COMPARATIVE BIOCONVERSION OF RICE LIGNOCELLULOSIC WASTE AND ITS AMENDMENTS BY TWO OYSTER MUSHROOMS (*PLEUROTUS* SPECIES) AND THE USE OF THE SPENT MUSHROOM COMPOST AS BIO-FERTILIZER FOR THE CULTIVATION OF TOMATO, PEPPER AND COWPEA

BY

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INTEGRI PROCEDAMUS

..

DATE

DECLARATION

I, the undersigned, WIAFE-KWAGYAN, MICHAEL, declare that am the author of this thesis, I do declare that this work is a result of my own research work carried out in the Department of Botany, University of Ghana Legon under the supervision of Prof. George Tawia Odamtten and Dr. Mary Obodai from January 2012 to July 2014. References made in this work have duly been acknowledged.

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DEDICATION

This thesis is dedicated first and foremost to the Lord Almighty God. I also dedicate it to my dear parents Mr. and Mrs Brenya Wiafe-Akenten as well as my mother Mrs. Beatrice Akua Korantemaa and my grandmother Madam Abena Serwaa. I dedicate this work to my late grandparents the late Mr. Kofi Adarkwa and late Mrs. Afia Owusua Adarkwa of blessed memory for their support and motivating me to pursue education in early life. Finally, to all my siblings Awo Asantewaa, Nana Adwoa, Ohemaa Akua, friends (especially Miss. Millicent Amponsah), dear ones who contributed in diverse ways to the success of this thesis deserve mention. I wish the surviving ones God's favour and blessings.

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ABSTRACT

Oyster mushrooms (*Pleurotus* species) have been an important food item in both developed and developing countries serving an invaluable resource for nutrition, health and disease prevention. Although several agricultural wastes lignocellulose have been used for cultivation of oyster mushrooms worldwide and in Ghana, the most utilized is the *wawa* (*Triplochiton scleroxylon*) sawdust and its amendments for the cultivation of *Pleurotus ostreatus* in Ghana. However, a longer period of 28 days is required for fermentation of substrates by resident microorganisms to make it suitable for bioconversion. In this thesis, seven differently formulated composts of rice waste (rice straw (RS), rice husk (RH), rice bran (RB) and its amendments; 1. Rice straw only; 2. Rice straw +1% $CaCO_3$ +10% rice bran; 3. Rice straw +1% $CaCO_3$ +10% rice bran+ 5, 10 0 or 15% of rice bran (RB); 4. Rice straw amended with Rice husk (1:1w/w) + 1% $CaCO_3$ +10% RB + rice bran+ 5, 10 or 15% of rice bran; 5. Engelberg mixture only; 6. Engelberg mixture + 1% $CaCO_3$ +10% rice bran) were tested for efficiency by *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31. The seventh composted medium was wawa sawdust for comparison purposes only. The compost formulation methods, composting, bagging, sterilization, spawning were done using the standard International Methods. The formulated substrates were composted for 0-12 days before utilization. The sterilized bags were incubated in the Mushroom House at the Food Research Institute (CSIR) Ghana. The assessment of resident mycoflora in the raw materials and composted substrates was done by using the Standard Decimal Serial Dilution Technique on two media (Cooke's and DRBC medium). At the end of the prescribed incubation period the following parameters were determined; surface mycelial density, total number of fruit bodies after 3 flushes, total number of pinheads formed, total yield and biological efficiency, record of stipe length, pileus width, average weight of fruit bodies of the mushroom and correlation between stipe length, pileus length and Biological Efficiency were calculated. To ascertain the influence of the formulated composts on the nutritional quality of the substrates and the sporophore formed, proximate analysis (nutrient quality and mineral elements concentration (Cu, Zn, Mg, Mn, Fe, Pb, Na, K, P and Ca) were determined using International Standard Methods in the Mushroom Industry. The use of spent mushroom compost from (*P. eous* strain P-31) as a bio-fertilizer was tested under

greenhouse conditions using tomato (*Lycopersicon esculentum*), pepper (*Capsicum annum*) and cowpea (*Vigna unguiculata*). The plants were grown in pots contained soil only (0%) and soil: compost mixtures to give 5-100% Spent Mushroom Compost proportions. Data was collected for 8 weeks at 30±2°C on plant height, leaf area, number of leaves, chlorophyll content, dry matter accumulation by shoot and root and in the case of cowpea, nodulation and nodule quality were assessed. The species of fungi resident in the seven differently formulated and composted substrates used for the cultivation of *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31 varied but belong to the genera *Aspergillus, Fusarium, Cladosporium, Curvularia, Rhizoctonia, Rhizopus*, *Rhodotorula, Trichoderma, Penicillium, Neosartorya, Byssochlamys, Geotrichum,* other *Yeasts* and *Mycelia sterilia.* In each compost substrate *Aspergillus* species predominated followed by *Penicillium*. The most frequently encountered species were *A. flavus, A. fumigatus, Rhodotorula sp*., *Penicillium citrinum* and *Trichoderma harzianum.* Culture metabolites of *A. flavus, P. citrinum* and *T. harzianum* variably depressed radial and vegetative growth of *P. ostreatus* and *P. eous* in concentrations (1:1- 1:10v/v dilutions). The effect was severer on *P. ostreatus* than *P. eous*. The best pH was 5.4-7.0. Surface mycelium density, spawn run period (weeks), number of fruit bodies and pinheads per flush, total yield and Biological Efficiency as well as the stipe length and pileus width of the two *Pleurotus* species on the different compost was influenced by the composition of the substrate. Generally, *P. eous* strain P-31 performed better than *P. ostreatus* strain EM-1 on the same substrates by all the criteria used to ascertain substrate performance. The correlation coefficient R^2 value of the stipe length and pileus width against Biological Efficiency showed a high correlation R^2=0.94 for all but one substrate. R^2 value for fruit bodies on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran was lower R^2<0.66 indicative of slower growth. In all instances growth on the same substrates by *P. eous* was superior to that of *P. ostreatus.* The best growth of *P. eous* was on uncomposted or short composted period (0-8 days) substrates. The Biological Efficiency for *P. eous* was 72.8 to 76.4% on rice straw and husk and yield of 211.0 and 221.5g while *P. ostreatus* performed better with a BE of 60.8 and 63.3% and yield of 175.8 and 183.5g. There was no advantage in the amendments of the rice straw substrates since the uncomposted rice substrate and the same composted for 4 days yielded higher weight and fruiting bodies and higher BE of 55.7 to 75.6% than what was obtained in the remaining

