6400

ASSESSMENT OF GROWTH AND YIELD OF OIL PALM MUSHROOM, *VOLVARIELLA VOLVACEA* ON SOME LOCAL AGRICUTLURAL WASTES

By

Daniel Kofi Ackah B.Sc. (Hons) Agriculture

A Thesis submitted to

The Department of Environmental Science,

Kwame Nkrumah University of Science and Technology

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

College of Science

SEPTEMBER, 2011

DECLARATION

I hereby declare that this submission is my own work towards the M.Sc. and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Daniel Kofi Ackah _____ 17/1/2013

(PG20067506) Signature Date

Certified by:

Mr. Alfred K. Apetorgbor _____ 17/01/2013

(Supervisor) Signature Date

Rev. S. Akyeampong _____ 17/01/2013

(Head of Department) Signature Date

DEDICATION

I dedicate this work to my lovely mother and family.

ABSTRACT

The edible mushroom *Volvariella volvacea* (oil palm mushroom) has been shown to be a potential crop to conserve and protect the environment by reducing agricultural/industrial wastes and to provide valuable food supplements for man due to its nutritional and medicinal values. This study was carried out to assess the growth and yield of *V. volvacea* on some local agricultural wastes (rice straw, cocoa pod, corn cob, corn husk, oil palm bunch stalk and oil palm female spikelets). The low and the high bed methods of cultivation were used. *V. volvacea was* cultivated on single non-composted and composted substrates, mixed non-composted and composted substrates, as well as their supplementation with lime ($CaCO_3$) and dry *Leucaena leucocephala* leaves. The chemical composition of the substrates were determined before and after cultivation of the mushroom. Each of the single substrates supported growth and yield of *V. volvacea*. The unsupplemented non-composted oil palm bunch stalk gave the highest mean yield of 118.00g fruitbodies/kg substrate with Biological Conversion Efficiency (BCE) of 11.77% followed by the compost from oil palm bunch stalk with 45.33g fruitbodies/kg substrate and BCE of 4.51% and the unsupplemented non-composted rice straw with 40.00g fruitbodies/kg substrate and BCE of 4.00%. The non-composted cocoa pod and oil palm bunch stalk mixture as well as composted oil palm female spikelets and corn cob mixture did not yield any fruitbodies. The hemicellulose, cellulose and lignin contents of the substrates reduced after colonization of *V. volvacea.* Protein and moisture contents of the substrate increased while the crude fat, crude fibre, ash, carbohydrates and pH contents decreased after colonization of *V. volvacea.* It is recommended that farmers cultivate *V. volvacea* using unsupplemented oil palm bunch stalk and rice straw where these substrates are available.

TABLE OF CONTENTS

TITLE PAGE **PAGE**

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

R.H.	Relative humidity
CO_2	Carbon dioxide
BCE	Biological Conversion Efficiency
OPBS	Oil palm bunch stalk
CP	Cocoa pod
CC	Corn cob
RS	Rice straw
CH	Corn husk
OPFS	Oil palm female spkelets
Lu	*Leucaena leucocephala*
Li	Lime

ACKNOWLEDGEMENTS

The promises of God are indeed yes and yes and amen. May He reign forever for the bountiful favour bestowed upon me throughout this work.

I am thankful to my supervisor, Mr. Alfred K. Apetorgbor and his wife Dr. (Mrs) Mary M. Apetorgbor for their immeasurable guidance, assistance and support towards the successful completion of this project.

I express my heartfelt gratitude to my mother Madam Victoria Agoro and to all my siblings for their encouragement and unrelenting assistance given me throughout the programme.

I give special thanks to my fiancée, Miss Sandra Adomako, a dear one for her encouragement and motivation.

I acknowledge the helping hand given me by Mr. Emmanuel Odame of the Horticultural Department of Kwame Nkrumah University of Science and Technology in gathering materials for this work.

To all my course mates I say thank you for the numerous support and encouragement which kept me on throughout this project.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Mushrooms represent one of the world's greatest untapped resources of nutritious food. Production of edible mushrooms may be the only currently economical biotechnology for lignocellulose organic waste recycling that combines the production of protein-rich food with the reduction of environmental pollution (Mshandete and Cuff, 2007).

Volvariella volvacea (Bull ex. Fr.) Singer, an edible basidiomycete, occurs in both tropical and subtropical regions of the world. The cultivation of this mushroom can be done on various commonly available organic wastes as substrate (Onuoha *et al.*, 2009). The species grows naturally on dead leaves, dead wood, animal droppings and tree/wood stumps (Zoberi, 1972).

Most farm wastes contain cellulose residues. Fasidi and Kadiri (1993) reported that most materials with cellulose residues have been found to stimulate the growth of wood rotting fungi. These fungi are capable of degrading cellulose materials and ultimately oxidizing the lignin content of the materials. Enzymes from basidiomycetous fungi, particularly carbohydrases, are involved in lignocellulose degradation and act as pre-treatment agents to break down the cellulose to simpler molecules.

There is a very high incidence of malnutrition, especially of protein deficiency in developing countries. The situation is especially severe in Sub-Saharan Africa, which is the region with the highest prevalence of under-nourishment with one in three people deprived of access to

sufficient food. The problem of protein shortage in developing countries is an existing reality and will continue for the foreseeable future. Protein malnutrition will become even more acute since the supply of protein for the diet has not kept pace with population growth. In order to meet the deficit, most developing countries tend to import essential protein sources of food from abroad, spending large sums of their meagre foreign exchange earnings. Such a situation has forced planners and nutritionists to think about unconventional alternative sources of protein, such as mushrooms (Mshandete and Cuff, 2007).

1.2. STUDY OBJECTIVES

1.2.1 MAIN OBJECTIVE

The main objective of this project was to assess the growth and yield of *Volvariella volvacea* (oil palm mushroom) on some local agricultural wastes/residues.

1.2.2 SPECIFIC OBJECTIVES:

The specific objectives were:

i. To evaluate the growth and yield of *V. volvacea* on agricultural wastes/residues using the low and high bed methods.

ii. To determine the chemical composition of the substrates before and after cultivation of *V. volvacea*.

1.3. JUSTIFICATION

Mushroom cultivation is a direct utilization of their ecological role in the bioconversion of solid organic wastes generated from industries and agricultural products into edible biomass, which could also be regarded as a functional food or as a source of drugs and pharmaceuticals. According to Oei (1996), no arable land is needed in mushroom production.

Mushroom production can convert the vast lignocellulose waste materials into a wide diversity of fungal biomass products (edible or medicinal food, feed and fertilizers), protecting and minimizing environmental pollution (Fan *et al.*, 2006). The cultivation of mushrooms serves as the most efficient and economically viable biotechnology for the conversion of lignocellulose waste materials to high quality food and this will naturally open up new job opportunities especially in rural areas. Mushroom production contributes to products of commercial interest that can generate equitable economic growth that has already had an impact at national and regional levels (Nwanze *et al.*, 2008).

In most parts of West Africa, *V. volvacea* occurs as an innate member of the fungal flora, growing usually on oil palm trees after they have been felled and/or tapped for palm wine. It is common on decaying forest trees or on their sawdust. Predominant cultivation of this mushroom can be observed in the tropics and subtropics (Ofosu-Asiedu *et al.*, 1984).

Traditionally in Ghana, *Volvariella* species, locally called oil palm mushroom or "Domo" and many other varieties of mushrooms are picked in the wild in the forest region during the onset of the rainy season for use in soups and sources. This exceptional preference is due to the seasonal appearance of this crop coupled with the inordinate demand for meat substitutes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History

Mushroom cultivation can be traced back to the 1712 century in Europe but it was not until the 18th century in France that modern mushroom cultivation techniques began to form. Outside of Paris, cultivation was done in limestone quarries where they produced "champignons de Paris (http://delfresh.com/html/history.html).

According to the Hieroglyphics (4600 years ago), ancient Egyptians believed that mushrooms are plants of immortality. The delicious flavour of mushrooms intrigued the pharaohs of Egypt so much, that they decreed that mushrooms were food for royalty and that no commoner could ever touch them. This assured them the entire supply of mushrooms. In various other civilizations throughout the world including Russia, China, Greece, Mexico and Latin America, mushroom rituals were carried-out. Many people believed that mushrooms had properties that could help in finding lost objects and lead the soul to the realm of the gods (http://www.foodreference.com/html/art-mush-history417.html).

France was the leader in the formal cultivation of mushrooms. Some accounts say that Louis XIV was the first mushroom grower. During his time, mushrooms were grown in special caves near Paris set aside for this unique form of agriculture. From France, the gardeners of England found mushrooms a very easy crop to grow which required little labour, investment and space.

Mushroom cultivation began gaining popularity in England with more experimentation with spawn and publicity in journals and magazines. In the late 19th century, mushroom production made its way across the Atlantic to the United States where curious home gardeners in the East tried their luck at growing this new and unknown crop. However, growers had to depend on spawn imported from England which, by the time it reached the U.S., was of poor quality. In 1891, the first book on mushroom cultivation was published (http://www.foodreference.com/html/art-mush-history417.html).

Many years before the development of mushroom production in the United States and Europe, the Chinese had long been growing mushrooms but of different species. Over the centuries mushrooms, especially the wild forms or "toadstools," became the object of fear and distrust, because of mushroom poisoning. Fungophobia was reported not only in America but also in Great Britain (Quimio *et al.,* 1990).

2.2 Morphology

The main body of the mushroom is subterranean, or lives on dead trees and living tree roots and can vary in size from a few inches to several miles wide. The fruiting bodies of fungi (mushroom) sprout out of the ground overnight. (http://www.starchefs.com/features/mushrooms/html/more mushrooms.shtml).

The mushroom species usually grown commercially attains a height of 5 to 10 cm and has a fleshy cap of about 2 to 10 cm across. When the mushroom is ripe, the cap is white or slightly brownish above and pink on the underside. With age, the entire fruiting body changes to dark

brown. In the young mushroom the margin of the cap is joined to the stem by a membranous collar, which breaks at maturity to expose the gills on the undersurface of the cap (Microsoft Encarta, 2009).

Volvariella volvacea has volva as a distinctive feature that resembles a classic Amanita, except that an annulus is lacking, hence the name *V. volvacea.* The volva or cap is 5–15 cm broad, egg-shaped at first and soon expands to form a broadly convex shape. The cap is smoky brown or cigar-brown or blackish brown. It is darker when young, though it fades in age and/or with exposure to light. The margin is radially ridged with the gills free. The stem is usually 4-20 cm long, 1.0-1.5 cm thick and is white to yellowish. It is solid and smooth with the base encased in a thick volva (Oei, 1996).

2.3 Importance of mushroom culture

2.3.1 Utilization of mushroom as food

Mushrooms contain many essential amino acids; white button mushrooms, for example, contain more protein than kidney beans. Shiitake mushrooms are less nutritious, but are still a good source of protein (http://attra.ncat.org/attra-pub/PDF/mushroom.pdf).

Mushrooms also contain some unsaturated fatty acids, provide several of the B vitamins, and vitamin D. Some even contain significant amounts of vitamin C, as well as the minerals potassium, phosphorus, calcium, and magnesium. Mushrooms are low in total fat content and

have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a healthy food. Furthermore, mushrooms contain significant amounts of carbohydrates and fibres (Oei, 1996).

2.3.2 Medicinal properties of mushrooms

Apart from being a relatively much cheaper source of protein compared with animal proteins, mushrooms could also hold special attraction. For thousands of years, mixtures of mushrooms have been used for healing purposes and may be recommended for people with cholesterol-related ailments (Jonathan *et al*., 2006). The mushrooms are mainly recommended to diabetic and anaemic persons, owing to their high folic acid content. Some have demonstrated antibiotic activities, others are reputed to be anti-allergic and some are used for soft and comfortable surgical dressing while others are used for anesthesia. Some mushrooms are used as a powder or tincture for swollen glands, epilepsy and against various other diseases. The sclerotia of *Pleurotus tuber-regium* are usually harvested from decaying logs; the dark brown exterior is peeled off and the white compact mycelia tissue used for food or medicine (Isikhuemhen and LeBauer, 2004). Recent scientific studies have shown that sclerotia of *P. tuber-regium* contain polysaccharides and other compounds with positive medicinal benefits (Gregori *et al.*, 2007).

2.3.3 Agricultural waste management

Organic waste constitutes about 60-70% of the materials in the waste stream in Ghana and it is increasingly becoming a major source of environmental and disease control effort. Primarily, organic waste (through copious discharge of leachates), is a major source of contamination in urban water supply and environmental pollution when left unattended. It putrefies easily under the hot tropical temperatures and generates considerable quantities of leachates and obnoxious odour. Under such circumstances, organic waste may also act as an important breeding site for disease causing vermins including insects and rodents, which are vectors of diseases such as cholera, diarrhoea, dysentery and typhoid fever. Therefore, the management of organic waste in Ghana is a key strategy for urban environmental health promotion and disease control efforts.

.

Mushroom-forming fungi are gaining global popularity in both liquid fermentation of industrial effluents and many lignocellulosic wastes such as waste papers, banana and plantain leaves, peelings of some food stuffs, sawdust of different tree origins and oil palm fruit fibres (Osemwegie *et al.*, 2002). There are huge potential socio-economic benefits associated with the effective and efficient bioconversion of agro-industrial wastes to valued edible sporocarps (Chang and Miles, 1991). The growth of mushroom production industries and the use of agro-industrial based substrates as the major raw materials may provide a partial solution to the nation's waste management problems and pollution challenges, poverty and rising youth unemployment. The potential use of spent substrates in crop farming as soil conditioner and/or mycorrhization practices have also been emphasized by Wasser (2007).

Mushrooms are grown, not directly on soil as are other crops, but on organic substrates, either raw or composted. These substrates are mostly waste materials from farms, plantations, or factories. These otherwise useless by-products can be recycled to produce additional food in the form of mushrooms for human consumption.

