix XVI

Two-way ANOVA for Table 8 a: Ash content of substrate before inoculation

Factor	Type	Levels	Values
ash	fixed	4	1, 2, 3, 4

Analysis of Variance for ash

Source	DF	SS	MS	F	P
a sh	3	612.75	204.25	163400.15	0.000
Error	4	0.00	0.00		
Total	7	612.76			

S = 0.0353553 R-Sq = 100.00% R-Sq(adj) = 100.00%

Appendix XVII

Two-way ANOVA for Table 8 a: pH of substrate before inoculation

Factor	Type	Levels	Values
pH	fixed	4	1, 2, 3, 4

Analysis of Variance for ph

Source	DF	SS	MS	F	P
p h	3	0.38570	0.12857	467.52	0.000
Error	4	0.00110	0.00027		
Total	7	0.38680			

S = 0.0165831 R-Sq = 99.72% R-Sq(adj) = 99.50%

Appendix XVIII

Two-way ANOVA for Table 8 b: Fat content of substrate before inoculation

Factor	Type	Levels	Values
subst	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for fat res

Source	DF	SS	MS	F	P
subst	3	6586.0	2195.3	23145.48	0.000
treat	1	385.3	385.3	4062.59	0.000
subst*treat	3	432.1	144.0	1518.45	0.000
Error	8	0.8	0.1		
Total	15	7404.2			

S = 0.307977 R-Sq = 99.99% R-Sq(adj) = 99.98%

Appendix XIX

Two-way ANOVA for Table 8 b: Substrate carbohydrate content before inoculation

Factor	Type	Levels	Values
subst	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for cabo res

Source	DF	SS	MS	F	P
subst	3	84.896	28.299	27.34	0.000
treat	1	24.185	24.185	23.36	0.001
subst*treat	3	9.361	3.120	3.01	0.094
Error	8	8.281	1.035		
Total	15	126.723			

S = 1.01743 R-Sq = 93.47% R-Sq(adj) = 87.75%

Appendix XX

Two-way ANOVA for Table 8 b: Substrate protein content before inoculation

Factor	Type	Levels	Values
subst	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for pro res

Source	DF	SS	MS	F	P
subst	3	19.1608	6.3869	417.32	0.000
treat	1	1.3644	1.3644	89.15	0.000
subst*treat	3	2.9598	0.9866	64.46	0.000
Error	8	0.1224	0.0153		
Total	15	23.6074			

S = 0.123712 R-Sq = 99.48% R-Sq(adj) = 99.03%

Appendix XXI

Two-way ANOVA for Table 9: Lignolytic content before inoculation

Factor	Type	Levels	Values
lignin	fixed	2	1, 2
substr	fixed	4	1, 2, 3, 4
treatmt	fixed	2	1, 2

Analysis of Variance for RES

Source	DF	SS	MS	F	P
lignin	1	20038.1	20038.1	9455.81	0.000
substr	3	3193.7	1064.6	502.36	0.000
treatmt	1	54.6	54.6	25.75	0.000
lignin*substr	3	3208.9	1069.6	504.74	0.000
lignin*treatmt	1	58.3	58.3	27.51	0.000
substr*treatmt	3	7.3	2.4	1.14	0.357
Error	19	40.3	2.1		
Total	31	26601.0			

S = 1.45572 R-Sq = 99.85% R-Sq(adj) = 99.75%

Appendix XXII

Two-way ANOVA for Table 10 a and 10 b: Substrate lignolytic content after harvesting

Factor	Type	Levels	Values
lignin_1	fixed	2	1, 2
locality	fixed	2	1, 2
substr_1	fixed	4	1, 2, 3, 4
treatmt_1	fixed	2	1, 2

Analysis of Variance for RES_1

Source	DF	SS	MS	F	P
lignin_1	1	28936.9	28936.9	22399.39	0.000
locality	1	111.3	111.3	86.13	0.000
substr_1	3	6458.1	2152.7	1666.37	0.000
treatmt_1	1	70.0	70.0	54.16	0.000
lignin_1*locality	1	111.3	111.3	86.14	0.000
lignin_1*substr_1	3	6458.0	2152.7	1666.34	0.000
lignin_1*treatmt_1	1	69.9	69.9	54.13	0.000
locality*substr_1	3	17.6	5.9	4.55	0.007
locality*treatmt_1	1	0.4	0.4	0.29	0.591
substr_1*treatmt_1	3	13.7	4.6	3.53	0.022
Error	45	58.1	1.3		
Total	63	42305.4			

S = 1.13660 R-Sq = 99.86% R-Sq(adj) = 99.81%

Appendix XXIII

Two-way ANOVA for Table 11: Dry matter reading of mushroom after harvesting

Factor	Type	Levels	Values
SOURCE.	fixed	2	1, 2
SUBSTRATE	fixed	4	1, 2, 3, 4
TREATMENT	fixed	2	1, 2