substrates (BE values 53.3 to 72.4%). Crude protein, ash, total carbohydrates, crude fibre contents of the fruit body were higher in *P. eous* than in *P. ostreatus* on the same substrates but were all within the reported values for *Pleurotus* species. Only fat content of both species (5.97- 17.8%) was higher than the reported limits of 1.6- 5.0%. The following minerals, Ca, Fe, P, Mg, Na, K were detected in higher quantities in *P. eous* than *P. ostreatus* but was influenced by the composition of the compost. The values were within the reported permissible limit by WHO (1982). Heavy metals Cu, Fe, Mg, Mn, Pb and Zn were found in the fruit bodies of both *Pleurotus* species in small quantities lower than their reported permissible limit by WHO (1982). The PCR performed with ITS 1 and ITS 4 primers to characterise the ITS region of mushroom samples grown in different substrates showed that the two *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31 produced the same characteristic band size of 650bp. However, the gel profile using restrictive enzymes *Hha I*, *Hinf I, Rsa I* and *Hae III* showed that *Hae III* restrictive endonuclease was effective in separating *P. ostreatus* from *P. eous* thus proving that the two *Pleurotus* were genetically different species. The Spent Mushroom Compost (SMC) from *P. eous* used a bio-fertilizer at 0- 100% SMC mixture with soil, variably supported better growth and development of tomato, pepper and cowpea under greenhouse conditions. Height of plant (cm), Leaf area (mm^2), chlorophyll content (CCI), total dry weight and the number of leaves formed were enhanced by low concentration (5-20% SMC). Concentration beyond 30% SMC variably depressed the selected developmental criteria used in assessing growth presumably because of the preponderance of high micro and macro-nutrient (Ca, Cu, Fe, K, Mg, Mn, Na, Pb, P and Zn). The advantage of the use of SMC for crop cultivation is discussed and the practical and economic value of using uncomposted and short composted period 0-8 day(s) substrate for cultivating *P. eous* as compared to *wawa* sawdust which requires 28 days composting before use is highlighted and future studies suggested.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Mushrooms are macrofungi with distinctive fruiting bodies, fleshy in nature, and sometimes tough umbrella-like sporophores which bear their basidia (spore-bearing structures) on the surface of gills or plates (lamellae). The distinctive fruiting bodies, can either occur above (epigeous) or below (hypogenous) the soil and are large enough to be seen with the unaided eye and can be picked with the hand. The gills of the mushroom cap produce numerous spores which are released into the soil and blown by wind to other substrates to commence the reproductive cycle. Fig. 1 summarises the life cycle of oyster mushroom.

Hawksworth (1991) and Kirk *et al*. (2001) estimated that there are over 1,500, 000 species of fungi on earth but approximately 45,000 species of mushrooms have been described. Out of these, 2,000 species are considered edible whereas 46 species are cultivated and accepted as food. A total of 10 species have become popular among countries where there is high consumption of mushrooms. Table 1 shows the estimated annual production of cultivated macrofungi in the world (Chang, 1999). Interestingly, 10% of these mushrooms are considered poisonous (about 4,500 species) and 30 species are very toxic and lethal in their effect on humans (Arailde, 2013).

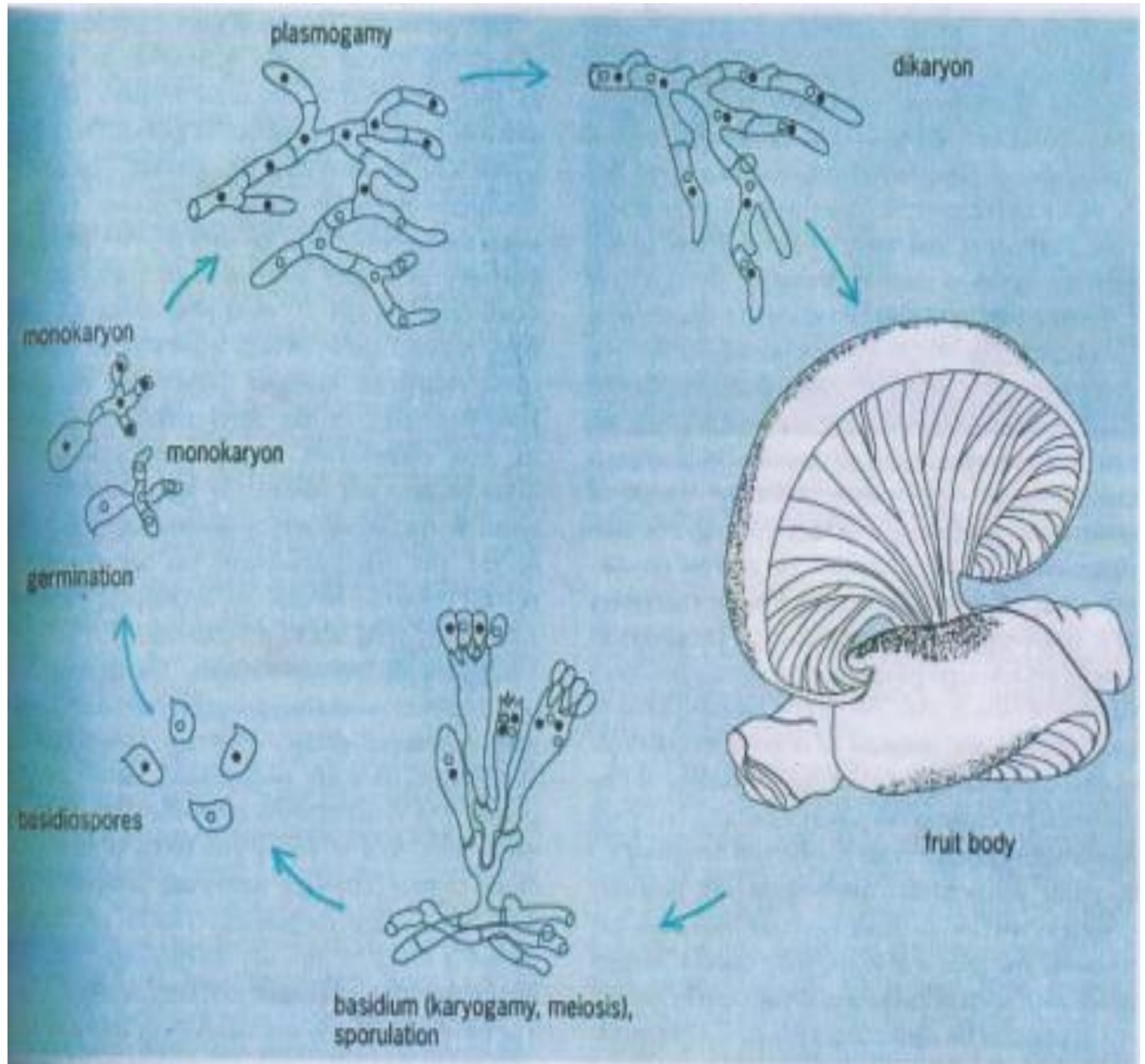


Fig 1: Life cycle of the oyster mushroom *Pleurotus* sp. (Martinez-Carrera, 1998)

Table 1: Annual production of cultivated macrofungi (Data after Chang, 1999)

Fungus	**Class**	**Quantity (thousands of tonnes)**
Agaricus bisporus	Agaricales	1,956
Lentinus edodes	Poriales	1,564
Pleurotus spp	Poriales	876
Auricularia spp	Auriculariales	485
Flammulina velutipes	Agaricales	285
Volvariella volvacea	Agaricales	181
Tramella spp	Tremallales	130
Hypsizygus marmoreus	Agaricales	74
Pholiota nameko	Agaricales	56
Grifola frondosa	Poriales	33
Others		521
Total		6,161

Majority of the mushrooms belong to the Phylum Basidiomycota: Order Agaricales. However, some like the truffles (*Tuber melanosporum*) and morels (*Morchella* spp.) belong to the Ascomycota (Alexopolous *et al*. 1996). Ecologically, mushrooms can be classified into three groups: the mycorrhizae, the saprophytes, and the parasites (Stamets, 1993; Ingale and Ramteke, 2010; Aroye, 2011).