One of the most intriguing opportunities offered by mushroom mycelia in the area of bioconversion is the exploitation of their ability to degrade pollutants, many of which are highly carcinogenic, released into the environment as a consequence of human activity. Mushrooms have been studied as a potential crop to reduce agricultural solid wastes and increase domestic mushroom production. Despite the fact that the mushroom extracts substantial amounts of nutrients from the substrate, the spent substrates can be used as fodder, as a soil conditioner and fertilizer and in bioremediation (Bisaria *et al.,* 1997). The cultivation of edible mushrooms like *V. volvacea* on these wastes may thus be a value added process capable of converting these materials, which are otherwise considered to be wastes, into food and feed (Bisaria *et al.,* 1997).

2.4 Life cycle of fungi

In nature fungi multiply by producing millions of spores. When a spore settles in a suitable environment, it can germinate and branch to form a mycelium. The mycelium growing out of a single spore (of a heterothallic mushroom) is *haploid* and generally not capable of sexual reproduction. When two sexually compatible mycelia meet, they may fuse to form the so-called secondary mycelium, which is *diploid*. There are some mushroom species, however, which are capable of forming fruiting bodies on mycelium which has grown from a single basidiospore. These are termed *homothallic*. Two types of homothallism are recognized: primary and

secondary. In primary homothallism only a single uninucleate spore is involved. In secondary homothallism the spores carry at least two nuclei (Oei, 1996).

Heterothallism is thus a different mechanism: the mycelium from a single spore has to fuse with the mycelium originating from a compatible spore. The mating system may require one or two compatibility factors. It is important for scientists who perform breeding experiments to recognize the specific sexual pattern of the chosen mushroom species. *Volvariella volvacea* reproduces sexually through the primary homothallism pattern (Oei, 1996).

2.5. Ecological conditions

Conditions necessary for the growth of *Volvariella volvacea* include:

a) Ventilation

Air flow over a mushroom bed will increase the water evaporation rate. If the air flow is non-uniform in a mushroom house, some mushroom beds will become drier than others. Fresh air can be blown in growing rooms in order to reduce the CO_2 level, adjust the desired humidity and temperature inside the growing room. If there is a large difference between the outside air condition and the desired conditions inside the mushroom growing house, the fresh air has to be conditioned. If outside air is relatively cool, then heating it would lead to very dry air, which would have to be humidified before it is blown along the substrate (Oei, 1996).

b) Temperature

The mushrooms selected for cultivation at a given site should be able to grow at temperatures close to normal outside air temperatures. Three different temperatures are important:

- Air temperatures outside the growing room,
- Air temperature inside the growing room,
- The substrate temperature.

The substrate temperature is an important parameter in both mycelia growth and fruit body formation. The substrate itself produces heat, the amount of which depends on the activity of microbes in the substrate. It may be necessary to remove excess heat by introducing air of a lower temperature. If many micro-organisms are present in the substrate, the temperature of the substrate is likely to be higher than the ambient air temperature. This can be desirable because it increases evaporation, which is necessary to transport nutrients from the mycelium inside the substrate to the fruiting bodies. A high substrate temperature (>35˚C), however can trigger growth of thermophilic microflora. Microorganisms in this group thrive at temperatures between roughly 30-55˚C. When they start to grow, they produce more heat, which increases the substrate temperature. In this way the mycelium of the cultivated mushroom can be killed (Oei, 1996).

The temperature for *Volvariella volvacea* cultivation is around or just below 30˚C (Oei, 1996).

c) Relative humidity

A wide range of relative humidity have been found to be optimal for cultivation of *V. volvacea.* For example, Samajpati (1979) found 57- 60% R.H. to be optimal for *V.volvacea* in contrast with 90% R.H. prescribed by a previous worker as the best R.H. for cultivation of *V.volvacea* (Ho,1972). It was conjectured that the relative humidity may differ with varying amount of ventilation during cultivation (Ho,1972).

d) Light

Studies by Oei (1996) revealed that *V. volvacea* has no light requirement at the spawn run stage. However, for the primordial formation and fruit-body development a light requirement of 250 to 500 lus and 500 to 750 lus respectively is required for the pinhead initiation and cropping, diffused natural or direct growing glow-light fluorescent for 12-18 hours a day is required.

e) CO_2

According to Stamets (2000), for *V. volvacea*, a CO_2 concentration of 5000 to 10000 ppm is a requirement for the spawn run. The pinhead initiation and cropping stages require less than a 1000 ppm and less than 600 ppm, respectively.

f) P^H

The spores of *V. volvacea* require a suitable pH of 7.5 to germinate. The mycelia growth requires a suitable pH range of 4.5-10.5, but the best is from 7.2-8.0. When the pH value is less than 5.5, mycelia growth is very weak. Fruit body grows best in the pH range of 7.5-8.0 (Oei, 1996).

2.6 Yield of mushrooms

Yield of mushrooms is strongly dependent on the substrates, culture method used and also on the strain of the mushroom. Calculating the revenues from mushroom cultivation depends on the amount of mushrooms produced within a certain time on a certain amount of substrate. The period of time before harvesting depends on the particular mushroom, cultivation method and temperature.

Several different methods to measure yield are commonly used. The most accurate (in a scientific sense) is dry weight of mushroom versus dry weight of substrate. It requires, however, that both the substrate and mushroom are dried for comparison. A representative sample of the fresh substrate is dried in an oven under a relatively low temperature. The fresh mushrooms are dried in the same way. Another method which is more convenient for growers is the Biological Conversion Efficiency (BCE):

$$\text{BCE} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

This method is less accurate than the previous one because the percentage of water in the mushroom and the starting substrate is variable. This method however, has one advantage, that is, a grower can easily determine the expected yield from a given amount of dry organic waste material. Another commonly used measure is the wet weight of the mushroom versus the wet weight of the substrate. This method is easier for growers who buy prepared substrate (Oei, 1996).

According to Stamets (2001), substrates such as oil palm fibre, rice husk and sawdust contain essential macro elements (potassium, calcium, phosphorous, magnesium, nitrogen and sodium) for crop production and these nutrients combine in various substrates to stimulate fruit body formation and development of *V. volvacea*.

Akinyele and Akinyosoye (2005), carried out a study using different agricultural wastes (i.e. groundnut shell, oil palm pericarp, cassava peel, sorghum shaft, cotton waste, rice shaft, red afra dust, white afra dust, corn cob and rice husk as substrates) to assess the growth performance of *V. volvacea* on each substrate. They also mixed the substrates (rice husk + red afra dust, oil palm pericarp + groundnut shell, corn cob + groundnut shell, cassava peel + sorghum shaft, cotton waste + rice husk and oil palm pericarp + cassava peel) to see which combination could give the best mycelia extension. They found cotton wastes and rice husk + cotton wastes gave the maximum mycelia extension. According to Akinyele *et al.* (2011), proximate analysis of agricultural wastes after spawning with *V. volvacea*, increased protein and moisture contents while fat, crude fiber, ash and carbohydrate contents decreased due to the colonization by *V. volvacea* mycelium.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Substrates

The following agricultural waste materials/residues were used as substrates:

Corn cobs, corn husks, rice straw, oil palm bunch stalks and oil palm female spikelets which were obtained from farms at Ampabame near Ejisu whiles the cocoa pods were obtained from Kwaem near Juaben, all in the Ejisu–Juaben District of the Ashanti Region. The substrates were obtained in the fresh state and sun dried later on a concrete platform.

3.1.2 Substrate preparation

Drying of substrates

The corn husks, corn cobs, oil palm bunch stalks, oil palm female spikelets and cocoa pods were cut into pieces of about 2 cm manually.

They were spread evenly on a flat concrete platform and exposed to sunlight in the open until they were well dried (Plates 1 and 2).

3.1.3 Mushroom spawn

Volvariella volvacea mushroom was obtained from the wild, cultured on malt extract agar and spawn prepared on sorghum (*Sorghum vulgare*) grains.

Plate 1: Chopped cocoa pod being dried on concrete platform

Plate 2: Chopped oil palm bunch stalks being dried on concrete platform

3.1.4 Composting of substrates

Composting was done for the best performed single substrate (oil palm bunch stalk) in terms of yield as well as the mixed substrates (oil palm bunch stalk + corn cob). A bucket of water was sprinkled on the substrates to make them moist; They were then heaped and covered with large polyethylene sheets to shield them from rain. The substrates were turned over every three days for the two week period to ensure uniform composting (Plate 3).

Plate 3: A heap of oil palm bunch stalks ready for composting

3.1.5 Tying of substrates into bundles

The rice straws were tied into bundles of about 60 cm long and 10 cm in diameter. The bundled substrates were used only for the high bed method of preparation.

3.1.6 Soil bed preparation

The soil bed on which the mushroom beds were to be mounted were raised to a height of about 10 cm above the ground with a canal around it. It was then made moist by watering it to prevent it from absorbing water from the substrates.

3.2 Mushroom Cultivation

Two methods were employed:

i. The high bed method
ii. The low bed method

The high bed method is employed due to the nature of the substrate to be used. Substrates which are light in weight and difficult to compact are tied and arrange in compact to form a small hill after soaking the substrates in water for some hours. The low bed method uses a trapezoid type of wooden box where pieces of cut substrates are put into it and firm to compact on a prepared soil bed.

3.2.1 The high bed preparation:

The dry rice straw bundles were weighed and then soaked in water for ten hours to soften. bundles were then taken out of the water, and left for about ten minutes for excess water to drain off. The bundles were packed lengthwise, in layers with each layer

consisting of five bundles, with the butt ends to one side. Pieces of non-bundled rice straw materials were used to fill gaps between the layers (Plate 4).

Plate 4: A bed of rice straw bundles in the high bed method

Volvariella spawn was sprinkled onto the edges of the layer, about one centimeter from the edges. Another layer of bundles was piled on the first one but this time with the butt-ends to the opposite end. Spawn was again sprinkled onto the edges of the layer and the process repeated until four layers were piled. The spawn was sprinkled over the entire surface of the last layer. A thin layer of loose rice straw materials were sprinkled over the spawn. There were three replicates for the substrates. The beds were then covered with transparent polyethylene sheets to maintain high temperature and humidity. Water was poured in a canal made around the soil bed to keep the substrates moist. The set-ups were left for eight days to allow for growth of mycelia through the substrates. The polyethylene sheets were raised to about ten centimeters above the beds using forked sticks to support them at the end of the beds. This was to make room for the newly formed pinheads to develop into mushrooms.

3.2.2 The low bed preparation:

3.2.2.1 Single non-composted substrates

The dry chopped substrates (corn cobs, corn husks, cocoa pods, oil palm bunch stalk and oil palm female spikelets) were separately packed into a trapezoid wooden box (Base: 55cm x 38cm; Top: 44cm x 27cm; Height: 26cm) (Plate 5).

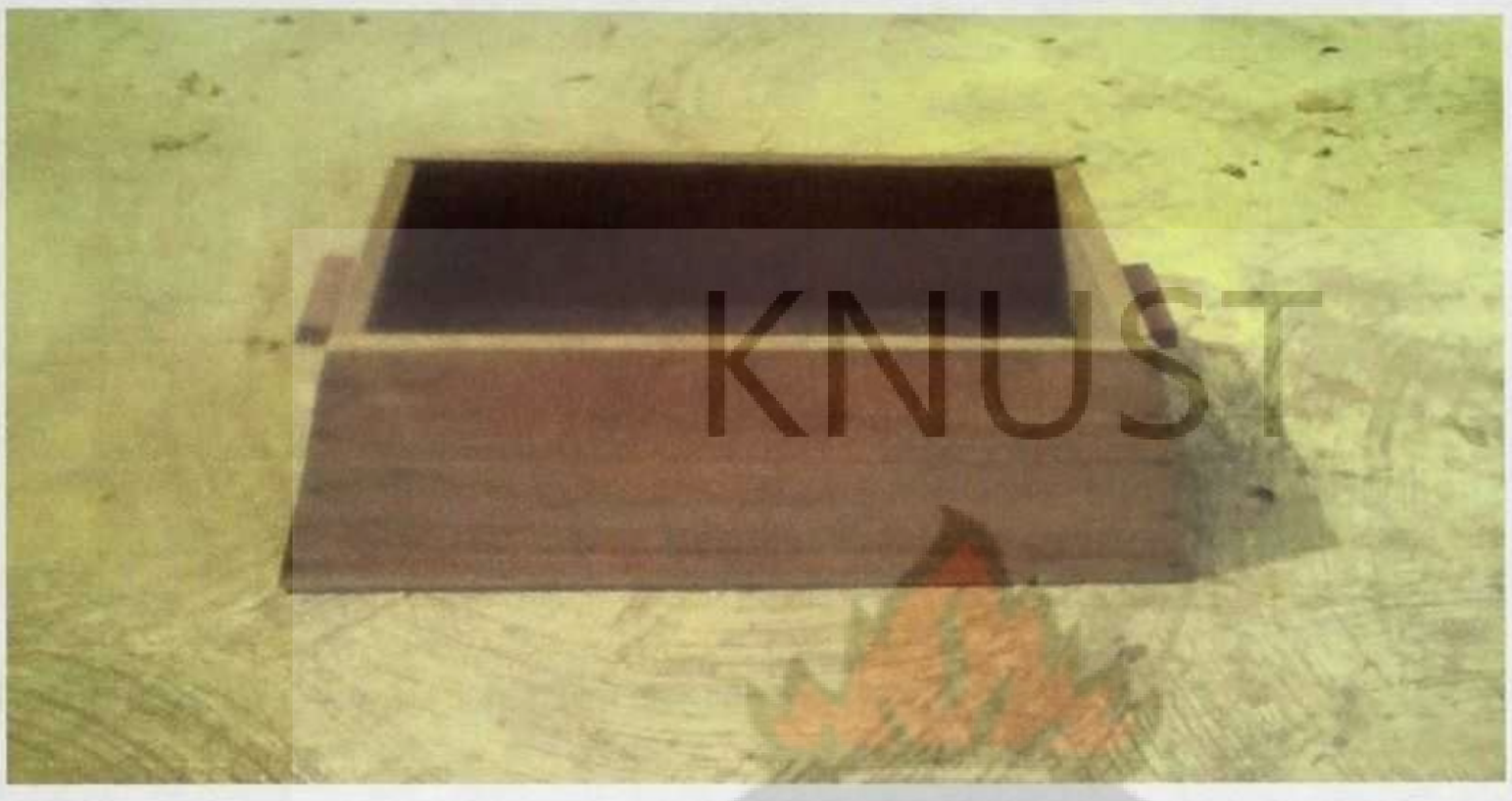

Plate 5: A trapezoid wooden box used for low bed preparation

Each dry substrate was carefully packed into the box to fill it. They were then removed and weighed. The substrates were separately packed into jute sacks and soaked in a drum of clean water. The soaked substrates were then taken out of the water after ten hours, arranged on an inclined surface for ten minutes for excess water to drain off.