Analysis of Variance for DRY M

Source	DF	SS	MS	F	P
SOURCE.	1	0.012	0.012	0.01	0.929
SUBSTRATE	3	59.857	19.952	14.00	0.000
TREATMENT	1	7.211	7.211	5.06	0.037
SOURCE.*SUBSTRATE	3	55.800	18.600	13.05	0.000
SOURCE.*TREATMENT	1	1.810	1.810	1.27	0.274
SUBSTRATE*TREATMENT	3	21.966	7.322	5.14	0.009
Error	19	27.074	1.425		
Total	31	173.728			

S = 1.19372 R-Sq = 84.42% R-Sq(adj) = 74.57%

Appendix XXIV

Two-way ANOVA for Table 11: Ash content reading of mushroom after harvesting versus source, substrate treatment

Factor	Type	Levels	Values
SOURCE.	fixed	2	1, 2
SUBSTRATE	fixed	4	1, 2, 3, 4
TREATMENT	fixed	2	1, 2

Analysis of Variance for ASH

Source	DF	SS	MS	F	P
SOURCE.	1	1.2129	1.2129	1.45	0.244
SUBSTRATE	3	1.6083	0.5361	0.64	0.598
TREATMENT	1	2.0352	2.0352	2.43	0.135
SOURCE.*SUBSTRATE	3	1.9331	0.6444	0.77	0.525
SOURCE.*TREATMENT	1	1.0047	1.0047	1.20	0.287
SUBSTRATE*TREATMENT	3	3.5408	1.1803	1.41	0.271
Error	19	15.9078	0.8373		
Total	31	27.2427			

S = 0.915015 R-Sq = 41.61% R-Sq(adj) = 4.73%

Appendix XXV

Two-way ANOVA for Table 11: Water absorption capacity reading of mushroom after harvesting versus source, substrate treatment

Factor	Type	Levels	Values
SOURCE.	fixed	2	1, 2
SUBSTRATE	fixed	4	1, 2, 3, 4
TREATMENT	fixed	2	1, 2

Analysis of Variance for WATER

Source	DF	SS	MS	F	P
SOURCE.	1	123.9	123.9	3.04	0.098
SUBSTRATE	3	22161.2	7387.1	181.08	0.000
TREATMENT	1	37.4	37.4	0.92	0.350
SOURCE.*SUBSTRATE	3	2810.3	936.8	22.96	0.000
SOURCE.*TREATMENT	1	83.8	83.8	2.05	0.168
SUBSTRATE*TREATMENT	3	1933.9	644.6	15.80	0.000
Error	19	775.1	40.8		
Total	31	27925.5			

S = 6.38704 R-Sq = 97.22% R-Sq(adj) = 95.47%

Appendix XXVI

Two-way ANOVA for Table 11: pH reading of mushroom after harvesting versus source, substrate treatment

Factor	Type	Levels	Values
SOURCE.	fixed	2	1, 2
SUBSTRATE	fixed	4	1, 2, 3, 4
TREATMENT	fixed	2	1, 2

Analysis of Variance for PH

Source	DF	SS	MS	F	P
SOURCE.	1	0.0306	0.0306	0.14	0.712
SUBSTRATE	3	4.2551	1.4184	6.51	0.003
TREATMENT	1	0.0026	0.0026	0.01	0.914
SOURCE.*SUBSTRATE	3	2.1298	0.7099	3.26	0.044
SOURCE.*TREATMENT	1	1.6517	1.6517	7.59	0.013
SUBSTRATE*TREATMENT	3	6.4362	2.1454	9.85	0.000
Error	19	4.1371	0.2177		
Total	31	18.6430			

S = 0.466628 R-Sq = 77.81% R-Sq(adj) = 63.79%

Appendix XXVII

Two-way ANOVA for Table 11: Accra and CSIR mushroom fat content after harvesting

Factor	Type	Levels	Values
source	fixed	2	1, 2
substrt	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for fat res

Source	DF	SS	MS	F	P
source	1	0.687	0.687	0.13	0.718
substrt	3	183.532	61.177	11.95	0.000
treat	1	181.594	181.594	35.48	0.000
source*substrt	3	79.645	26.548	5.19	0.009
source*treat	1	0.044	0.044	0.01	0.927
substrt*treat	3	171.290	57.097	11.16	0.000
Error	19	97.241	5.118		
Total	31	714.034			

S = 2.26229 R-Sq = 86.38% R-Sq(adj) = 77.78%

Appendix XXVIII

Two-way ANOVA for table 11: Accra and CSIR mushroom carbohydrate content after harvesting versus source, substrate, treatment

Factor	Type	Levels	Values
source	fixed	2	1, 2
substrt	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for cabo res