a) *Mycorrhizae Gourmet Mushrooms*

Mycorrhizal mushrooms form a mutually dependent, beneficial relationship with the roots of host plants, ranging from trees to grasses (Stamets, 1993). The mycelium can form an exterior sheath covering of roots of plants and they are called ectomycorrhizae or they can invade the interior of root cell of host plants and these are called endomycorrhizae. In either case, both organisms benefit from the symbiotic relationship (association) and plant growth is accelerated as the mycelium of the resident mushroom mycelium increases the plant absorption of nutrients, nitrogenous compounds and essential elements such as phosphorus, copper, and zinc. Plants with Mycorrhizal fungus partner can also resist diseases far better. For example, truffle orchards are well established in Europe (France, Spain, Italy) with the perigold black truffle *Tuber melanosporum* in association with oak *(Quercus ilex*) yielding tons of edible truffles for several thousands of dollars (Stamets,1993). The same is true for *Tuber gibbosum* in association with Douglas fir (*Pseudotsuga menziesii*) seedlings. Another example of mycorrhizal association producing wild collected mushrooms: Chanterelles (*Cantharellus cibarius*) form mycorrhizal association with trees. Chanterelles are one of the most popular collected wild mushrooms in the Northwest of North America. There are about 20 or more species throughout South and Central Africa including *Cantharellus cibarius* Fr, *C. congolensis* Beeli, *C. densifolius* Heinem., *C. longisporus* Heinem, *C. miniatescens*

Heinem, *C. pseudocibarius* P. Henn etc (Ryvarden *et al*. 1994). They occur in large quantities in favourable seasons and are edible and highly-prized mushroom worldwide available throughout the rainy season dried and stored for consumption and selling commercially on Africa markets or at road side (Ryvarden *et al*. 1994). One thing that has hampered their commercial production is their unique interdependence on soil yeasts *Rhodotorula* glutins which is crucial in stimulating spore germination of *Cantharellus s*pp. (Stamets, 1993; Ryvarden *et al*. 1994).

b) Saprophytic mushrooms: (The decomposers)

Most of the gourmet edible mushrooms are saprophytic, wood decaying fungi. These saprophytic macrofungi are the premier nutrient recyclers on earth. Their filamentous mycelial network is designed to weave between and through the cell walls of plants. Their mode of nutrition brings about the secretion of a wide range of enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (phenol oxidase) which can breakdown complex substances such as lignin as well as polysaccharides found in cellulose and hemicellulose (Chang and Miles, 1992; Aroye, 2011) after which the nutrients are absorbed. Thus macrofungi play a fundamental role with other microfungi and bacteria as well as other bio-deteriroagent (such as nematode, earthworm etc.) to rid the environment of litter, logs, agro-lignocellulose waste etc. (Aroye, 2011). The end result of their activity is the return of carbon, hydrogen, nitrogen and mineral salts back into the ecosystem in a form usable by plants, insects and mankind.

As decomposers, they can be delimited into three groups: Primary decomposers, secondary decomposers and tertiary decomposers.

i) Primary decomposers: They are those which degrade a twig, a blade of grass, chip of wood, log or a stump or agro-lignocellulosic material. Primary decomposers are typically fast growing, which quickly attach to substrates and decompose plant tissue. Most of the decomposers degrade wood and agro-lignocellulosic materials.

The oyster mushrooms (*Pleurotus* species) together with Shiitake (*Lentinula edodes*) and King Stropharia (*Stropharia rugoso-anmulata*) are primary decomposers and they have developed specific set of enzymes to break down lignocellulose. Once the enzymes have broken down the ligno-cellulose bondage to its full potential, other saprophytes set in utilizing their own ‘‘battery’’ of enzymes to reduce the material even further (Stamets, 1993).

ii) Secondary decomposers: These are mushrooms which rely on previous activity of other primary decomposing fungi to partially break down a substrate to a state wherein they can thrive. Secondary decomposers grow from composted material. Because mushrooms are heterotrophic organisms, they have to get all the nutritive elements from the substrate (called compost). Therefore, the compost plays a more comprehensive role in mushroom production than does soil in higher plants.

Composting is a process of controlled microbial succession in the substrate. The composting material is stacked up in large piles; the microenvironment conditions differ at different depths of the piles. During composting, a mixture of rich organic materials is converted into a stable medium which is suitable for the growth of a particular mushroom but not for the competing microorganisms (Oei, 1991). As plant residue is degraded by these microorganisms, the mass, structure and composition of the compost is reduced. Phenology of microorganisms including bacteria, actinomycetes, fungi and protozoa is different at different stages; different groups of microorganisms may dominate (Hayes, 1977).

The initial microflora may be mesophilic and utilizes the soluble organic carbohydrates and nitrogen. This is followed by the increased growth of more heat tolerant organisms and the release of carbon dioxide, ammonia, and a considerable amount of heat. At a latter stage of composting the temperature is higher and thermophilic microorganisms become dominant. Thermophilic organisms present throughout the compost are mainly responsible for the second stage of fermentation. Thereafter, the compost becomes selective and following spawning (planting the spawn onto the compost) mushroom mycelia grow out rapidly and ramify throughout the compost in a short time. Recently, Obodai and Odamtten (2013) gave a comprehensive review of the composting process and its pristine value in the process of mushroom production in Africa. Once microorganisms, especially actinomycetes, have completed their life cycle, the compost is susceptible to invasion by a select secondary decomposers.

iii) Tertiary decomposers: These are an amorphous group; the fungi represented by this group are typically soil dwellers. They survive in habitats that are years in

the making from the activity of primary and secondary decomposers such as fungi existing in these reduced substrates are remarkable in that their habitat appears inhabitable for most other mushrooms.

Sandhu and Sidhu (1980) identified certain fungi associated with the composting process. These contaminants can be divided into two well-defined groups: those attacking the mushrooms are called pathogens while those competing for the substrate are called indicators or competitors. Mushroom pathogens are either fungi, bacteria, viruses, or pests; indicators are always fungi. Sandhu and Sidhu (1980) identified *Aspergillus fumigatus* (Fres), *A. terreus* Thom, *Mucor pusillus* Lindt, *Penicillium* spp, *Rhizopus microsporus* (Saito) Schipper and Stalpers, *Trichoderma* spp and an agaric associated with the composting process. *A. fumigatus* and *A. terreus* predominated over others. Some microorganisms were found to be harmful to *Pleurotus* cultivation. *Trichoderma* spp cause problems (Cailleux and Diop, 1978). *Monilia* sp *Fusarium* and *Penicillium* grow more slowly than *P. ostreatus* (Saalback, 1978) and *Sclerotinum rolfsii* inhibited growth of *P. flabellatus* (Rajarathnam *et al*. 1997). Clearly, these species acted as antagonistic competitors of *Pleurotus*. Jandaik *et al*. (1998) showed that *Penicillium cyclopium* infection of *Pleurotus sajor-caju* fruit bodies soon after emergence from the substrate decreased yield by 50-75%. *Coprinus cinereus* (Schaeff), *A. fumigatus* and *A. niger* inhibited the growth *Volvariella volvacea*. The *Aspergilli* were not affected by *V. volvacea* but mutual inhibition seemed to exist between *V. volvacea* and *C. cinereus* (Chang-Ho, 1982).

Obodai (1992) showed that the phenology of fungal species in wawa (*Triplochiton scleroxylon*) sawdust during composting varied with the period of composting and the species of *Pleurotus* which utilized the substrate. Generally, thirteen fungal species belonging to eight genera (*Aspergillus, Cladosporium, Mucor, Neurospora*, *Paecilomyces, Penicillium, Rhizopus* and *Trichoderma*) were encountered. The fungal profile in rice straw differed. *Mucor pusillus* predominated followed by *Paecilomyces variotii* and *Penicillium oxalicum*. In addition, *Aspergillus flavus*, *A. terreus,* and *Cladosporium herbarum* were initially present in the rice straw substrate at varying frequencies but *A. terreus* and *C. herbarum* could not be detected within 3 months (Obodai, 1992).

Primary and secondary decomposers afford the opportunities for cultivation of mushrooms but the substrate must be selected carefully for mushroom increased productivity. The climate, available materials and the mushroom strain must all interplay for cultivation to result in success (Stamets, 1993).