The box was placed on the raised soil and the substrates packed into it in turns. Spawning was done after packing and compacting each of the substrates to one-third and two-thirds the height of the box. Spawn was sprinkled onto the edges of each level before the packing continued. The spawn was sprinkled over the entire surface of the third (last) layer. There were three boxes for each substrate. The boxes were then covered with transparent polyethylene sheets to maintain high temperature and humidity.

Water was poured in the canal made around the soil bed to keep the substrates moist (Plate 6).

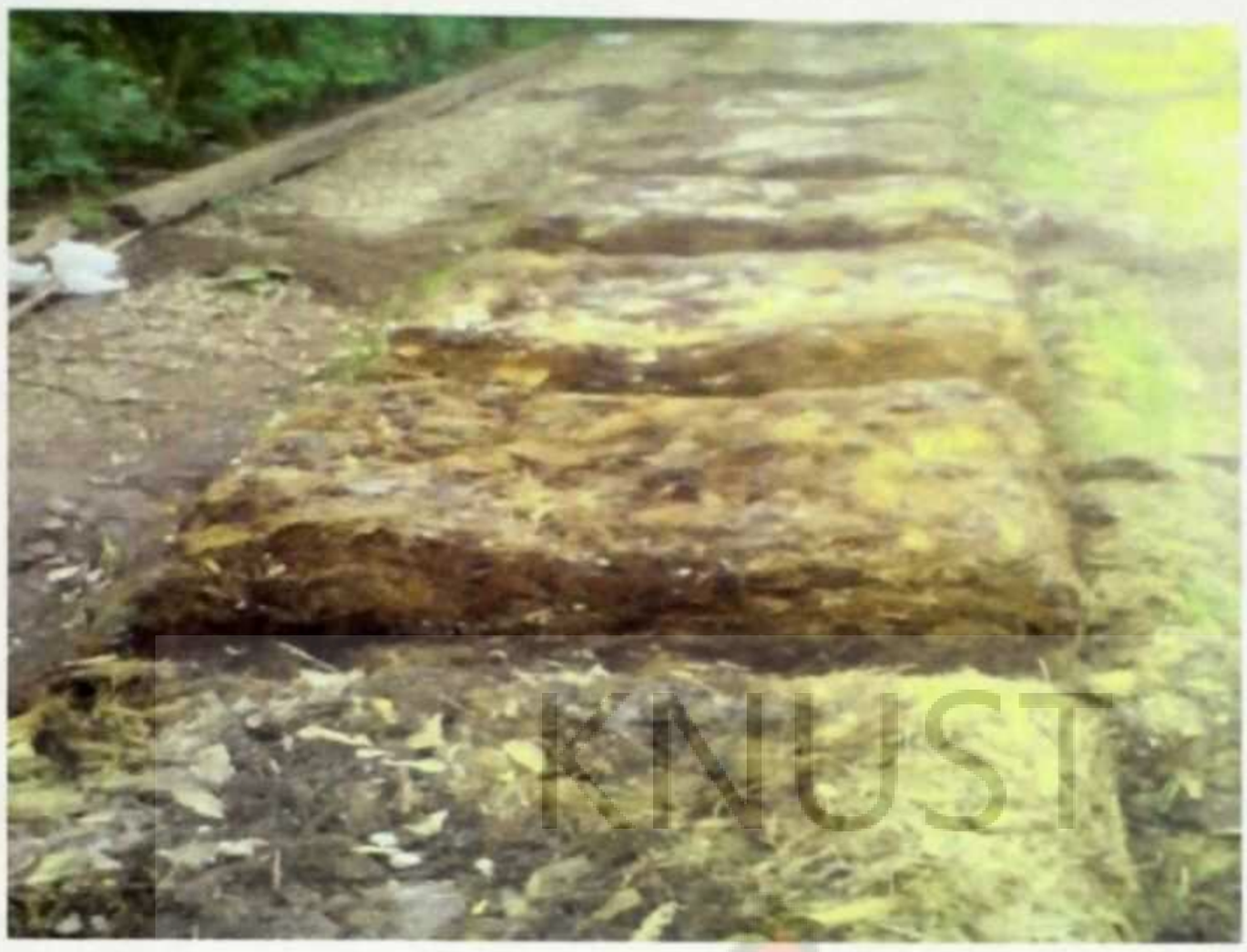

Plate 6: Low beds of oil palm bunch stalks in a row

The set up was left for eight days after which the polyethylene sheet was raised about ten centimeters above the beds using forked sticks to support it at each end of the beds. This was to give enough space for development of the mushrooms.

3.2.2.2 Mixed substrates

The six substrates were mixed (taken a half box full of each substrate) to obtain five combinations as follows:

Corn cob + oil palm bunch stalk

Cocoa pod + oil palm bunch stalk

Corn cob + rice straw

Oil palm female spikelets + corn cob

Rice straw + corn husk

The substrates were uniformly mixed after cutting the materials into pieces and drying them. The low bed method was used in preparing the beds as done for the single substrates. Three replicates were prepared for each mixed substrate.

3.2.3 Addition of supplements

Two additives (Dry leaves, *Leucaena leucocephala* and lime ($CaCO_3$)) were added as supplements to the best performed substrates from the single non-composted substrates (oil palm bunch stalk), mixed non-composted substrates (corn cob + rice straw), mixed composted substrates (corn cob + oil palm bunch stalk) and single composted substrates (oil palm bunch stalk). The leucaena leaves were to add protein to the substrates, this is due to it high protein content (the crude protein and total N content of leucaena leaves is about 25.9% and 4.2% respectively, the amino acid content total about 3266mg/gN) and lime to raise the pH of the substrates. Each bed (box full of substrates) was mixed with supplements weighing 0.25% dry weight of substrates. There were three replicates for each substrate.

3.3 Yield

The number of fruitbodies that developed on each substrate bed was counted and harvested just after maturing. Harvesting of fruitbodies was done at the elongation stage ensuring that the substrate was not disturbed. The fruit bodies were weighed using a top pan balance.

The Biological Conversion Efficiency (BCE) was determined using the method of Tschierper and Hartman (1977) from the mathematical relationship:

$$\text{BCE} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

3.4 Chemical analysis

The proximate composition (protein, ash, carbohydrates, crude fiber, crude fat and moisture),pH cellulose, hemi-cellulose and lignin contents of the substrates were determined before and after cultivation of the mushrooms.

3.5 Statistical analysis

Means of readings from the three replicates were determined and subjected to analysis of variance. Statistix 9 software was used for the analysis. Means were separated using the least significant difference (Completely Randomize Design) at the one percent (0.01) significant level.

CHAPTER FOUR

4.0 RESULTS

Growth and yield of *V. volvacea* on single and mixed substrates

Results for single non-composted, single composted, mixed non-composted, mixed composted substrates and their supplementation with lime ($CaCO_3$) and *Leucaena leucocephala* leaves on growth and yield performance of *V. volvacea* are presented below (Tables 1-32). Except the results on the rice straw in the single non-composted group which was from the high bed (Tables 1 and 2), all the others were from the low bed method.

4.1 Growth and yield of *V. volvacea* on single non-composted substrates

Table 1 represents vegetative growth of *V. volvacea* on single non-composted substrates. The rice straw gave the earliest appearance of pinheads in 13 days followed by corn cob and oil palm bunch stalk in 16 days with those on the corn husk being the last to appear in 28 days. The oil palm bunch stalk gave the highest mycelia growth and the highest number of pinheads (151) out of which 135 fruitbodies were harvested. This was followed by the corn husk which gave good mycelia growth, 71 pinheads out of which 42 fruitbodies were harvested and the rice straw which gave good mycelia growth with 55 pinheads out of which 50 fruitbodies were harvested. Oil palm female spikelets and the cocoa pod gave the poorest mycelia growth with three and two pinheads respectively and yielding one and two fruitbodies respectively.

The 118.00g/kg substrate of fruitbodies harvested from the oil palm bunch stalk with a biological conversion efficiency (BCE) of 11.77% was the best, compared to the other substrates in the group. This was followed by the rice straw and corn husk with

40.00g/kg and 36.67g/kg substrate of fruitbodies and BCE of 4.00% and 3.70% respectively. The oil palm female spikelet gave the poorest yield with 0.07g/kg substrate of fruitbodies and BCE of 0.01%.

There were significant differences in the mean yield of the fruitbodies from the substrates or their BCEs ($p \leq 0.01$) except between those of the rice straw and corn husk (Appendix A).

Results show a significant increase in percentage protein content for most of the substrates after cultivation following *Volvariella volvacea* mycelia colonization (Table 2). Rice straw, corn cob, corn husk, oil palm bunch stalk had their protein contents increasing from 7.90%, 5.30%, 3.60% and 7.90% before cultivation to 10.70%, 12.80%, 8.60% and 12.50% respectively after cultivation. The hemicellulose, cellulose, lignin, proximates and pH had their values either increasing or decreasing. For example, corn cob had 32.00% hemicellulose content before cultivation but this decreased to 23.00% after cultivation whiles corn husk had 15.00% hemicellulose content before cultivation which increased to 17.00% after cultivation. Rice straw had a cellulose content of 6.00% before cultivation but decreased to 5.00% after cultivation whiles that of oil palm bunch stalk increased from 4.00% to 5.00%.

Corn husk had lignin content of 13.00% before cultivation which increased to 22.00% after cultivation whiles oil palm bunch stalk had 48.00% before cultivation and decreased to 27.00 after cultivation. Rice straw had crude fiber content decreasing from 23.26% before cultivation to 16.50% after cultivation whiles that of corn cob increased

from 28.41% to 30.50% after cultivation. Corn husk had a crude fat content increasing from 1.00% before cultivation to 2.50% after cultivation. Oil palm female spikelets had crude fat content decreasing from 7.50% before cultivation to 2.00% after cultivation. Corn cob and cocoa pod had their pH values decreasing from 6.0 and 7.5 before cultivation to 5.5 and 7.0 after cultivation respectively. Rice straw, corn husk and oil palm bunch stalk had pH of 5.0 and remained unchanged after cultivation. Oil palm female spikelets had pH 6.0 before and after cultivation.

Table 1: Vegetative growth of *V. volvacea* on single non-composted substrates

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency(%)[2]
Rice straw	+++	13	55	50	40.00 ± 0.02	4.00 ± 0.16[b]
Cocoa pod	+	19	2	2	3.13 ± 0.01	0.31 ± 0.08[d]
Corn husk	+++	28	71	42	36.67 ± 0.06	3.70 ± 0.39[b]
Corn cob	++	16	18	15	13.33 ± 0.01	1.33 ± 0.09[c]
Oil palm bunch stalk	++++	16	151	135	118.00 ± 0.04	11.77 ± 0.25[a]
Oil palm female spikelets	+	22	3	1	0.07 ± 0.00	0.01 ± 0.00[d]
LSD at 1%		4.55	5.85	5.76	0.08	0.51
CV%		9.67	4.70	5.65	5,76	5.85

[1]Mycelial density : ++++ = Profuse mycelial growth

+++ = Moderate mycelial growth

++ = Scanty mycelial growth

+ = Very scanty mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 0.01% significant level

Table 2: Hemicellulose, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrates	Moisture	pH
Rice straw	30.00 (29.00)	6.00 (5.00)	13.00 (26.00)	7.90 (10.70)	23.26 (16.50)	0.50 (2.00)	15.00 (20.50)	76.60 (57.80)	14.00 (6.00)	5.0 (5.0)
Cocoa pod	5.00 (1.00)	7.00 (4.00)	42.00 (50.00)	9.80 (7.05)	41.00 (42.00)	3.00 (9.50)	25.00 (10.00)	62.20 (73.45)	12.00 (4.00)	7.5 (7.0)
Corn cob	32.00 (23.00)	7.00 (4.00)	41.00 (37.00)	5.30 (12.80)	28.41 (30.50)	2.00 (1.00)	5.50 (8.00)	87.20 (78.20)	12.50 (6.00)	6.0 (5.5)
Corn husk	15.00 (17.00)	4.00 (6.00)	13.00 (22.00)	3.60 (8.60)	23.08 (23.50)	1.00 (2.50)	27.00 (21.00)	68.40 (67.90)	8.50 (5.00)	5.0 (5.0)
Oil palm bunch stalk	13.00 (8.00)	4.00 (5.00)	48.00 (27.00)	7.90 (12.50)	53.41 (30.00)	1.50 (4.00)	15.00 (9.00)	75.60 (74.50)	15.50 (6.00)	5.5 (5.0)
Oil palm female spikelets	18.00 (9.00)	5.00 (7.00)	35.00 (39.00)	9.80 (6.10)	43.10 (31.50)	7.50 (2.00)	10.50 (9.50)	75.90 (78.70)	12.50 (4.00)	6.0 (6.0)

Values in parenthesis are those of the spent substrates

4.2 Growth and yield of *V. volvacea* on single composted substrates

Table 3 represents yield of *V. volvacea* on single composted substrates.