Source	DF	SS	MS	F	P
source	1	343.98	343.98	50.51	0.000
substrt	3	1032.78	344.26	50.55	0.000
treat	1	358.46	358.46	52.63	0.000
source*substrt	3	416.47	138.82	20.38	0.000
source*treat	1	32.87	32.87	4.83	0.041
substrt*treat	3	53.73	17.91	2.63	0.080
Error	19	129.40	6.81		
Total	31	2367.68			

S = 2.60966 R-Sq = 94.53% R-Sq(adj) = 91.08%

Appendix XXIX

Two-way ANOVA for Table 11: Accra and CSIR mushroom protein content after harvesting versus source, substrate, treatment

Factor	Type	Levels	Values
source	fixed	2	1, 2
substrt	fixed	4	1, 2, 3, 4
treat	fixed	2	1, 2

Analysis of Variance for pro res

Source	DF	SS	MS	F	P
source	1	150.624	150.624	112.19	0.000
substrt	3	102.451	34.150	25.44	0.000
treat	1	56.665	56.665	42.21	0.000
source*substrt	3	111.486	37.162	27.68	0.000
source*treat	1	1.001	1.001	0.75	0.399
substrt*treat	3	15.222	5.074	3.78	0.028
Error	19	25.509	1.343		
Total	31	462.959			

S = 1.15870 R-Sq = 94.49% R-Sq(adj) = 91.01%

Appendix XXX

Two-way ANOVA for Table 12: pH reading of substrates after harvesting

Factor	Type	Levels	Values
sourc	fixed	2	1, 2
subtr	fixed	4	1, 2, 3, 4
treamt	fixed	2	1, 2

Analysis of Variance for ph res

Source	DF	SS	MS	F	P
sourc	1	0.4925	0.4925	4.29	0.052
subtr	3	0.2086	0.0695	0.61	0.620
treamt	1	0.1070	0.1070	0.93	0.347
sourc*subtr	3	0.2178	0.0726	0.63	0.603
sourc*treamt	1	0.0520	0.0520	0.45	0.509
subtr*treamt	3	0.7014	0.2338	2.04	0.143
Error	19	2.1827	0.1149		
Total	31	3.9620			

S = 0.338941 R-Sq = 44.91% R-Sq(adj) = 10.11%

Appendix XXXI

Two-way ANOVA for Table 12: Substrate fat content after harvesting versus source_1, substrt_1, treat_1

Factor	Type	Levels	Values
source_1	fixed	2	1, 2
substrt_1	fixed	4	1, 2, 3, 4
treat_1	fixed	2	1, 2

Analysis of Variance for fat res_1

Source	DF	SS	MS	F	P
source_1	1	0.062	0.062	0.14	0.712
substrt_1	3	67.534	22.511	51.00	0.000
treat_1	1	65.809	65.809	149.09	0.000
source_1*substrt_1	3	38.138	12.713	28.80	0.000
source_1*treat_1	1	2.755	2.755	6.24	0.022
substrt_1*treat_1	3	18.207	6.069	13.75	0.000
Error	19	8.387	0.441		
Total	31	200.892			

S = 0.664384 R-Sq = 95.83% R-Sq(adj) = 93.19%

Appendix XXXII

Two-way ANOVA for Table 12: Substrate carbohydrate content after harvesting versus source_1, substrt_1, treat_1

Factor	Type	Levels	Values
source_1	fixed	2	1, 2
substrt_1	fixed	4	1, 2, 3, 4
treat_1	fixed	2	1, 2

Analysis of Variance for cabo res_1

Source	DF	SS	MS	F	P
source_1	1	0.296	0.296	0.17	0.687
substrt_1	3	733.625	244.542	138.32	0.000
treat_1	1	46.933	46.933	26.55	0.000
source_1*substrt_1	3	31.233	10.411	5.89	0.005
source_1*treat_1	1	0.140	0.140	0.08	0.781
substrt_1*treat_1	3	11.287	3.762	2.13	0.130
Error	19	33.591	1.768		
Total	31	857.106			

S = 1.32965 R-Sq = 96.08% R-Sq(adj) = 93.61%

Appendix XXXIII

Two-way ANOVA for Table 12: Substrate protein content after harvesting_1 versus source_1, substrt_1, treat_1 after harvesting

Factor	Type	Levels	Values
source_1	fixed	2	1, 2
substrt_1	fixed	4	1, 2, 3, 4
treat_1	fixed	2	1, 2

Analysis of Variance for pro res_1

Source	DF	SS	MS	F	P
source_1	1	21.735	21.735	28.49	0.000
substrt_1	3	60.604	20.201	26.48	0.000
treat_1	1	12.273	12.273	16.09	0.001
source_1*substrt_1	3	23.026	7.675	10.06	0.000
source_1*treat_1	1	4.326	4.326	5.67	0.028
substrt_1*treat_1	3	11.365	3.788	4.96	0.010
Error	19	14.497	0.763		
Total	31	147.825			

S = 0.873487 R-Sq = 90.19% R-Sq(adj) = 84.00%