Mushrooms as food extend back to Paleolithic times and crosses all cultural boundaries. Highly prized by the Greeks, mushroom consumption in European nations has deep traditional roots. The Agaric, a pre-Scythian people from Samartia (now Poland and Western Russia) held mushrooms in high esteem and used them medicinally (Stamets and Chilton, 1983). The early Greeks held a similar fascination for fungi and apparently worked them into their religious rituals, to the extent that to discuss the use of these sacraments violated strong taboos (Stamets and Chilton, 1983). Japan and China have used mushrooms for thousands of years and to date have prized highly a variety of mushroom species

including, *Pleurotus* (oyster mushrooms) *Volvariella volvacea* (oil palm mushroom), *Lentinula edodes* (shiitake), *Flammulina velutipes* (Eroke, Winter mushroom), *Agaricus brunnescens* (button mushroom) to mention but a few (Stamets, 1993).

In the New World, the Aztec and Mazatec Indians of Mexico used mushrooms for both their healing and divining properties (Stamets and Chilton, 1983). In Africa, rural people are less consecutive than most Europeans in the range of mushroom species they regularly consume, all of which are perfectly adaptable for use in international cuisine and for alternative forms of storage, such as freezing (Ryvarden *et al.,* 1994). Greater varieties of species than those that are traditionally harvested (like *Termitomyces clypeatus, T. letestui, T. microcarpus, T. schimperi, T. titanicus*) are eaten in Southern and Central Africa such as species of *Amanita*, *Cantharellus*, *Lacturius, Russula* (Ryvarden *et al.,* 1994). The edible mushrooms collected in the wild in Ghana have been documented by Obodai and Apetorgbor, 2001; 2008 and Motey (2006). Twenty four (24) different species of mushrooms have been documented from the Western Region of Ghana; 18 are edible, 6 are medicinal. The edible mushrooms include *Termitomyces letestui, Volvariella volvacea, Corpinus disseminates, Catharellus sp., Mycena flavescence, Schizophylum commune, Auricularia sp., Agaricus sp.* all to the order Agaricales, Cantharellales, Tricholomatales, Schizophyllales and Auriculariales. The medicinal mushrooms are *Schizophyllum commune*, *Pleurotus tuber-regium, Auricularia auriculata, Ganoderma lucidum, Clavatia sp.* and *Daldinia concentrica* (Obodai and Apertogbor, 2001, 2008, 2009).

The overall importance of mushrooms in the national and local economics cannot be achieved without resorting to cultivation of those species which can be domesticated. It took

two centuries of experience in Western World to develop the techniques by which good crops of *Agaricus bisporus* can be grown, harvested and marketed and this concomitantly raised their nutritional status. In its present form *A. bisporus* is virtually a man-made species (Ryvarden *et al.,* 1994) or cultivar quite distinct from the original wild relatives. In the Far East, village-based industries have been modeled on ancient practices of mushroom cultivation. Species like *Volvariella volvacea* and *Lentinula edodes* (shiitake) have been artificially cultivated on commercial basis with great success. The substrates used for their cultivation varied and were arrived at after centuries of open cultivation on a wide range of agricultural waste materials. Table 2 shows some agricultural and industrial waste used in the cultivation of edible mushrooms.

Table 2: Some agricultural and industrial waste used in the cultivation of edible mushrooms

Agricultural and Industrial waste	Residues	Competing use
Grain harvesting Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks	Animal feed, burnt as fuel, compost, soil conditioner
Processed grains Corn, wheat, rice, soybean	Waste water, bran,	Animal feed
Fruit and vegetable harvesting	Seeds, peels, husks, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Sugar cane other sugar products	Bagasse	Burnt as fuel
Oils and oilseed plants Nuts, cotton seeds, olives, soybean etc.	Shells, husks, lint, fibre, sludge, press cake, wastewater	Animal feed, fertilizer, burnt fuel
Animal waste	Manure, other waste	Soil conditioners
Forestry-paper and pulp Harvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw-and plywood waste	Woodchips, wood shavings, saw Dust	Pulp and paper industries, chip and fibre board
Pulp & paper mills	Fibre waste, sulphite liquor	Reused in pulp and board industry as fuel
Lignocellulose waste from communities	Old newspapers, paper, cardboard, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Unutilised grass	Burnt

Data after Howard *et al*., (2003)

Oyster mushroom cultivation in Ghana

Rice straw is traditionally used for commercial production of the ''paddy straw mushroom'' *Volvariella volvacea* in South-East Asia. Cultivation of mushrooms on woody substrates, mainly logs and sawdust have refined the techniques of commercial production of *Lentinula edodes* (shiitake), several species of *Pleurotus*, notably *P. ostreatus* in the Far East (Chang and Quimio, , 1982; Oei, 1991; Zadrazil, 1996 etc.) and in Ghana (Obodai, 1992; Obodai *et al*. 2001, 2003, 2010). *P. ostreatus* has been produced commercially in Zimbabwe (Ryvarden and Masuka, 1994). Many technical journals and books are available to guide both small-scale and industrial entrepreneurs (Chang and Hayes, 1978; Chang and Quimio 1982; Oei, 1991; Stamets, 1993) in mushroom cultivation.

Since the commencement of the small-scale commercial cultivation of *Pleurotus* species (*P. ostreatus*, *P. sajor-caju* etc) in Ghana over 22 years ago, many composted agricultural lignocellulose have been tried. Oyster mushrooms are by far the easiest and least expensive to grow. Few other mushrooms demonstrate such adaptability, aggressiveness, and productivity as these species of *Pleurotus*. Wood decomposers such as species of *Pleurotus* grow on a wide range of forest and agricultural wastes than species from any other group. They thrive on almost all hard wood by-products (sawdust, paper, and pulp sludge), all cereal straws and corn cobs, on sugar cane bagasse, coffee residues (coffee grounds, hulls, stalks and leaves), banana fronds, cotton seed hulls, agave waste, soy pulp and on other materials too numerous to list. With the improved cultivation techniques and use of other *Pleurotus* species e.g. *P. eous*. Oyster mushrooms can best serve to reduce poverty and

hunger in Ghana and revitalize rural economies. One extraordinary thing about oyster mushrooms is their conversion of substrate mass into mushroom. Biological Efficiencies exceed 100%; some of the highest, if not the highest, in the world of cultivated mushrooms.

Biological Efficiency is defined as:

$$B.E = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100\ (\%)$$

In the course of decomposing dry straw, nearly 50% of the mass is liberated as gaseous carbon dioxide; 20% is lost as residual water, 20% remain as ‘‘spent compost’’ and 10% is converted into dry mushroom. This yield can be also expressed as a 25% conversion of the net mass of substrate into fresh mushrooms. One caution is that this formula is affected by the stage at which mushrooms are harvested (Stamets, 1993).

As aforementioned, many agricultural waste materials have been tried for the cultivation of *Pleurotus* spp in Ghana with varying success. Recently, Obubuafo (2009) assessed the effect of fermentation of *Chromolaena odorata* on the cultivation of oyster mushrooms (*Pleurotus ostreatus* and *P. eous*) in Ghana and concluded that even though results indicated significant differences among the average treatments for the parameters measured, the use of *Chromolaena odorata* as substrate for mushroom production on a larger scale may not be economically viable (Obubuafo, 2009).