Cultivation on the single composted substrate was done only for oil palm bunch stalk due to it performance among the other substrate in the non-composted substrates group. The substrate showed late appearance of pinheads (23 days). It gave good mycelia colonization with 47 pinheads out of which 34 fruitbodies were harvested. The 45.33g/kg substrate of fruitbodies with BCE of 4.51% was less than that harvested on the single non-composted oil palm stalk bunch.

The data suggests that the differences in the mean yield of fruitbodies harvested from the non-composted oil palm bunch stalk and the composted oil palm bunch stalk or their BCEs were statistically significant ($p<0.01$) (Appendix B).

The hemicellulose, lignin, crude protein and crude fiber contents increased from 21.05%, 31.00%, 9.00% and 31.12% before cultivation to 22.00%, 32.00% 10.50% and 32.00% respectively after cultivation whiles ash and carbohydrate contents decreased from 15.00% and 74.00% before cultivation to 14.00% to 73.50% respectively after cultivation. The pH decreased from 5.5 before cultivation to 5.0 after cultivation (Table 4).

Table 3: Vegetative growth of *V. volvacea* on single composted substrates

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
Oil palm bunch stalk	++++	16	151	135	118 ± 0.04	11.77 ± 0.20[a]
Composted oil palm bunch stalk	+++	23	47	34	45.33 ± 0.03	4.51 ± 0.25[b]
LSD at 1%		8.26	11.38	10.52	0.12	0.86
CV%		11.27	3.06	3.33	2.69	2.81

[1]Mycelial density = ++++ : Profuse mycelial growth +++: Moderate mycelial growth

[2]Values in the same column followed by different letters in superscript are different at the 1% significant level

Table 4: Hemicellulose, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS	21.05 (22.00)	4.50 (4.50)	31.00 (32.00)	9.00 (10.50)	31.12 (32.00)	2.00 (2.00)	15.00 (14.00)	74.00 (73.50)	4.00 (3.00)	5.5 (5.0)

OPBS = Oil palm bunch stalk

Values in parenthesis are those of the spent substrates

4.3 Growth and yield of *V. Volvacea* on mixed non-composted substrates

Substrates used for the cultivation of the single non-composted substrates were mixed to obtain five combinations. Table 5 represents yield of *V. volvacea* on the mixed non-composted substrates. Pinheads on corn cob + oil palm bunch stalk were the first to appear in 18 days followed by those on corn cob + rice straw, oil palm female spikelets + corn cob and rice straw + corn husk in 20, 22 and 22 days respectively. The cocoa pod and oil palm bunch stalk mixture produced no pinheads. The corn cob + rice straw mixture showed the highest mycelia colonization with mean of 70 pinheads which produced 35 fruitbodies. This was followed by rice straw + corn husk and corn cob + oil palm bunch stalk with mean of 65 and 33 pinheads out of which 13 and 12 fruitbodies respectively were harvested. The 16.00g/kg substrate of fruitbodies harvested from the corn cob + rice straw mixture with BCE of 1.58% was the best, compared to those from the other substrates in the group. This was followed by the rice straw and corn husk mixture which gave 15.33g/kg substrate of fruitbodies and BCE of 1.53%. The cocoa pod + oil palm bunch stalk mixture was the poorest mixture with no fruitbodies.

The data suggests that there was a significant difference ($p<0.01$) in the mean yield of fruitbodies produced by the substrates or their BCEs except those from the rice straw + corn cob and rice straw + corn husk. The difference between oil palm bunch stalk + corn cob and oil palm female spikelets + corn cob and also between cocoa pod + oil palm bunch stalk and oil palm female spikelets + corn cob were not statistically significant ($p>0.01$) (Appendix C).

There were increases in the percentage crude protein, crude fiber and carbohydrate content from 10.94%, 19.80% and 69.06% to 11.31%, 20.92% and 70.15% after

cultivation respectively whiles those of hemicellulose, lignin and ash decreased from 29.00%, 23.00% and 18.00% before cultivation to 25.00%, 15.00% and 15.00% respectively after cultivation (Table 6). The pH value decreased from 5.5 before cultivation to 5.0 after cultivation.

Table 5: Vegetative growth of *V. volvacea* on mixed non-composted substrates

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + OPBS	+++	18	33	12	5.33 ± 0.01	0.55 ± 0.07[b]
CP + OPBS	-	0	0	0	0.00 ± 0.00	0.00 ± 0.00[c]
CC + RS	+++	20	70	35	16.00 ± 0.04	1.58 ± 0.30[a]
OPFS + CC	++	22	12	8	2.67 ± 0.002	0.26 ± 0.02[c]
RS + CH	+++	22	65	13	15.33 ± 0.01	1.53 ± 0.12[a]
LSD at 1%		4.53	11.99	7.91	0.06	0.37
CV%		10.51	12.87	22.46	18.71	18.64

[1]Mycelial density : +++ = Moderate mycelial growth ++ = Scanty mycelial growth - = No mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level

CC = Corn cob, OPBS = Oil palm bunch stalk, CP = Cocoa pod, RS = Rice straw, CH = Corn husk, OPFS = Oil palm female spikelet

Table 6: Hemicellulose, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
RS + CC	29.00 (25.00)	4.00 (4.00)	23.00 (15.00)	10.94 (11.31)	19.80 (20.92)	2.00 (2.00)	18.00 (15.00)	69.06 (70.15)	6.00 (3.00)	5.5 (5.0)

RS = Rice straw CC= Corn cob

Values in parenthesis are those of the spent substrates

4.4 Growth and yield of *V. volvacea* on mixed composted substrates

The substrates used for the cultivation in the mixed non-composted substrates were the same mixed substrates used but here the substrates were composted. Table 7 represents yield of *V. volvacea* on mixed composted substrates. The corn cob + oil palm bunch stalk gave the earliest appearance of pinheads in 16 days followed by the cocoa pod + oil palm bunch stalk and corn cob + rice straw which produced pinheads in 18 days. Rice straw + corn husk mixture was the last to produce pinheads in 21days. Oil palm female spikelets + corn cob did not produce any pinheads.

The corn cob + oil palm bunch stalk showed the highest mycelia colonization with mean of 41 pinheads out of which 9 fruitbodies were harvested. This was followed by the cocoa pod + oil palm bunch, corn cob + rice straw and rice straw + corn husk which produced 20, 15 and 5 pinheads out of which 4, 8 and 2 fruitbodies were harvested respectively. The 7.33g/kg substrate of fruitbodies and 0.73% BCE produced by the corn cob + oil palm bunch stalk was the best, compared to the other substrates in the group. This was followed by the corn cob + rice straw mixture with 4.00g/kg substrate of fruitbodies and BCE of 0.40%. The corn cob + oil palm bunch stalk mixture was the poorest, producing no fruitbodies.

The data suggests that there were significant differences ($p<0.01$) in the fruitbodies produced from all the substrates or their BCEs except those from the cocoa pod + oil palm bunch stalk and rice straw + corn husk (Appendix D).

There were increases in percentage hemicellulose, cellulose, lignin, crude protein, crude fibre, crude fat and ash contents of the substrates before cultivation from 22.00%,

4.00%, 25.00%, 10.43%, 28.23%, 2.00% and 10.00% to 24.00%, 5.00%, 28.00%, 11.00%, 29.05%, 3.00% and 11.00% after cultivation, respectively whiles carbohydrate and moisture contents decreased from 77.57% and 6.00% before cultivation to 75.00% and 3.00% after cultivation, respectively. The pH value decreased from 5.5 before cultivation to 5.0 after cultivation (Table 8).

Table 7: Vegetative growth of *V. volvacea* on mixed composted substrates

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + OPBS	+++	16	41	9	7.33 ± 0.006	0.73 ± 0.04[a]
CP + OPBS	++	18	20	4	2.27 ± 0.01	0.26 ± 0.06[c]
CC + RS	++	18	15	8	4.00 ± 0.07	0.40 ± 0.05[b]
OPFS + CC	-	0	0	0	0.00 ± 0.00	0.00 ± 0.00[d]
RS + CH	+	21	5	2	1.33 ± 0.005	0.13 ± 0.04[c]
LSD at 1%		2.31	6.68	1.89	0.02	0.12
CV%		6.21	15.87	15.43	14.50	14.56

[1]Mycelial density :

+++ : Moderate mycelia growth

++ : Scanty mycelia growth

+ : Very scanty mycelia growth

- : No mycelia growth

[2]Values bearing the same letters in the same colum n in superscript are not different at the 1% significant level

CC = Corn cob, OPBS = Oil palm bunch stalk, CP = Cocoa pod, RS = Rice straw, CH = Corn husk, OPFS = Oil palm female spikelets

Table 8: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS+CC	22.00 (24.00)	4.00 (5.00)	25.00 (28.00)	10.43 (11.00)	28.23 (29.05)	2.00 (3.00)	10.00 (11.00)	77.57 (75.00)	6.00 (3.00)	5.5 (5.0)

CC = Corn cob, OPBS = Oil palm bunch stalk

Values in parenthesis are those of the spent substrates

4.5 Growth and yield of *V. volvacea* on single non-composted substrates supplemented with *Leucaena leucocephala* leaves

Oil palm bunch stalk which gave the best yield among the single non-composted substrates was mixed with *L. leucocephala* leaves to assess the performance of the yield compared to the one without *L. leucocephala* leaves. The appearance of pinheads on the supplemented substrate delayed and showed up in 21 days. The supplemented substrates gave minimal colonization of mycelia with a mean of 9 pinheads which produced 9 fruitbodies. The 10.00g/kg substrate of fruitbodies and BCE of 1.0% was less than the 118g/kg substrate of fruitbodies with a BCE of 11.77% harvested from the non-composted substrate (Table 9).

The data suggests that there were significant differences ($p<0.01$) in the mean yield of fruitbodies harvested from the two substrates or their BCEs (Appendix E).

There was decrease in percentage hemicellulose, cellulose, lignin, crude fiber, crude fat and carbohydrate contents from 7.00%, 7.00%, 32.00%, 39.05%, 3.50% and 76.37% before cultivation to 5.00%, 6.00%, 31.00%, 26.37%, 1.00% and 72.93%, respectively whiles crude protein and ash contents increased from 9.13% and 11.00% to 10.07% and 16.00%, respectively. The pH value remained unchanged at 5.5 before and after cultivation (Table 10).

Table 9: Vegetative growth of *V. volvacea* on single non-composted substrates supplemented with *L. leucocephala* leaves

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	++++	16	151	135	118.00 ± 0.04	11.77 ± 0.20[a]
OPBS + Lu	+	21	9	9	10.00 ± 0.03	1.00 ± 0.23[b]
LSD at 1%		6.86	10.41	8.54	0.13	0.90
CV%		9.87	3.45	3.16	3.58	3.74

[1]Mycelial density: ++++ = Profuse mycelial growth + = Very scanty mycelial growth

[2]Values bearing the different letters in the same column in superscript are different at the 1% significant level

OPBS = Oil palm bunch stalk Lu: *Leucaena leucocephala* leaves

Table 10: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS + Lu	7.00 (5.00)	7.00 (6.00)	32.00 (31.00)	9.13 (10.07)	39.05 (26.37)	3.50 (1.00)	11.00 (16.00)	76.37 (72.93)	4.00 (3.00)	5.5 (5.5)

OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves

Values in parenthesis are those of the spent substrates

4.6 Growth and yield of *V. volvacea* on single composted substrates supplemented with *L. leucocephala* leaves

Oil palm bunch stalk which gave the best yield among the single non-composted substrates was also composted and mixed with *L. leucocephala* leaves to assess the yield performance compared to the one without *L. leucocephala* leaves. Pinheads appeared earlier in 21 days. The substrates gave a lower mycelia colonization with a mean of 28 pinheads out of which 15 fruitbodies were harvested. The 12.00g/kg substrate of fruitbodies with BCE of 1.20% harvested was less than the 44.00g/kg substrate of fruitbodies and BCE 4.49% harvested from the single composted oil palm bunch stalk without any supplement (Table 11).

The data suggests that there were significant differences ($p<0.01$) in the mean yield of fruitbodies harvested from the substrates or their BCEs (Appendix F).

There was a decrease in the percentage hemicellulose, cellulose, lignin, crude fiber, crude fat and ash contents from 21.00%, 6.00%, 29.00%, 33.82%, 2.00% and 12.00% before cultivation to 11.00%, 4.00%, 23.00% 21.00% 0.05% and 10.00% respectively after cultivation whiles the crude protein and carbohydrate contents increased from 9.19% and 76.81% to 12.2% and 77.25% respectively. The pH value remained unchanged at 5.0 before and after cultivation (Table 12).

Table 11: Vegetative growth of *V. volvacea* on single composted substrates supplemented with *L. leucocephala* leaves

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	+++	22	46	33	44.00 ± 0.09	4.49 ± 0.63[a]
OPBS + Lu	++	21	28	15	12.00 ± 0.02	1.20 ± 0.16[b]
LSD at 1%		12.28	20.82	13.11	0.25	1.73
CV%		15.07	15.03	14.64	15.90	16.15

[1]Mycelial density: +++ = Moderate mycelial growth ++ = Scanty mycelial growth

[2]Values bearing the different letters in the same column in superscript are different at the 1% significant level

OPBS = oil palm bunch stalk Lu= *Leucaena leucocephala* leaves

Table 12: Hemicellulose, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrates	Composition of substrates (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS + Lu	21.00 (11.00)	6.00 (4.00)	29.00 (23.00)	9.19 (12.25)	33.82 (21.00)	2.00 (0.50)	12.00 (10.00)	76.81 (77.25)	5.00 (4.00)	5.0 (5.0)

OPBS = Oil palm bunch stalk Lu= *Leucaena leucocephala* leaves

Values in parenthesis are those of the spent substrates

4.7 Growth and yield of *V.volvacea* on mixed non-composted substrates supplemented with *L. leucocephala* leaves

Among the five non-composted substrate mixtures, rice straw + corn cob gave the best yield. The mixture was mixed with *L. leucocephala* leaves to assess the performance compared to the one without *L. leucocephala* leaves. Pinheads appeared earlier (in 17 days). The mixture gave a lower mycelia colonization with 49 pinheads out of which 28 fruitbodies were harvested. The 30.67g/kg substrate of fruitbodies with BCE of 3.06% was better than the 16.00g/kg substrate of fruitbodies with BCE of 1.58% from the non-composted mixture of rice straw + corn cob without *L. leuccocephala* leaves (Table 13).

The data however suggests that the differences in the mean yield of fruitbodies harvested from the two substrates or their BCEs are not statistically significant ($p>0.01$) (Appendix G).

There was decrease in the percentage hemicellulose, cellulose, crude fiber and crude fat contents from 21.00%, 6.00%, 26.26% and 2.00% before cultivation to 18.00%, 2.00%, 25.74% and 1.50% respectively after cultivation. There was increase in percentage crude protein and carbohydrate contents from 10.94% and 75.06% before cultivation to 11.19% and 75.31% respectively after cultivation. The pH value remained unchanged at 6.0 before and after cultivation (Table 14).

Table 13: Vegetative growth of *V. volvacea* on mixed non-composted substrates supplemented with *L. leucocephala* leaves

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
RS + CC	+++	20	70	35	16.00 ± 0.04	1.58 ± 0.30[a]
RS + CC + Lu	+++	17	49	28	30.67 ± 0.14	3.06 ± 0.95[a]
LSD at 1%		8.54	31.04	28.46	0.40	2.65
CV%		12.18	13.92	24.29	29.98	30.08

[1] Mycelial density: +++ = Moderate mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level
CC = Corn cob, RS = Rice straw Lu= *Leucaena leucocephala* leaves

Table 14: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrates used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
RS + CC + Lu	21.00 (18.00)	6.00 (2.00)	31.00 (31.00)	10.94 (11.19)	26.26 (25.74)	2.00 (1.50)	12.00 (12.00)	75.06 (75.31)	5.00 (5.00)	6.0 (6.0)

RS = Rice straw CC = Corn cob Lu = *Leucaena leucocephala* leaves

Values in parenthesis are those of the spent substrates

4.8 Growth and yield of *V. volvacea* on mixed composted substrates supplemented with *L. leucocephala* leaves

Among the five composted substrate mixtures, the corn cob + oil palm bunch stalk gave the best yield. This mixture was mixed with *L. leucocephala* leaves to assess the performance compared to the one without *L. leucocephala* leaves. The appearance of pinheads delayed, coming in 23 days. The mixture gave lower mycelia colonization with a mean of 23 of pinheads out of which 17 fruitbodies were harvested. The 14.67g/kg substrate of fruitbodies with BCE of 1.45% harvested was better than 7.33g/kg substrate of fruitbodies and BCE of 0.73% harvested from the composted mixture of corn cob + oil palm bunch stalk without *L. leucocephala* leaves (Table 15).

The data however suggests that the differences in the mean yield of fruitbodies harvested from the two substrates or their BCEs are not statistically significant ($p>0.01$) (Appendix H).

There was decrease in the hemicellulose, cellulose, lignin, crude fiber, ash and carbohydrate contents from 19.00%, 7.00%, 31.00%, 28.70%, 15.00% and 70.81% before cultivation to 3.00%, 5.00%, 13.56%, 26.26%, 14.00% and 70.74% respectively after cultivation whiles crude protein contents increased from 12.69% to 13.56%. The pH value decreased from 6.0 before cultivation to 5.5 after cultivation (Table 16).

Table 15: Vegetative growth of *V. volvacea* on mixed composted substrates supplemented with *L. leucocephala* leaves

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + OPBS	+++	16	41	9	7.33 ± 0.006	0.73 ± 0.05[a]
CC + OPBS + Lu	++	23	23	17	14.67 ± 0.08	1.45 ± 0.55[a]
LSD at 1%		2.17	13.29	14.72	0.22	1.47
CV%		2.96	11.05	29.37	35.39	35.43

[1] Mycelial density +++: Moderate mycelial growth ++: Scanty mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level
CC = Corn cob, OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves

Table 16: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS+CC+ Lu	19.00 (3.00)	7.00 (5.00)	31.00 (13.00)	12.69 (13.56)	28.70 (26.26)	1.50 (1.50)	15.00 (14.00)	70.81 (70.74)	3.00 (3.00)	6.0 (5.5)

OPBS = Oil palm bunch stalk CC = Corn cob Lu = *Leucaena leucocephala* leaves

Values in parenthesis are those of the spent substrates

4.9 Growth and yield of *V. volvacea* on single non-composted substrates supplemented with lime ($CaCO_3$)

Oil palm bunch stalk which gave the best yield among the single non-composted substrates was mixed with lime to assess the performance of the yield compared to the one without lime. The appearance of pinheads delayed, coming in 25 days. It gave very minimal mycelia colonization with 11 pinheads out of which 4 fruitbodies were harvested. The 4.67g/kg substrate of fruitbodies and BCE of 0.43 harvested was less than 118.00g/kg substrate of fruitbodies and BCE of 11.77% harvested from non-composted oil palm bunch stalk without lime (Table 17).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are statistically significant ($p<0.01$) (Appendix I).

There was decrease in hemicellulose, lignin, crude protein, crude fiber crude fat, ash and moisture contents of the substrates from 30.00%, 25.00%, 13.38%, 35.18%, 1.0% 13.00% and 5.000% before cultivation to 14.00%, 19.00%, 11.38%, 31.86%, 0.50% 12.00% and 3.00% respectively after cultivation whiles carbohydrate content increased from 72.62% to 76.12%. The cellulose content remained unchanged after cultivation. The pH value decreased from 6.0 before cultivation to 5.5 after cultivation (Table 18).

Table 17: Vegetative growth of *V. volvacea* on single non-composted substrates supplemented with lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	++++	16	151	135	118.00 ± 0.04	11.77 ± 0.25[a]
OPBS + Li	++	25	11	4	4.67 ± 0.05	0.43 ± 0.35[b]
LSD at 1%		10.74	15.50	11.49	0.17	1.15
CV%		13.83	5.09	4.40	4.89	5.01

[1] Mycelial density: ++++ = Profuse mycelial growth ++++: = Scanty mycelial growth

[2]Values bearing different letters in the same column in superscript are different at the 1% significant level

OPBS = Oil palm bunch stalk Li = Lime

Table 18: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS + Li	30.00 (14.00)	5.00 (5.00)	25.00 (19.00)	13.38 (11.38)	35.18 (31.86)	1.00 (0.50)	13.00 (12.00)	72.62 (76.12)	5.00 (3.00)	6.0 (5.5)

OPBS = Oil palm bunch stalk Li = Lime

Values in parenthesis are those of the spent substrates

4.10 Growth and yield of *V. volvacea* on single composted substrates supplemented with lime ($CaCO_3$)

Oil palm bunch stalk which gave the best yield among the single non-composted substrates was composted and mixed with lime to assess the yield performance compared to the one without lime. The appearance of pinheads delayed, and came in 24 days. The mixture gave a lower mycelia colonization with 9 pinheads out of which 4 fruitbodies were harvested. The 2.67g/kg substrate of fruitbodies and BCE of 0.27% was less than the 44.00g/kg substrate of fruitbodies and BCE of 4.42% from the single composted oil palm bunch stalk without lime (Table 19).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are statistically significant ($p<0.01$) (Appendix J).

There was an increase in the percentage carbohydrate, crude protein and crude fiber contents from 69.31%, 11.69% and 32.14% before cultivation to 70.81%, 12.69% and 35.60% respectively after cultivation while there was a decrease in the hemicellulose, cellulose, lignin, crude fat, ash and moisture contents from 19.00%, 5.00%, 28.00%, 1.00%, 18.00% and 5.00% before cultivation to 8.00% 3.00%, 25.00%, 0.50%, 16.00% and 3.00%, respectively after cultivation. The pH value remained unchanged 6.5, before and after cultivation (Table 20).

Table 19: Vegetative growth of *V. volvacea* on single composted substrates supplemented with lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	+++	22	46	33	44.00 ± 0.09	4.42 ± 0.61[a]
OPBS + Li	+	24	9	4	2.67 ± 0.02	0.27 ± 0.14[b]
LSD at 1%		16.24	18.48	13.11	0.25	1.67
CV%		18.65	17.99	19.03	18.88	18.88

[1]Mycelial density +++: Moderate mycelial growth +: Very scanty mycelial growth

[2]Values bearing different letters in the same column in superscript are different at the 1% significant level

OPBS = Oil palm bunch stalk Li = Lime

Table 20: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrates	Moisture	pH
OPBS + Li	19.00 (8.00)	5.00 (3.00)	28.00 (25.00)	11.69 (12.69)	32.14 (35.60)	1.00 (0.50)	18.00 (16.00)	69.31 (70.81)	5.00 (3.00)	(6.5) (6.5)

OPBS = Oil palm bunch stalk Li = Lime

Values in parenthesis are those of the spent substrates

4.11 Growth and yield of *V. volvacea* on mixed non-composted substrates supplemented with lime ($CaCO_3$)

The rice straw and corn cob mixture which gave the best result from the mixed non-composted group was mixed with lime to assess the performance. The pinheads appeared earlier in 17 days. The mixture gave minimal mycelia colonization with 40 pinheads out of which 30 fruitbodies were harvested. The 22.00g/kg substrate of fruitbodies and BCE of 2.20% was better than the 16.00g/kg substrate of fruitbodies and BCE of 1.58% harvested from the non-composted rice straw + corn cob mixture without lime (Table 21).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are not statistically significant ($p>0.01$) (Appendix K).

There was increase in percentage crude protein and carbohydrate contents from 9.19% and 73.81% before cultivation to 10.06% and 76.44% respectively after cultivation but there was decrease in the percentage hemicellulose, cellulose, lignin, crude fiber, moisture and crude fat contents from 25.00%, 3.00%, 32.00%, 31.37%, 5.00% and 4.00% before cultivation to 11.00%, 2.00%, 30.00% 27.78%, 2.00% and 0.50%, respectively after cultivation. Percentage ash content remained the same at 13.00%. The pH value decreased from 6.5 before cultivation to 6.0 after cultivation (Table 22).

Table 21: Vegetative growth of *V. volvacea* on mixed non-composted substrates supplemented with lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + RS	+++	20	70	35	16.00 ± 0.04	1.58 ± 0.30[a]
CC + RS+ Li	+++	17	40	30	22.00 ± 0.03	2.20 ± 0.22[a]
LSD at 1%		10.41	27.67	19.05	0.15	0.98
CV%		14.83	13.42	15.67	13.81	13.77

[1]Mycelial density +++: Moderate mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level

RS = rice straw, CC = corn cob Li = lime

Table 22: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	%Hemicellulose	%Cellulose	%Lignin	%Crude protein	%Crude fiber	%Crude fat	%Ash	%Carbohydrate	%Moisture	pH
RS + CC + Li	25.00 (11.00)	3.00 (2.00)	32.00 (30.00)	9.19 (10.06)	31.37 (27.78)	4.00 (0.50)	13.00 (13.00)	73.81 (76.44)	5.00 (2.00)	6.5 (6.0)

RS = Rice straw CC = Corn cob Li = Lime

Values in parenthesis are those of the spent substrates

4.12 Growth and yield of *V. volvacea* on mixed composted substrates supplemented with lime ($CaCO_3$)

The corn cob + oil palm bunch stalk mixture which gave the best result from the mixed composted group was mixed with lime to assess the performance. The appearance of pinheads on the supplemented substrates delayed and appeared in 25 days. The substrates gave minimal mycelia growth with 11 pinheads out of which 7 fruitbodies were harvested. The 6.67g/kg substrate of fruitbodies and BCE of 0.64% was less than the 7.33g/kg substrate of fruitbodies and BCE of 0.73% harvested from the composted corn cob + oil palm bunch stalk mixture without any supplement (Table 23).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are not statistically significant ($p>0.01$) (Appendix L).

There were decreases in percentage hemicellulose, cellulose, lignin, crude fiber, crude fat, moisture and ash contents from 24.00%, 9.00%, 41.00%, 34.00%, 8.00%, 5.00% and 11.00% before to 23.00%, 3.00%, 35.00%, 30.37%, 1.00%, 2.00% and 7.00% respectively after cultivation but there were increases in percentage crude protein and carbohydrate contents from 9.63% and 71.37% before cultivation to 10.94% and 81.06% respectively, after cultivation. The pH value remained unchanged at 5.5 before and after cultivation (Table 24).