Taxonomy of *Pleurotus*

The Genus *Pleurotus* has been hard to place from the taxonomic point of view. *Lentinus* and *Pleurotus* were placed under the Agaricales (Singer, 1975) in their own Family Pleurotaceae, or even in the Tricholomataceae, or by splitting the Family Polyperaceae in such a way that it can be considered under both Agaricales and Aphyllophorales. Later, Singer, (1976) put the genus into Polyporaceae family along with *Lentinus* and Pegler (1986) included them under separate family Lentinacaeae of the Aphyllophorales. Tricholomataceae is a very large family composed primarily of white-spored species. Examples include *Termitomyces, Flammulina, Marasmius, Clitocybe, Tricholoma, Panellus, Laccaria, Omphalotus* and *Armillaria*. Singer (1986) considered *Lentinus* and *Pleurotus* in this family. Hibbett and Vilgalys (1993) also placed *Lentinus* and *Pleurotus* in the Tricholomataceae.

Recently, a group of mushroom scientists (OECD, 2005) have followed the preposition made by Singer (1986) which divides the genus *Pleurotus* into six sections: Sect. *Lepiotarii* (Fr.) Pilat, Sect. *Calyptrati* Sing., Sect. *Pleurotus* Sing., *Tuber-regium* Sing: *Pleurotus ostreatus* was placed in the section *Pleurotus* based on the absence of veil and with monomitic hyphal system.

The current taxonomic classification of *Pleurotus ostreatus* is as below:

KINGDOM: Fungi; DIVISION: Basidiomycota; CLASS: Hymenomycetes; ORDER: Agaricales

FAMILY: Polyporaceae; GENUS: *Pleurotus*; SCIENTIFIC NAME: *Pleurotus ostreatus* (OECD, 2005). *P. eous* falls under the same category as *P. ostreatus*.

Species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects (OECD, 2005). Mating compatibility studies have demonstrated the existence of discrete intersterility groups in *Pleurotus* to distinguish one species from the other. For example, the following are distinct species of *Pleurotus* currently cultivated on semi-commercial and commercial basis for human consumption. *Pleurotus citrinopiletus*, Singer (Golden Oyster Mushroom); *P. cystidiosus* O.K. Miller (The Abalone Mushroom or Maple Oyster); *P. djamor* (Fries) Boedjin sensu latu (The Pink Oyster Mushroom); *P. eryngii* (De Candole ex Fr.) Quelet sensu latu (The King Oyster, or Boletus of the Steppe); *P. pulmonarius* (Fries) Quelet (The Indian Oyster often mistakenly called *P. sajor-caju* and misapplied by cultivators and rather similar to *P. ostreatus*). *P. euosmus* (Berkeley apud Hussey) Saccardo (The Tarragon Oyster Mushroom) distinguished from *P. ostreatus* by its odour (tarragon) and spore size of 12-14μ substantially larger than the 7.5-11.0μ spore-size of the *P. ostreatus* (Stamets, 1993).

P. eous (Berkeley) Saccardo is a discretely separate species from *P. euosmus* and is more closely allied to the pink *P. djamor* varieties than to the grey brown *P. ostreatus* but its fruiting body is white although initially introduced to Ghana, its cultivation has not caught up with that of *P. ostreatus*. *P. eous* is included in this study to assess its growth condition, vigour of productivity and nutritional status on rice straw and husk. The interfertility or DNA studies can also help authenticate the growth performance parameters and nutritional quantities of *P. ostreatus* and *P. eous*.

Molecular characterisation and relationship in the genus *Pleurotus*

There is some information on the molecular characterisation of *Pleurotus* species. Larraya *et al*. (1999) identified incompatibility alleles and characterised molecular markers genetically linked to the A incompatibility locus of *P. ostreatus*. Ruiz-Duėnas *et al*. (1999) also carried out molecular characterisation of a novel peroxidase isolated from *P. eryngii*. Subsequent work by Gonzalez and Labarère (2000) studied the phylogenetic relationship of *Pleurotus* species according to the sequence and secondary structure of the mitochondrial small sub-unit rRNA, V4, V6 and V9 domains. Recently, Moussa (2009) worked on the molecular characterisation of the phenol-oxidase (pox 2) gene from *P. ostreatus*. The morphomolecular characters of *P. ostreatus* in relation to luminosity and temperature of fructification was investigated by Marino *et al*. (2003) and they showed that molecular characterisation of Pos 98/37 strain was 30% similar to the remaining seven strains. Menoli *et al*. (2010) examined morphological and molecular identification of four Brazilian commercial isolates of *Pleurotus* spp and cultivation on corn cob and found that the identification was based on the morphology of the basidiomata obtained and on sequencing of the LSU rDNA gene. In the cultivation experiment, the isolates of two *P. ostreatus* and one *P. djamour* reacted differently to the substrates. One isolate showed high growth on the substrate containing charcoal.

Recently, Ingale and Ramteke (2010) showed that *P. eous* cultivated on different agro-wastes (soybean straw, paddy straw, wheat straw) and their combination in 1:1 proportion gave a high protein content of 46%. The highest mineral content was found in *P. eous* as compared to *P. sajor-caju* and *P. florida* and also gave a high Biological Efficiency of 76.76 as compared to 60.72% and 56.64% for *P. sajor-caju* and *P. florida* respectively.

P. ostreatus has been cultivated and studied extensively, but there is hardly any information in the pertinent literature on growth performance of *P. eous* on lignocellulose from agricultural waste in Ghana. Furthermore the growth performance and the comparative biological efficiency (yield) of *P. ostreatus* and *P. eous* on rice waste (husk and straw) have not been studied in Ghana. It would also be informative to use molecular characterisation to elucidate any differences in performance of the two under the same substrate and environmental conditions.

Rice waste as a substrate for mushroom cultivation in Ghana

Materials for composting a mushroom substrate are diverse and plentiful. Most by-products from agriculture and forestry industry can make up a base medium for mushroom culture. This base medium often called ‘‘fruiting substrate’’ is often supplemented with a carbohydrate and a protein rich additive to enhance yield and with lime / calcium carbonate ($CaCO_3$) or inorganic compound to amend the pH to suitable level for optimal growth of the mushroom. Potential substrates include wood wastes, paper products, cereal straws, grain hulls, corn cobs, coffee plants and wastes, tea leaves, sugarcane bagasse, banana fronds, seed hulls (cotton seed and oil-rich seeds), hulls of almonds, walnuts, sunflower, pecans and peanuts, soybean meal, soybean waste, rice straw, oil palm pericarp waste, chicken manure, molasses, artichoke waste, cactus waste, yucca, agave. The list is endless and is spell out by Stamets (1993), Oei (1991), Chang and Quimio (1982) and Ingale and Ramteke (2010).

The compost plays a more comprehensive role in mushroom production than soil does in higher plant growth. A good substrate should have; i) a suitable physical condition(s) which will provide good anchorage for the mushrooms and will at the same time maintain good

aeration and water holding capacity. ii) a good chemical condition which will release some nutrients from the raw materials of the compost during the fermentation and pasteurization and iii) a proper condition for microbial activities which will help improve both the physical and chemical conditions for mushroom growth. There is a great demand for the available world supply of mushroom protein especially in Africa.

Obodai (1992) used rice straw, maize stover, wawa (*Triplochiton scleroxylon*) sawdust, oil palm pericarp, cocoa shells and banana (*Musa* spp.) as substrate for cultivating *Pleurotus ostreatus*, *P. sajor-caju* (currently called *P. pulmonarius*) and *Volvariella volvacea* with some success. Since then some other substrates have been used for the cultivation of *P. ostreatus* and many papers have been published from Ghana in this regard e.g. Obodai *et al* (2010; 2011; 2014); Obodai and Odamtten (2013). The use of wawa sawdust although worthwhile has become a non-sustainable economic venture for the mushroom growers in Ghana. Rice wastes products stand out as potential candidate for use in the cultivation of mushrooms in Ghana. Rice (*Oryza sativa* L.) is now the world's most economically important food crop which provides two-third of calories intake of more than three billion people in Asia, provides 80% of the dietary calories in Bangladesh, Indonesia and one-third of nearly 1.5 billion people in Africa and Latin America (Khush, 2005). Rice (*Oryza sativa* L.) provides 20% of per capita energy and 13% of protein consumed worldwide (Juliano, 1994). Rice is therefore considered to be one of the world's most important cereal crop which serves as a primary source of food and calories for about half of the world (Khush, 2005).