Table 23: Vegetative growth of *V. volvacea* on mixed composted substrates supplemented with lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + OPBS	+++	16	41	9	7.33 ± 0.006	0.73 ± 0.05[a]
CC + OPBS + Li	++	25	11	7	6.67 ± 0.08	0.64 ± 0.51[a]
LSD at 1%		16.02	17.83	14.88	0.20	1.36
CV%		20.96	18.24	48.47	51.59	51.95

[1]Mycelial density: +++ = Moderate mycelial growth ++ = Scanty mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level

OPBS = Oil palm bunch stalk, CC = Corn cob Li = lime

Table 24: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS+CC+Li	24.00 (23.00)	9.00 (3.00)	41.00 (35.00)	9.63 (10.94)	34.00 (30.37)	8.00 (1.00)	11.00 (7.00)	71.37 (81.06)	5.00 (2.00)	5.5 (5.5)

OPBS = Oil palm bunch stalk CC = Corn cob Li = Lime

Values in parenthesis are those of the spent substrates

4.13 Growth and yield of *V. volvacea* on single non-composted substrates supplemented with *L. leucocephala* leaves and lime ($CaCO_3$)

L. leucocephala leaves and lime were added to oil palm bunch stalk which was the best substrate from the single non-composted substrate group to assess the yield performance. The appearance of pinheads on the supplemented substrates delayed, coming in 19 days. The substrate gave minimal mycelia colonization with 26 pinheads out of which 11 fruitbodies were harvested. The 6.00g/kg substrate of fruitbodies and BCE of 0.60% was less than the 118.00g/kg substrate of fruitbodies and BCE of 11.77% harvested from the single non-composted oil palm bunch stalk without any supplement (Table 25).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are statistically significant ($p<0.01$) (Appendix M).

There was increase in percentage crude protein and carbohydrate content from 11.38% and 68.12% before cultivation to 13.13% and 71.87% respectively after cultivation and decrease in percentage hemicellulose, cellulose, lignin, crude fiber, crude fat, moisture and ash contents from 14..00%, 3.00%, 23.00%, 37.56%, 8.50%, 5.00% and 12.00% before cultivation to 12.00%, 2.00%, 22.00%, 30.00%, 5.00%, 1.00% and 10.00%, respectively after cultivation. The pH value remained unchanged at 6.5 before and after cultivation (Table 26).

Table 25: Vegetative growth of *V. volvacea* on single non-composted substrates supplemented with *L. leucocephala leaves* and lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	++++	16	151	135	118.00 ± 0.04	11.77 ± 0.25[a]
OPBS + Lu + Li	++	19	26	11	6.00 ± 0.04	0.60 ± 0.27[b]
LSD at 1%		8.26	9.58	14.87	0.14	0.98
CV%		12.56	2.88	5.43	4.11	4.22

[1]Mycelial density: ++++ = Moderate mycelial growth ++ = Scanty mycelial growth

[2]Values bearing different letters in the same column in superscript are different at the 1% significant level

OPBS = Oil palm bunch stalk Li = Lime Lu = *Leucaena leucocephala* leaves

Table 26: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS +Lu +Li	14.00 (12.00)	3.00 (2.00)	23.00 (22.00)	11.38 (13.13)	37.56 (30.00)	8.50 (5.00)	12.00 (10.00)	68.12 (71.87)	5.00 (1.00)	6.5 (6.5)

OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves Li = Lime

Values in parenthesis are those of the spent substrates

4.14 Growth and yield of *V. volvacea* on single composted substrates supplemented with *L. leucocephala* leaves and lime ($CaCO_3$)

L. leucocephala leaves and lime were added to the single composted oil palm bunch stalk to assess the yield compared to the one cultivated without any supplement. The appearance of pinheads on the supplemented substrates delayed, and came in 24 days. The substrate gave very minimal mycelia colonization with 9 pinheads out of which 7 fruitbodies were harvested. The 8.00g/kg substrate of fruitbodies and BCE of 0.77% was less than the 44.00g/kg substrate of fruitbodies and BCE of 4.42% harvested from the single composted oil palm bunch stalk without any supplements (Table 27).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are statistically significant ($p<0.01$) (Appendix N).

There was decrease in percentage hemicellulose, cellulose, lignin, crude protein, and carbohydrate contents from 15.00%, 5.00%, 27.00%, 10.44% and 74.06% before cultivation to 10.00%, 3.00%, 24.00%, 9.74% and 73.76% respectively after cultivation but there was increase in percentage crude fiber and crude fat contents from 27.50% and 0.50% before cultivation to 32.85% and 1.50% respectively after cultivation. Percentage ash and moisture content remained unchanged at 15.00% and 2.00% respectively. The pH value increased from 6.0 before cultivation to 6.5 after cultivation (Table 28).

Table 27: Vegetative growth of *V. volvacea* on single composted substrates supplemented with *L. leucocephala* and lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
OPBS	+++	22	46	33	44.00 ± 0.09	4.42 ± 0.61[a]
OPBS + Lu + Li	+	24	9	7	8.00 ± 0.05	0.77 ± 0.37[b]
LSD at 1%		10.74	12.27	15.19	0.28	1.89
CV%		12.34	11.95	20.21	19.45	19.42

[1]Mycelial density: +++ = Moderate mycelial growth + = Very scanty mycelial growth

[2]Values bearing different letters in the same column in superscript are different at the 1% significant level

OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves Li: Lime

Table 28: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPBS + Lu + Li	15.00 (10.00)	5.00 (3.00)	27.00 (24.00)	10.44 (9.74)	27.50 (32.85)	0.50 (1.50)	15.00 (15.00)	74.06 (73.76)	2.00 (2.00)	(6.0) (6.5)

OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves Li = Lime

Values in parenthesis are those of the spent substrates

4.15 Growth and yield of *V. volvacea* on mixed non-composted substrates supplemented with *L. leucocephala* leaves and lime ($CaCO_3$)

L. leucocephala leaves and lime were added to non-composted rice straw + corn cob mixture which gave the best yield among the mixed non-composted group to assess the yield compared to that of the non-composted rice straw + corn cob mixture without any supplements. The appearance of pinheads on the supplemented substrates was earlier (in 16 days). The substrate gave a little lower mycelia colonization with 66 pinheads out of which 51 fruitbodies were harvested. The 45.33g/kg substrate of fruitbodies and BCE of 4.53% was more than the 16.00g/kg substrate of fruitbodies and BCE of 1.58% harvested from the non-composted rice straw + corn cob mixture without supplements (Table 29).

The data suggests that the differences in the mean weight of fruitbodies harvested from the two substrates or their BCEs are statistically significant ($p<0.01$) (Appendix O).

There was decrease in percentage hemicellulose, lignin, crude fiber, moisture and ash contents from 35.00% 32.00%, 31.00%, 4.00% and 17.00% before cultivation to 23.00%, 24.00%, 23.62%, 2.00% and 10.00% respectively after cultivation but there was increase in percentage cellulose and carbohydrate contents from 1.00% and 71.94% before cultivation to 5.00% and 78.94, respectively whiles percentage crude protein and crude fat remained unchanged after cultivation. The pH increased from 6.0 before cultivation to 6.5 after cultivation (Table 30).

Table 29: Vegetative growth of *V. volvacea* on mixed non-composted substrates supplemented with *L. leucocephala* and lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + RS	+++	20	70	35	16.00 ± 0.04	1.58 ± 0.30[b]
CC + RS + Lu + Li	+++	16	66	51	45.33 ± 0.08	4.53 ± 0.54[a]
LSD at 1%		12.56	21.32	23.53	0.24	1.62
CV%		18.39	8.91	14.61	14.11	14.13

[1]Mycelial density: +++ = Moderate mycelial growth

[2]Values bearing different letters in the same column in superscript are different at the 1% significant level

RS = Rice straw CC = Corn cob Lu = *Leucaena leucocephala* leaves Li = Lime

Table 30: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
RS + CC +Lu + Li	35.00 (23.00)	1.00 (5.00)	32.00 (24.00)	10.06 (10.06)	31.00 (23.62)	1.00 (1.00)	17.00 (10.00)	71.94 (78.94)	4.00 (2.00)	6.0 (6.5)

RS = Rice straw CC = Corn cob Lu = *Leucaena leucocephala* leaves Li = Lime

Values in parenthesis are those of the spent substrates

4.16 Growth and yield of *V. volvacea* on mixed composted substrates supplemented with *L. leucocephala* leaves and lime ($CaCO_3$)

Leucaena leucocephala leaves and lime were added to composted corn cob and oil palm bunch stalk mixture to assess the yield of *V. volvacea* compared to the yield without any supplements. The appearance of pinheads on the supplemented substrate delayed, coming in 19 days. The substrate showed a little higher mycelia colonization with 39 pinheads out of which 17 fruitbodies were produced. The 14.00g/kg substrate of fruitbodies and BCE of 1.43% was more than the 12.00g/kg substrate of fruitbodies harvested with BCE of 1.19% from the corn cob + oil palm bunch stalk mixture without supplements (Table 31).

The data suggests that the differences in the mean yield of fruitbodies harvested from the two substrates or their BCEs are not statistically significant ($p>0.01$) (Appendix P).

There was increase in percentage ash and carbohydrate contents from 13.00% and 74.69% before cultivation to 14.00% and 75.87% respectively after cultivation. There was decrease in the hemicellulose, cellulose, lignin, crude protein, moisture and crude fibre contents from 18.00%, 8.00%, 35.00%, 11.81%, 5.00% and 28.43% before cultivation to 16.00%, 1.00%, 25.00%, 9.63%, 2.00% and 25.89% respectively after cultivation. The percentage crude fat content remained unchanged at 0.50%. The pH increased from 6.0 before cultivation to 6.5 after cultivation (Table 32).

Table 31: Vegetative growth of *V. volvacea* on mixed composted substrates supplemented with *L. leucocephala* leaves and lime ($CaCO_3$)

Substrate	Mycelial density[1]	Mean number of days for pinheads to appear	Mean number of pinheads	Mean number of fruitbodies harvested	Mean yield (g/kg substrate)	Biological Conversion Efficiency (%)[2]
CC + OPBS	+++	18	38	14	12.00 ± 0.12	1.19 ± 0.80[a]
CC + OPBS + Lu + Li	+++	19	39	17	14.00 ± 0.08	1.43 ± 0.55[a]
LSD at 1%		11.99	14.48	29.64	0.39	2.59
CV%		17.39	10.00	50.33	52.30	52.52

[1]Mycelial density: +++: Moderate mycelial growth

[2]Values bearing the same letters in the same column in superscript are not different at the 1% significant level

CC = Corn cob OPBS = Oil palm bunch stalk Lu = *Leucaena leucocephala* leaves Li = Lime

Table 32: Hemicelluloses, Cellulose, Lignin, pH and Proximate composition of fresh and spent substrate used in cultivation of *V. volvacea*

Substrate	Composition of substrate (%)									
	Hemicellulose	Cellulose	Lignin	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrate	Moisture	pH
OPB + CC + Lu + Li	18.00 (16.00)	8.00 (1.00)	35.00 (25.00)	11.81 (9.63)	28.43 (25.89)	0.50 (0.50)	13.00 (14.00)	74.69 (75.87)	5.00 (2.00)	6.0 (6.5)

OPBS = Oil palm bunch stalk CC = Corn cob Lu = *Leucaena leucocephala* leaves Li = Lime

Values in parenthesis are those of the spent substrates

CHAPTER FIVE

5. DISCUSSION

The study showed that the composition of substrates greatly influenced on yield and BCE of *Volvariella volvacea*. Considering all the substrates, rice straw and oil palm bunch stalk from the single non-composted substrates recorded the highest mean yield of fruitbodies and biological conversion efficiency. This could be due to the nutrient materials present in the substrates contain. *V. volvacea* mycelia grows very well on a wide range of cellulosic wastes. Fasidi (1996) reported that rice husk is good for the production of *Volvariella esculenta* because of its richness in oils and vitamins which are good stimulants for high mushroom yield. Unsupplemented non-composted rice straw and oil palm bunch stalk wastes supported maximum mycelia growth of *V. volvacea*. The growth could be due to the wide range of nutrients present in these substrates which can support growth of microorganisms. This agrees with the findings of Isikhuemhen (2004) who reported that *V. volvacea* can be cultivated on unsupplemented agricultural wastes.

Various combination of the agricultural wastes also supported appreciable growth of the mycelia. For instance a combination of rice straw and corn cob gave the highest BCE in the mixed non-composted substrates. This may be due to the physical nature exhibited by the substrates after mixing. Chang (1983) suggested that looseness, high proportion of cellulose and compactness on wetting of wastes allow good growth of mushrooms.

Supplemented substrates have additional nutrients for microorganisms to perform better on substrates. However, this was not seen when oil palm bunch stalk was mixed with *Leucaena leucocephala* leaves which gave mean yield and BCE lower than that from the

unsupplemented single non-composted oil palm bunch stalk. This could be due to a rise in the substrate temperature from the growth of moulds. Supplementation has been reported to cause a rise in substrate temperature, possibly due to faster metabolic activities triggered by extra nitrogen. Royse and Schisler (1986) observed overheating (from 30°C to 47°C) in bags where a nitrogen-rich supplement was applied without benomyl (fungicide) treatment and proposed that it could be due to the growth of competitor moulds. Addition of lime ($CaCO_3$) as a supplement to correct acidic conditions did not have much effect on yield performance compared to those from the unsupplemented substrates. Fasidi (1996) reported that *Volvariella esculenta* was able to tolerate pH range of 3 – 10.

The effects of physical and chemical properties of substrates on yield and BCE have been investigated in *Volvariella volvacea* (Peksen and Yakupoglu, 2009). The result of proximate composition of various agricultural wastes before and after growth of *V. volvacea* mycelia showed a significant increase in the protein content of agricultural wastes after growth of *V. volvacea* compared to the counterparts not inoculated with *V. volvacea.* This could be due to increase in the nitrogen and other nutritional components as well as secretions from the *V. volvacea.* Some authors reported that, the colonization of wastes by fungi results in increase in their nutritional values (Zadrazil, 1993). This may be due to secretion of certain extracellular enzymes which are proteinaceous in nature into the waste during their breakdown and its subsequent metabolism (Kadiri,1999). Protein increase could also be as a result of hydrolysis of starch to glucose and its subsequent use by same organism as a carbon source to synthesize fungal biomass rich in protein (Bender, 1970).