Currently, Asia is the top rice producer; China being the world largest followed by India, Thailand, Bangladesh, Indonesia, Japan, Brazil, Vietnam, Myanmar (Burma) and Philippines (FAOSTAT, 2012). In terms of production in Sub-Saharan Africa rice is the fourth most important cereal after sorghum, maize and millet and it occupies 10% of the total land under cereal production and account for 15% of the total cereal production (FAOSTAT, 2006). Ghana, like most rice-growing countries has adopted three cultivation methods which rice farmers employ to grow their rice. These are valley-bottom rice, rain-fed upland rice and controlled flooding rice production (Norman and Otoo, 2003). Valley-bottom rice production is where farmers usually plant rice in small quantities in valley-bottoms. Much of this rice is consumed at festivals or sold between household and so never reach the market. The rice produced this way is virtually ignored in all agricultural statistics but the husk and straw are discarded or burned. Rain-fed upland rice is grown mainly in mountainous areas of the Volta Region, between Volta Lake and the Togo Border. The rice area stretches between Ho and Nkwanta, not excepting the recent Avehime and Afife rice farms. In recent times, however, the introduction of new systems of rice cultivation has changed this pattern. Valley-bottom production has been adopted, and rice is now cultivated widely in low land sites without irrigation. Controlled flooding system is the third rice cultivation method where the farmers depend on pumps, dams, elaborate water- channelling and mechanical harvesting as well as focusing on high-input rice varieties (Norman and Otoo, 2003). Map 1 shows the rice cultivating areas in Ghana (shown as green) and Table 3 shows rice production Figures for Ghana for the past eighteen years.

There is therefore a huge potential for the use of rice lignocellulose (the straw, husk and bran on a sustainable basis as compost (substrate) for mushroom production in Ghana. For

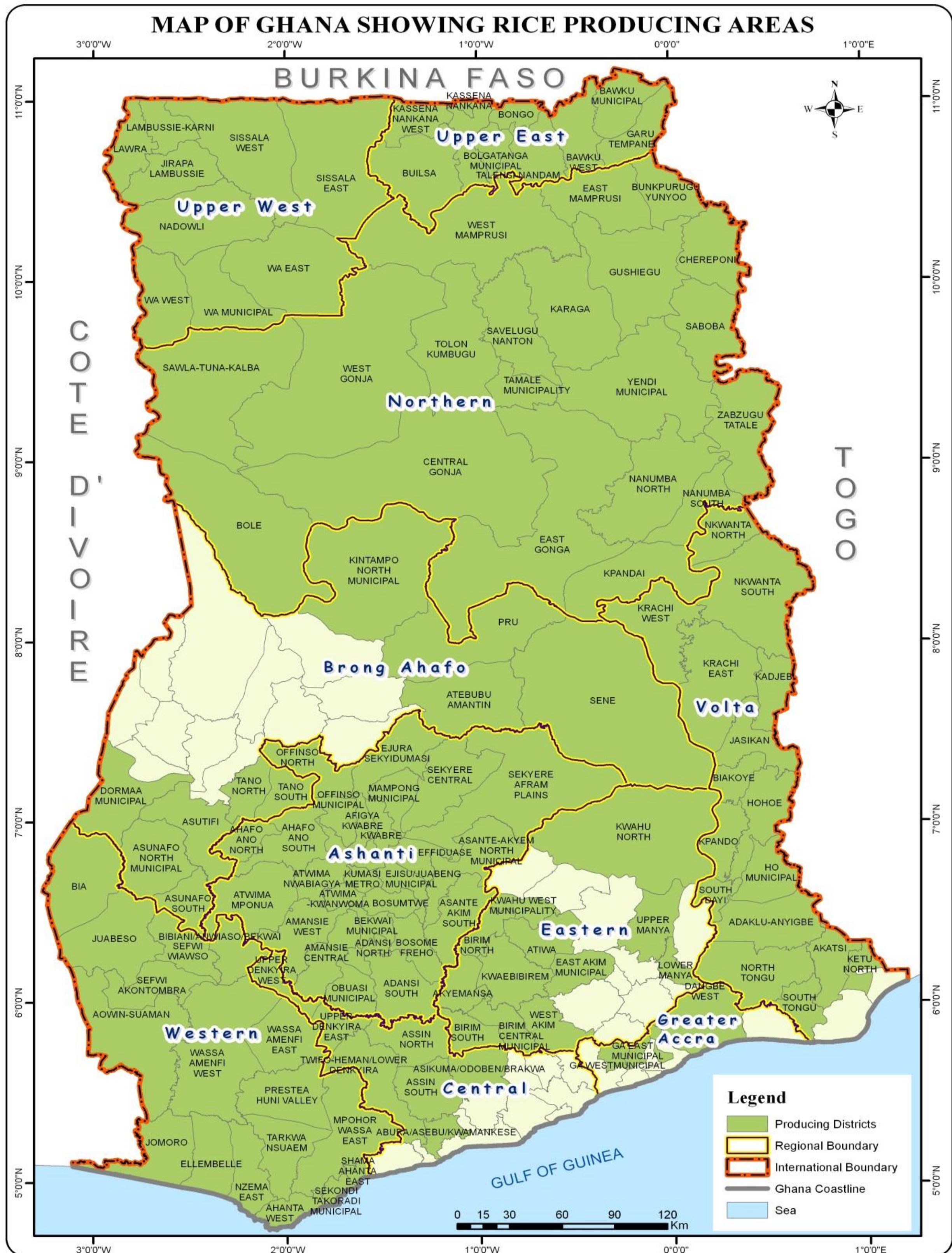
example, in 2008, the world paddy rice production was 661 million tons; 132 million tons of rice husk alone were produced (Paranthaman *et al*. 2009) and this quantity increases every year. It is estimated that more than 4.2 million tonnes of agricultural residues are generated yearly in Ghana. In 2008 alone, out of the total 5.2 million tonnes of agro-waste, rice waste

constituted approximately 360,000 tonnes (Ebo and Jaromirá 2013). The rice grain is covered with a woody structure which is called the husk or hull, which is indigestible and is removed in the first step during processing in order to make rice edible. Fig 2 shows the structure of the rice straw, husk and the kernel which is the main grain for human consumption. After milling rice, the husk and bran layers are removed while the white kernel is freed of impurities. However, appropriate methods of waste management of rice straw and husk is a major challenge as they are considered to be a poor feed for animals due to high silica content (Krishna *et al*. 2004).