There was a reduction in cellulose, hemicellulose and lignin content of most of the substrates after colonization of *V. volvacea*. This reduction could be due to the fact that the cellulose, hemicellulose and lignin contents of most of the substrates have been hydrolysed, decomposed or utilized by the *V. volvacea*. This is in agreement with the findings of Yung and Yee (1977) that *V. volvacea* could decompose and utilize hemicellulose and cellulose very efficiently. Cellulose and hemicellulose components of plant cell walls are intimately associated with lignin moiety (Kirk, 1985). According to Deacon (1980) *V. volvacea* hydrolyses cellulose and hemicellulose leaving the lignin intact. This is due to its inability to synthesize lignin degrading enzymes. This was however not in agreement with the results found for most of the substrates used in this work where the lignin contents were, reduced to some degree for most of the substrates.

There were no considerable changes in the fat and ash contents of the substrates. Increase in the ash content observed in some substrates could be due to the inorganic mineral elements present. The ash content is always a rough measure of the inorganic mineral elements in the samples. It is unlikely that the microorganisms might have used some of the minerals for their metabolic activities. This corroborated the work of Frazier and Westhoff (1978) who reported that all living organisms require some mineral elements to maintain some metabolic functions. The crude fibre content of most of the substrates before cultivation was less than the contents after cultivation of *V. volvacea*. This could be due to the nature of the substrates and how they were utilized by particular species of fungi. This was supported by the findings of Safari-Sinegani *et al*. (2005) who found that the biodegradation of plant residues is dependent on the plant and fungus species and that there was a crude fiber loss in wheat, barley and rice straws after treating with the fungus *Phaenerochaete chrysosporium*.

Most of the substrates experienced a decrease in moisture content after *V. volvacea* colonization. The decrease in the moisture content may be due to the absorptive nature of the substrates after *V. volvacea* colonization. This is however, not in agreement with the findings of Akinyele (2003) who noted increase in moisture content observed in substrates after mycelia colonization may be due to the processes of forming slurry and activities and/or influence of *Volvariella volvacea*, which potentiate water retention within the fermentation medium.

There was also appreciable decrease in the carbohydrate content of most of the substrates after cultivation of *V. volvacea.* This observation might have occurred due to the ability of *V. volvacea* to utilize carbohydrate in the production of cellulase and amylase. Howard *et al.* (2003) reported the ability of mushrooms to hydrolyze carbohydrates in bioconversion process for various biochemical pathways.

Most of the substrates had a drop in pH values. This could be due to the carbon dioxide released during the growth of *V. volvacea.* Reports by some researchers indicated a drop in pH could be attributed to high organic carbon content, mineralization of organic acid by acid forming bacteria as well as the large quantities of carbon dioxide released during the composting process (Beck-Friis *et al.*, 2003). The gradual decline in pH observed in all the substrates during the growth period was likely caused by fungal activities. Safari-Sinegani *et al.* (2005) reported that the lower the fungal activity the higher the pH value meaning less carbon dioxide will be reeased. In addition, they said basidiomycetes decreased pH of cultures especially, wood and rice cultures.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The chemical and physical properties of the various substrates influenced the yield and growth of *Volvariella volvacea.* The observed differences in the yield from the substrates may be due to the different percentages or contents of cellulose materials and other essential nutrients. Unsupplemented single non-composted oil palm bunch stalk was the best substrate for the growth and yield of *V. volvacea* followed by the unsupplemented single non-composted rice straw and the poorest being cocoa pod and oil palm female spikelets.

6.2 RECOMMENDATIONS

- With reference to the results obtained from the cultivation of *V. volvacea* on the agricultural wastes, it is recommended that the oil palm bunch stalk and rice straw be used for the production of *V. volvacea.*
- There should be a better way of cutting the substrates than the manual one to save time and energy. (Suggestion: a machine should be used to do the cutting)
- Mushroom growing should be integrated into our farming system due to its environmental waste management.

REFERENCES

Akinyele, B.J. (2003). *In vitro* nutritional studies on *Volvariella volvacea* (Bull. Ex Fr.) Sing, an edible mushroom. Ph.D. Thesis, Federal University of Technology, Akure, Nigeria, pp. 157.

Akinyele, B.J. and Akinyosoye, F.A. (2005). Effect of *Volvariella volvacea* cultivaton on the chemical composition of agrowastes. African Journal of Biotechnology 4 (9): 979-983.

Akinyele, B.J., Olaniyi, O.O. and Arotupin, D.J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by *Volvariella volvacea*, an edible mushroom. *Res. J. Microbiol.* 6: 63-70.

Beck-Friis, B., Smars, S., Jonsson, H., Eklind, Y. and Kirchmann, H. (2003). Composting of source separated household organic wastes at different oxygen and understanding of the emission dynamics. *Compost Science and Utilization* 11: 41-50.

Bender, P.F. (1970). Under-utilized resources as animal feedstuffs. National Academic Press, Washington, D.C pp.100.

Bisaria, R., Madan, M. and Vasudevan, P. (1997). Utilization of agro-residues as animal feed through bioconversion. *Bioresour. Technol.*, *59*:5-8.

Chang, S.T. (1983). Prospect of *Volvoriella volvacea* cultivation. *Mushroom Newsletter for the Tropics,* 4(2) : 5-8.

Chang, S.T. and Miles, P.G. (1991). Recent trends in world production of cultivated edible mushrooms. *The Mushroom Journal, 503:* 15-18.

Deacon, J.W. (1980). Introduction to modern mycology. ELBS, UK. pp. 61 – 62.

Fan, L., Pan, H., Soccol, T., Pandey, A. and Soccol, C. S. (2006). Advances in mushroom research in the last decade. Food Technol. Biotechnol. 44(3): 303-331.

Fasidi, I. O. (1996). Studies on *Volvariella esculenta* (Mass) Singer: Cultivation on agricultural wastes and proximate composition of stored mushrooms. *Food Chemistry*: *55*(2): 161 – 163.

Fasidi, I.O. and Kadiri, M. (1993). Use of agricultural wastes for the cultivation of *Lentinus subnudus* in Nigeria. *Revita Biol. Trop., 41*: 411-415.

Frazier, C. N. and Westhoff, C. D. (1978). Food microbiology. 3rd Edn., Mc Graw Hill Inc., India, pp. 540.

Gregori, A., Svagelj, M. and Pohleven, J. (2007). Cultivation techniques and medicinal properties of *Pleurotus* spp. Food Technol. Biotechnol. 45(3): 238-249.

Ho, M.S. (1972). Straw mushroom cultivation in plastic house. *Mushroom Science, 8*:257-263.

Howard, R.L.I., Abotsi, E., Van-Rensburg, E.L.I.J. and Howard, S. (2003). Lignocellulose biotechnology issues of bioconversion and enzyme production. Rev. *Afr. J. Biotechnol., 2*: 602-619.

Isikhuemhen, O.S. (2004). Volunteer trainer to Imo ADP extension staff on mushroom production using locally available substrates (palm fibre and sawdust). A seminar. 27th – 30th Dec., 2004. Owerri, Imo State, Nigeria.

Isikhuemhen, O.S. and LeBauer, D. S. (2004). Growing *Pleurotus tuberregium*. *Mushworld Publication, 11*: 264-274.

Jonathan, G., Adetolu, A., Ikpebivie, O. and Donbebe, W. (2006). Nutritive value of common wild edible mushrooms from southern Nigeria. Global J. Biotechnol. Biochem 1(1): 16-21.

Kadiri, M. (1999). Changes in intracellular and extracellular enzyme activities of *Lentinus subnudus* during sporophore development. *Biosciences Res. Comm. 11*(2):127-130.

Kirk, T.K. (1985). The discovery and promise of lignin degrading enzymes. In: New Horizons for Biotechnological Utilization of Forest Resources. Marcus Wallenberg Foundation Symposium, pp.27 – 42.

Microsoft Encarta (2009). © 1993-2008 Microsoft Corporation.

Mshandete, A. M. and Cuff, J. (2007). Proximate and nutrient composition of three types of indigenous edible wild mushrooms grown in Tanzania and their utilization prospects. *African Journal of Food, Agriculture, Nutrition and Development, 7*(6):2-6.

Nwanze, P. I., Ameh, J. B., and Umoh, V. J. (2008). The effect of the interaction of various spawn grains and oil types on carpophore dry weight, stipe length and stipe and pileus diameters of *Lentinus squarrosulus* (Mont.) Singer. *Afr. J . Food Agric. Nutr. Dev., 8*(4): 480-491.

Oei, P. (1996). Mushroom cultivation with special emphasis on appropriate techniques for developing countries. Tool Publications. Leiden, The Netherlands, pp. 2, 46, 102-110.

Ofosu-Asiedu, A., Schmidt, O. and Liese, W. (1984). Growth studies of *Volvariella volvacea* for cultivation on wood waste. *Material and Organism, 19*:241-251.

Onuoha, C. I., Oyibo, G. and Ebibila, J. (2009). Cultivation of *Volvariella volvacea* (mushroom) using some agrowaste materials. *Journal of American Science* 5(5):135-138.

Osemwegie, O.O., Isikhuemhen, O.S., Onyolu, O.J. and Okhuoya, A.J. (2002). Cultivation of a selected sporophore-only-producing strain of the edible and medicinal mushroom, *Pleurotus tuberregium* (Fr.) Singer (Agaricomycetideae) on waste paper and plantain peelings. *International Journal of Medicinal Mushrooms, 4*: 343-348.

Peksen, A. and Yakupoglu, G. (2009). Tea waste as a supplement for the cultivation of *Ganoderma lucidum*. *World J. Microbiol. Biotechnol. 25*(4): 611-618.

Quimo, T.H., Chang, S.T. and Royse, D.J. (1990). Technical guidelines for mushroom growing in the tropics. Food and Agriculture Organisation of the United Nations, Rome. pp. 106.

Royse, D.J. and Schisler, L.C. (1986). Effect of benomyl application and spawn rate supplementation on yield and size of selected genotypes of *Pleurotus* spp. In: Proc. Int. Scientific and Technical aspects of cultivating edible fungi. Penn State University, Elsevier Science Publishers, Amsterdam, pp. 109-115.

Safari-Sinegani, A.A., Emmtiazi, G., Hajrasuliha, S., and Shariatmadari, H. (2005). Biodegradation of some agricultural residues by fungi in agitation submerged cultures. *African Journal of Biotechnology, 4*(10): 1058-1061.

Samajpati, N. (1979). Cultivation method of paddy straw mushroom in India. *Mushroom Science, 10*(2):629-633.

Stamets, P. (2000). Growing gourmet and medical mushrooms. Ten Speed Press, Berkeley, Toronto pp. 341-348.

Stamets, P. (2001). A novel approach to farm waste management. *Mushroom Journal*, Winter pp. 22.

Tshieper, H.J. and Hartman, K. (1977). A comparison of different mushroom growing methods, Mushroom Journal 60:404-416.

Wasser, P.S. (2007). A book review. Mycelium running: How mushrooms can help save the world. *Herbalgram, 76*: 50-57.

Yung, C.H. and Yee, T.N. (1977). Comparative study of the physiology of *Volvariella volvacea* and *Coprinus cinereus*. Transactions of the British Mycological Society pp:167-172.

Zadrazil, F. (1993). Conversion of lignocellulosic wastes into animal feed with white fungi. *Proceedings of the International Conference of Mushroom Biology*, London pp. 151-161.

Zoberi, M.H. (1972). Tropical macrofungi: Some common species. Macmillian, London pp: 158.

http://wwwattra.ncat.org/attra-pub/PDF/mushroom.pdf. Mushroom cultivation and marketing. Visited 12/9/2009.

http://wwwdelfresh.com/html/history.html. History of mushrooms. Visited 12/9/2009.

http://www.foodreference.com/html/art-mush-history417.html. Mushroom history. Visited 12/9/2009.

http://www.starchefs.com/features/mushrooms/html/more_mushrooms.shtml.

Marvelous Mushroom Histroy. Visited 12/9/2009

APPENDICES

APPENDIX A

Analysis of variance on growth and yield of *Volvariella volvacea* on single non-composted substrates

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	5	0.00000	0.00000	M	M
Error	12	0.00000	0.00000		
Total	17	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	5	409.778	81.9556	24.59	0.0000
Error	12	40.000	3.3333		
Total	17	449.778			

Grand Mean 18.889 CV 9.67

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	5	48538.9	9707.79	1765.05	0.0000
Error	12	66.0	5.50		
Total	17	48604.9			

Grand Mean 49.944 CV 4.70

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	5	37881.8	7576.36	1420.57	0.0000
Error	12	64.0	5.33		
Total	17	37945.8			

Grand Mean 40.889 CV 5.65

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	5	6.47150	1.29430	1399.75	0.0000
Error	12	0.01110	0.00092		
Total	17	6.48260			

Grand Mean 0.5281 CV 5.76

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	5	286.960	57.3921	1353.41	0.0000
Error	12	0.509	0.0424		
Total	17	287.469			

Grand Mean 3.5200 CV 5.85

APPENDIX B

Analysis of variance on growth and yield of *Volvariella volvacea* on single composted substrates

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	60.1667	60.1667	12.45	0.0243
Error	4	19.3333	4.8333		
Total	5	79.5000			

Grand Mean 19.500 CV 11.27

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	16328.2	16328.2	1781.25	0.0000
Error	4	36.7	9.2		
Total	5	16364.8			

Grand Mean 98.833 CV 3.06

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	15301.5	15301.5	1953.38	0.0000
Error	4	31.3	7.8		
Total	5	15332.8			

Grand Mean 84.167 CV 3.33

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	1.78215	1.78215	1645.06	0.0000
Error	4	0.00433	0.00108		
Total	5	1.78648			

Grand Mean 1.2217 CV 2.69

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	78.9888	78.9888	1511.27	0.0000
Error	4	0.2091	0.0523		
Total	5	79.1979			

Grand Mean 8.1383 CV 2.81

APPENDIX C

Analysis of variance on growth and yield of *Volvariella volvacea* on mixed non- composted substrates