Managing methods of rice straw and husk waste

Three major management methods are used to dispose off these wastes namely soil incorporation, commercial utilization and open burning. Open burning is the commonest method used in Ghana to eliminate this residue because it is the quickest and less expensive management. Open burning is not environmentally friendly as it is one of the major causes of air pollution and emission of greenhouse gases (GMGs) such as CO_2 and N_2O (nitrous oxide), ammonia (NH_3), nitrogen oxide (NOx), particles (smoke carbon) and particulate matter leading to global warming and health concerns. These GMGs reduce the number and activity of soil microbes (Kirkby, 1999) thereby affecting soil fertility as well as its subsequent adverse effects on crops and other farm produce. The magnitude of carbon and

nutrient loss during burning is influenced by the quantity of residue burned and the intensity of the fire. Complete burning of rice husk, straw and bran at 470ºC in muffle furnace resulted in 80-100, 20-25, 20-21 and 4-80% losses of Nitrogen (N), Phosphorus (P), Potassium (K) and Sulphur (S) respectively (Sharma and Mishra, 2001; Krishna *et al*. 2004). The alternative of residue incorporation in soil in turn causes methane emission from rice fields, contributing negatively to climate change as well. Therefore the use of rice lignocellulose for mushroom cultivation particularly *P. ostreatus* and *P. eous* in Ghana is a viable alternative as mushrooms can be grown under different climatic conditions on cheap, readily available waste material. This represents a possible solution to environmental pollution management and at the same time providing supplementary plant protein for the rural poor and also serve as poverty alleviation strategy for rural dwellers.

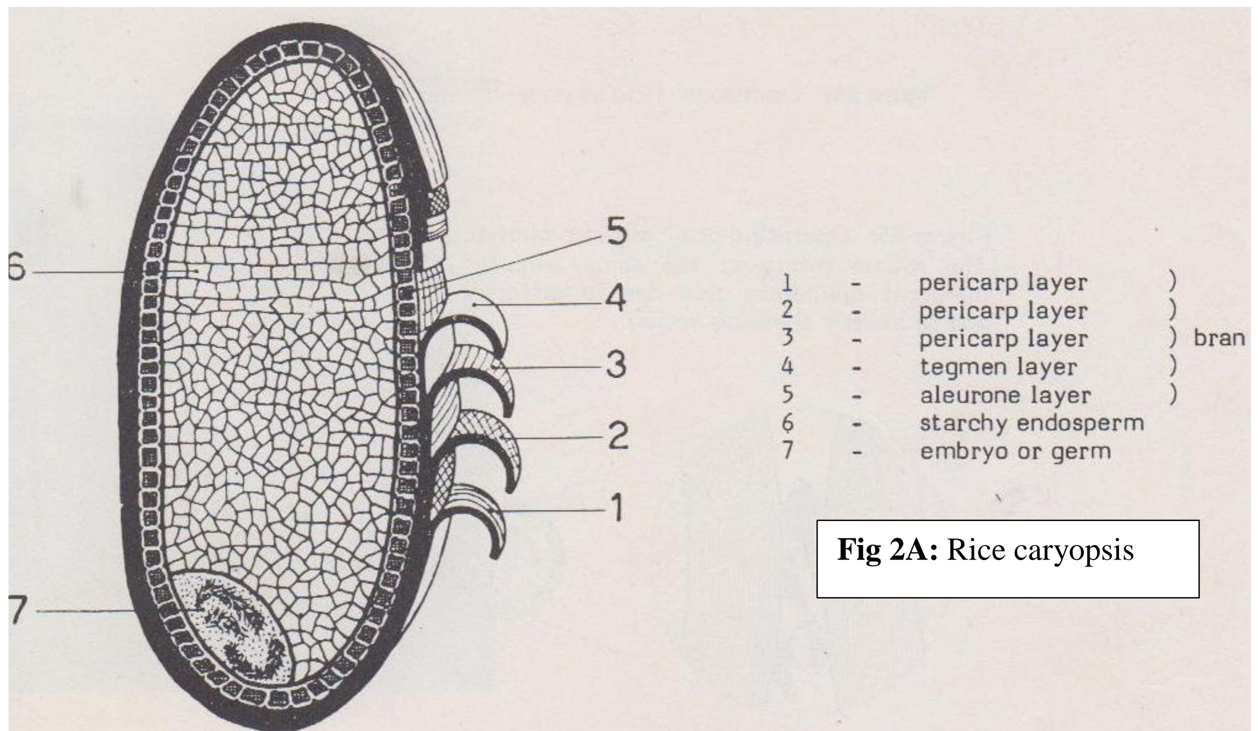


Map 1: The rice cultivating Districts in Ghana

Table 3: Rice production figure for Ghana for the past eighteen years (1995-2013)

Year	Total Domestic Production (metric tonnes)		Production available for human consumption	Estimated available quantity of waste per annum (metric tonnes)
	Paddy Rice	**Milled Rice**		
1995	-	202,000	141,400	60,600
1996	-	203,000	142,100	60,900
1997	-	197,000	137,900	59,100
1998	-	194,000	135,800	58,200
1999	-	168,000	117,600	50,400
2000	-	247,000	160,550	86,450
2001	-	273,000	237,783	35,217
2002	-	168,000	137,340	30,660
2003	243,000	146,000	117,000	29,000
2004	-	145,090	116,070	29,020
2005	-	142,000	113,600	28,400
2006	-	150,000	120,000	30,000
2007	185,340	148,272	118,000	30,272
2008	-	181,000	157,600	23,400
2009 / 2010	391,000	234,600	204,000	30,600
2010 / 2011	-	294,962	256,617	38,345
2011 / 2012	-	278,962	242,195	36,767
2012 / 2013	481,000	311,897	288,750	23,147
2013	569,524	364,000	305,905	58,095

Data after Statistics, Research and Information Department; Ministry of Food and Agriculture (SRID, MOFA, 2014)



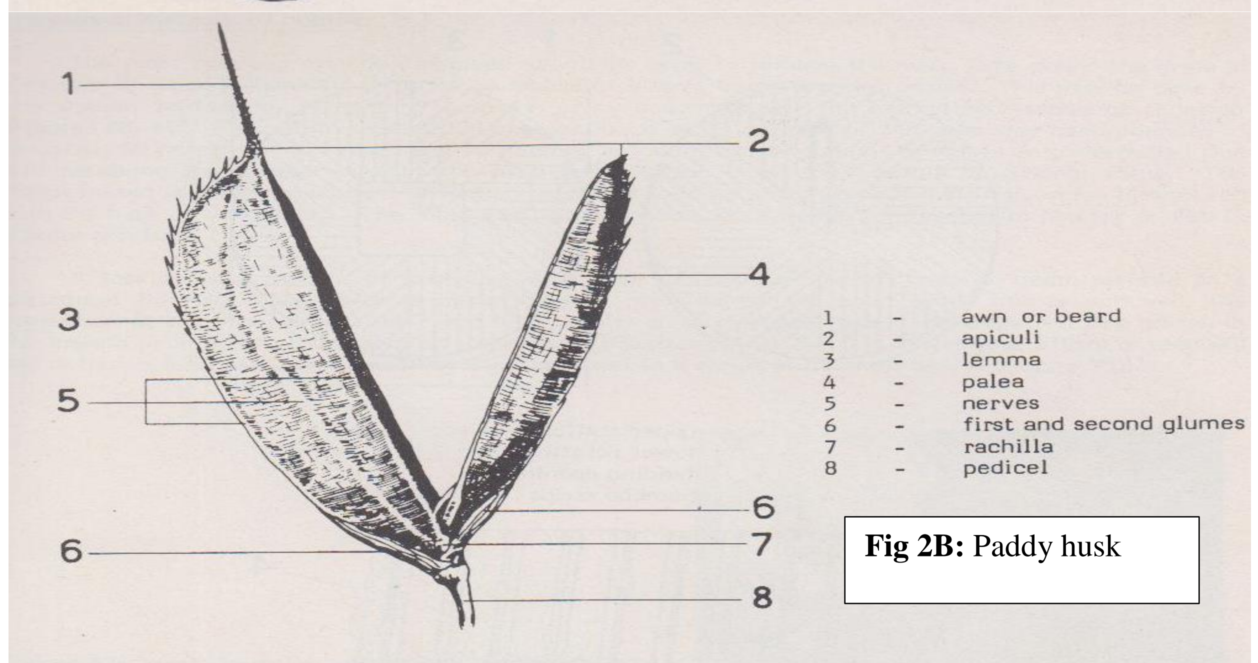


Fig. 2: The structure of the rice / paddy husk and the kernel which is the main grain for human consumption. (Diagram after FAO, 1983)

Composting of substrate for mushroom cultivation

Generally, elemental structure of rice waste include cellulose (30%), hemicellulose (20%), lignin (20%), water (10%) and silica (7.4%) (Krishna *et al*. 2004). The main components of rice straw are hemicellulose (26-30%), cellulose (38-41%), lignin (15%) and water soluble polysaccharides (8%), mineral ash (15%) containing high silicate and other metal oxides (IRRI, 2009). Due to the nature of these biopolymers a complex microbial community is necessary for the degradation of these components in order to enhance utilization by mushroom to produce high and mineral salts for improved human nutrition.