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	4	0.00000	0.00000	M	M
Error	10	0.00000	0.00000		
Total	14	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	4	1074.67	268.667	87.61	0.0000
Error	10	30.67	3.067		
Total	14	1105.33			

Grand Mean 16.667 CV 10.51

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	4	11455.3	2863.83	133.41	0.0000
Error	10	214.7	21.47		
Total	14	11670.0			

Grand Mean 36.000 CV 12.87

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	4	1986.27	496.567	53.20	0.0000
Error	10	93.33	9.333		
Total	14	2079.60			

Grand Mean 13.600 CV 22.46

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	4	0.14529	0.03632	75.06	0.0000
Error	10	0.00484	0.00048		
Total	14	0.15013			

Grand Mean 0.1176 CV 18.71

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	4	6.43353	1.60838	75.44	0.0000
Error	10	0.21320	0.02132		
Total	14	6.64673			

Grand Mean 0.7833 CV 18.64

APPENDIX D

Analysis of variance on yield of *Volvariella volvacea* on mixed composted substrates

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	4	0.00000	0.00000	M	M
Error	10	0.00000	0.00000		
Total	14	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	4	815.600	203.900	254.88	0.0000
Error	10	8.000	0.800		
Total	14	823.600			

Grand Mean 14.400 CV 6.21

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	4	3034.27	758.567	113.78	0.0000
Error	10	66.67	6.667		
Total	14	3100.93			

Grand Mean 16.267 CV 15.87

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substract	4	181.600	45.4000	85.12	0.0000
Error	10	5.333	0.5333		
Total	14	186.933			

Grand Mean 4.7333 CV 15.43

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	4	0.02245	0.00561	118.08	0.0000
Error	10	0.00048	0.00005		
Total	14	0.02293			

Grand Mean 0.0475 CV 14.50

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	4	0.99097	0.24774	117.97	0.0000
Error	10	0.02100	0.00210		
Total	14	1.01197			

Grand Mean 0.3147 CV 14.56

APPENDIX E

Analysis of variance for yield of *Volvariella volvacea* on single non-composted substrates + Leucaena leaves

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	28.1667	28.1667	8.45	0.0438
Error	4	13.3333	3.3333		
Total	5	41.5000			

Grand Mean 18.500 CV 9.87

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	30104.2	30104.2	3926.63	0.0000
Error	4	30.7	7.7		
Total	5	30134.8			

Grand Mean 80.167 CV 3.45

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	23688.2	23688.2	4584.81	0.0000
Error	4	20.7	5.2		
Total	5	23708.8			

Grand Mean 71.833 CV 3.16

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	3.92203	3.92203	3329.87	0.0000
Error	4	0.00471	0.00118		
Total	5	3.92674			

Grand Mean 0.9582 CV 3.58

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	173.989	173.989	3053.34	0.0000
Error	4	0.228	0.057		
Total	5	174.217			

Grand Mean 6.3817 CV 3.74

APPENDIX F

Analysis of variance for yield of *Volvariella volvacea* on single composted substrates + Leucaena leaves

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	2.6667	2.6667	0.25	0.6433
Error	4	42.6667	10.6667		
Total	5	45.3333			

Grand Mean 21.667 CV 15.07

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	468.167	468.167	15.27	0.0174
Error	4	122.667	30.667		
Total	5	590.833			

Grand Mean 36.833 CV 15.03

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	468.167	468.167	38.48	0.0034
Error	4	48.667	12.167		
Total	5	516.833			

Grand Mean 23.833 CV 14.64

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.34993	0.34993	77.89	0.0009
Error	4	0.01797	0.00449		
Total	5	0.36790			

Grand Mean 0.4215 CV 15.90

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	16.2361	16.2361	76.95	0.0009
Error	4	0.8440	0.2110		
Total	5	17.0801			
Grand Mean		2.8450	CV 16.15		

APPENDIX G

Analysis of variance for yield of *Volvariella volvacea* on mixed non-composted substrates + Leucaena leaves

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	16.6667	16.6667	3.23	0.1469
Error	4	20.6667	5.1667		
Total	5	37.3333			

Grand Mean 18.667 CV 12.18

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	640.667	640.667	9.40	0.0374
Error	4	272.667	68.167		
Total	5	913.333			

Grand Mean 59.333 CV 13.92

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	73.500	73.5000	1.28	0.3208
Error	4	229.333	57.3333		
Total	5	302.833			

Grand Mean 31.167 CV 24.29

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.07729	0.07729	6.97	0.0575
Error	4	0.04434	0.01108		
Total	5	0.12163			

Grand Mean 0.3512 CV 29.98

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	3.43527	3.43527	6.94	0.0580
Error	4	1.98113	0.49528		
Total	5	5.41640			

Grand Mean 2.3400 CV 30.08

APPENDIX H

Analysis of variance for yield of *Volvariella volvacea* on mixed composted substrates + Leucaena leaves

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	88.1667	88.1667	264.50	0.0001
Error	4	1.3333	0.3333		
Total	5	89.5000			

Grand Mean 19.500 CV 2.96

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	486.000	486.000	38.88	0.0034
Error	4	50.000	12.500		
Total	5	536.000			

Grand Mean 32.000 CV 11.05

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	96.000	96.0000	6.26	0.0666
Error	4	61.333	15.3333		
Total	5	157.333			

Grand Mean 13.333 CV 29.37

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.01591	0.01591	4.64	0.0976
Error	4	0.01372	0.00343		
Total	5	0.02964			

Grand Mean 0.1655 CV 35.39

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	0.71415	0.71415	4.69	0.0964
Error	4	0.60953	0.15238		
Total	5	1.32368			

Grand Mean 1.1017 CV 35.43

APPENDIX I

Analysis of variance for yield of *Volvariella volvacea* on single non-composted substrates + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	112.667	112.667	13.80	0.0206
Error	4	32.667	8.167		
Total	5	145.333			

Grand Mean 20.667 CV 13.83

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	29400.0	29400.0	1729.41	0.0000
Error	4	68.0	17.0		
Total	5	29468.0			

Grand Mean 81.000 CV 5.09

Source	DF	SS	MS	F	P

ANOVA for mean number of fruitbodies harvested

substrate	1	25480.2	25480.2	2730.02	0.0000
Error	4	37.3	9.3		
Total	5	25517.5			

Grand Mean 69.500 CV 4.40

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	4.34350	4.34350	2167.24	0.0000
Error	4	0.00802	0.00200		
Total	5	4.35152			

Grand Mean 0.9158 CV 4.89

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	192.667	192.667	2064.29	0.0000
Error	4	0.373	0.093		
Total	5	193.040			

Grand Mean 6.1000 CV 5.01

APPENDIX J

Analysis of variance for yield of *Volvariella volvacea* on single composted substrates + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	4.1667	4.1667	0.22	0.6612
Error	4	74.6667	18.6667		
Total	5	78.8333			

Grand Mean 23.167 CV 18.65

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	2016.67	2016.67	83.45	0.0008
Error	4	96.67	24.17		
Total	5	2113.33			

Grand Mean 27.333 CV 17.99

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	1232.67	1232.67	101.32	0.0005
Error	4	48.67	12.17		
Total	5	1281.33			

Grand Mean 18.333 CV 19.03

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.58219	0.58219	132.18	0.0003
Error	4	0.01762	0.00440		
Total	5	0.59981			

Grand Mean 0.3515 CV 18.88

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	25.9168	25.9168	132.24	0.0003
Error	4	0.7839	0.1960		
Total	5	26.7007			

Grand Mean 2.3450 CV 18.88

APPENDIX K

Analysis of variance for yield of *Volvariella volvacea* on mixed non-composted substrates + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	16.6667	16.6667	2.17	0.2144
Error	4	30.6667	7.6667		
Total	5	47.3333			

Grand Mean 18.667 CV 14.83

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	1320.17	1320.17	24.37	0.0078
Error	4	216.67	54.17		
Total	5	1536.83			

Grand Mean 54.833 CV 13.42

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	32.667	32.6667	1.27	0.3223
Error	4	102.667	25.6667		
Total	5	135.333			

Grand Mean 32.333 CV 15.67

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.01279	0.01279	8.32	0.0448
Error	4	0.00615	0.00154		
Total	5	0.01893			

Grand Mean 0.2838 CV 13.81

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	0.57042	0.57042	8.41	0.0441
Error	4	0.27127	0.06782		
Total	5	0.84168			

Grand Mean 1.8917 CV 13.77

APPENDIX L

Analysis of variance for yield of *Volvariella volvacea* on mixed composted substrates + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	130.667	130.667	7.19	0.0551
Error	4	72.667	18.167		
Total	5	203.333			

Grand Mean 20.333 CV 20.96

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	1350.00	1350.00	60.00	0.0015
Error	4	90.00	22.50		
Total	5	1440.00			

Grand Mean 26.000 CV 18.24

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	8.1667	8.1667	0.52	0.5102
Error	4	62.6667	15.6667		
Total	5	70.8333			

Grand Mean 8.1667 CV 48.47

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.00047	0.00047	0.16	0.7104
Error	4	0.01178	0.00294		
Total	5	0.01224			

Grand Mean 0.1052 CV 51.59

ANOVA for Biological Conversion Efficiency

Source	**DF**	**SS**	**MS**	**F**	**P**
substrate	1	0.02042	0.02042	0.16	0.7138
Error	4	0.52647	0.13162		
Total	5	0.54688			

Grand Mean 0.6983 CV 51.95

APPENDIX M

Analysis of variance for yield of *Volvariella volvacea* on single non-composted substrates + Leucaena leaves + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	8.1667	8.16667	1.69	0.2635
Error	4	19.3333	4.83333		
Total	5	27.5000			

Grand Mean 17.500 CV 12.56

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	23437.5	23437.5	3605.77	0.0000
Error	4	26.0	6.5		
Total	5	23463.5			

Grand Mean 88.500 CV 2.88

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	22940.2	22940.2	1464.27	0.0000
Error	4	62.7	15.7		
Total	5	23002.8			

Grand Mean 72.833 CV 5.43

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	4.21682	4.21682	2893.85	0.0000
Error	4	0.00583	0.00146		
Total	5	4.22265			

Grand Mean 0.9283 CV 4.11

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	187.042	187.042	2745.90	0.0000
Error	4	0.272	0.068		
Total	5	187.314			

Grand Mean 6.1833 CV 4.22

APPENDIX N

Analysis of variance for yield of *Volvariella volvacea* on single composted substrates + Leucaena leaves + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	4.1667	4.16667	0.51	0.5145
Error	4	32.6667	8.16667		
Total	5	36.8333			

Grand Mean 23.167 CV 12.34

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	2016.67	2016.67	189.06	0.0002
Error	4	42.67	10.67		
Total	5	2059.33			

Grand Mean 27.333 CV 11.95

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	962.67	962.667	58.94	0.0015
Error	4	65.33	16.333		
Total	5	1028.00			

Grand Mean 20.000 CV 20.21

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.44991	0.44991	78.55	0.0009
Error	4	0.02291	0.00573		
Total	5	0.47282			

Grand Mean 0.3892 CV 19.45

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	20.0568	20.0568	78.97	0.0009
Error	4	1.0159	0.2540		
Total	5	21.0727			

Grand Mean 2.5950 CV 19.42

APPENDIX O

Analysis of variance for yield of *Volvariella volvacea* on mixed non-composted substrates + Leucaena leaves + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	28.1667	28.1667	2.52	0.1874
Error	4	44.6667	11.1667		
Total	5	72.8333			

Grand Mean 18.167 CV 18.39

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	28.167	28.1667	0.88	0.4024
Error	4	128.667	32.1667		
Total	5	156.833			

Grand Mean 67.833 CV 8.36

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	400.167	400.167	10.22	0.0330
Error	4	156.667	39.167		
Total	5	556.833			

Grand Mean 42.833 CV 14.61

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.29349	0.29349	70.01	0.0011
Error	4	0.01677	0.00419		
Total	5	0.31026			

Grand Mean 0.4588 CV 14.11

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	13.0538	13.0538	69.91	0.0011
Error	4	0.7469	0.1867		
Total	5	13.8007			

Grand Mean 3.0583 CV 14.13

APPENDIX P

Analysis of variance for yield of *Volvariella volvacea* on mixed composted substrates + Leucaena leaves + lime

ANOVA for dry weight of substrates

Source	DF	SS	MS	F	P
substrate	1	0.00000	0.00000	M	M
Error	4	0.00000	0.00000		
Total	5	0.00000			

Grand Mean 15.000 CV 0.00

ANOVA for mean number of days for pinheads to appear

Source	DF	SS	MS	F	P
substrate	1	2.6667	2.6667	0.26	0.6355
Error	4	40.6667	10.1667		
Total	5	43.3333			

Grand Mean 18.333 CV 17.39

ANOVA for mean number of pinheads

Source	DF	SS	MS	F	P
substrate	1	4.1667	4.1667	0.28	0.6242
Error	4	59.3333	14.8333		
Total	5	63.5000			

Grand Mean 38.500 CV 10.00

ANOVA for mean number of fruitbodies harvested

Source	DF	SS	MS	F	P
substrate	1	10.667	10.6667	0.17	0.7000
Error	4	248.667	62.1667		
Total	5	259.333			

Grand Mean 15.667 CV 50.33

ANOVA for mean weight of fruitbodies

Source	DF	SS	MS	F	P
substrate	1	0.00184	0.00184	0.17	0.6985
Error	4	0.04239	0.01060		
Total	5	0.04423			

Grand Mean 0.1968 CV 52.30

ANOVA for Biological Conversion Efficiency

Source	DF	SS	MS	F	P
substrate	1	0.08402	0.08402	0.18	0.6956
Error	4	1.89847	0.47462		
Total	5	1.98248			

Grand Mean 1.3117 CV 52.52