Physical, chemical and microbial phenology associated with composting

The physical composition and physical characteristics of various substrates used in the cultivation of *Pleurotus* spp vary during the composting process. For example, nitrogen content was low; rice straw contained 0.9g / 100g sample while that in wawa sawdust decreased from 0.2±0.1g / 100g to 0.16±0.09g/ 100g after 28 days composting. Cellulose and hemicellulose composition decreased as composting progressed in wawa sawdust so did crude fibre, organic matter and crude protein (Obodai, 1992; Obodai *et al*. 2010). Lignin content on the other hand increased with days of composting (Obodai, 1992; Obodai *et al*. 2010). The yield of seven strains of oyster mushrooms (*Pleurotus* spp) grown on composted sawdust (*Triplochiton scleroxylon*) have been tested with reasonable success.

Recently, Obodai and Odamtten (2013) reviewed the use of the process of composting in environmental bioremediation, bio-fertilizer application, nutrient recycling and preparation of substrate for mushroom cultivation as a means of boosting mushroom protein supplement in rural and urban nutrition. They recommend it as an environmentally friendly technology for the future in Africa. Sandhu and Sidhu (1980) identified certain fungi associated with the spawning and composting process. They are called contaminants although some are yield enhancing organisms such as several thermophilic fungi and bacteria e.g. *Humicola, Torula, Actinomyces*, *Streptomyces*, *Pseudomonas* and *Bacillus* species. Generally the underlisted genera were encountered in the compost; *Alternaria, Aspergillus, Bacillus* (Bacteria), *Botrytis*, *Chaetomium, Chrysosporium, Cladosporium*, *Coprinus, Dactylium, Epicoccum, Fusarium, Geotrichum, Monilia, Mucor, Mycelia sterilia* (sterile mycelium), *Mycogyne*, *Neurospora, Papulospora, Penicillium, Rhizopus, Scopulariopsis, Sepedium, Trichoderma, Trichothecium, Verticillium* and *Yeasts* (Stamets, 1993).

Different contaminants are associated with different stages of the compost preparation and mushroom cultivation. Grain cultures for the spawn preparation are contaminated by air-borne spores from a source where the grains were harvested and stored (Ivanovich-Biserka, 1972). In the compost culture, the major contributors for contamination are the materials used, the spawn and the workers or the aeromycoflora of the facility. In general, mushroom pathogens are not as numerous as the competitor fungi; though pathogens can be more detrimental to yield in mushroom cultivation.

During composting, the fungal succession in the compost is influenced by such factors as chemical reaction, aeration, temperature and nutritional factors (Chang-Ho, 1982). Stamets, (1993) reported that the number of microorganisms decreased during fermentation process

in wheat straw composted for 30 days. In rice straw, mesophilic members of the Phylum (Division) Zygomycota and Deuteromycota predominated. *Aspergillus* and *Mucor* multiplied quickly but soon disappeared from the compost (Chang-Ho, 1982).

Obodai (1992) showed that *Mucor pusillus* predominated in rice straw used for the cultivation of *Pleurotus* spp followed by *Paecilomyces varioti* and *P. oxalicum. A. flavus, A. terreus* and *Cldosporium herbarium* were initially present but could not be detected within 3 months. The environmental and nutritional conditions created during composting selectively favour certain fungi to the detriment of others and such competition may partly explain why the fungal profile before and after fruiting differ from one substrate to another.

Supplementation of basal substrate during composting

Supplements added to substrate may or may not benefit mushrooms. If it does benefit the mushroom it acts by enriching the growth of the microorganism for satisfactory composting. Rice bran, chicken manure and other agricultural by-products are commonly mixed into the compost base to increase nitrogen level and to improve texture and quality (Chang and Miles, 1987). The best cumulative yield of three *Pleurotus* spp (*P. ostreatus, P. sajor-caju* (Honkong) and *P. sajor-caju* (Mauritius) was obtained in 21days composted wawa sawdust supplemented with 5% *Leuceana leucocephala* (legume) leaves. Similar results were obtained when the same compost was supplemented with 15% cocoa nib dust. Varying proportions of supplements of substrate may be required to attain high cumulative and optimal biological efficiency (which is a measure of fruiting production) on the compost. This supplementation method has not been tried for rice husk and straw which are candidate compost to be used for the cultivation of *P. ostreatus* and *P. eous* in this present thesis. It is

conjectured that probably the supplements added may influence the growth and phenology of microorganisms which may result in beneficial or detrimental chemical and physical environment for growth and production of fruit bodies by the mushroom.

The Physical and Environmental Parameters for Cultivation

Geographical and climatic differences between tropical areas do not always allow the transfer of knowledge into new region. It is therefore necessary to investigate, using local environmental conditions in order to arrive at data which will provide defined techniques for particular species which allow cultivation without extra cost.

When *Pleurotus* spp were cultivated in a substrate of initial pH 5.0 to 6.5, the pH dropped to 4.4 and 5.6 and a maximum quantity of fruiting bodies harvested (Hong, 1978). Initial pH of 4.5 to 6.5 was optimal for *P. flabellatus* (Srivastava and Bano, 1970). *P. ostreatus*, *P. sapidus,* and *P. florida* NRRL 3526 growing on wash-pasteurised wheat straw of initial pH 6.1 decreased to 5.1 in 5 weeks and to pH 4.8 after 10 weeks and remained optimal at pH 4.8 for 18 weeks. Sugimori *et al*. (1971) reported an optimum pH of 4 to 5 for *P. ostreatus*. In Ghana the optimal pH for maximum yield of *P. ostreatus* has been found to be pH 5.5 to 6.5 (Narh *et al*. 2011) but this may vary with the different strains of *P. ostreatus* in Ghana. The optimum pH of *P. eous* for growth and fruiting bodies formation has not been determined for Ghana. In Japan *Pleurotus* is grown on a mixture of hardwood dust and bran at pH of 6.8-7.0 (Stamets and Chilton, 1983). Quimio (1978) reported an optimum temperature of 28-30ºC for maximum growth of *P. flabellatus*; *P. ostreatus* could grow best at 25.6ºC (Block *et al*. 1958; Zadrazil, 1978) and will fruit at less than 20.0ºC. In Ghana the optimum growth of *P. ostreatus* was 30ºC whereas that of *P. eous* has not been exhaustively

investigated. However, Uddin *et al*. (2011) showed that *P. eous* (Berk) Sacc., prefers temperature range from 21-35ºC and a humidity of 65-100%. The temperature, relative humidity and light preferences of eleven (11) species of *Pleurotus* species have been provided by OECD (2005), Kang (2004) and Stamets (1993) and are presented in Table 4.

Table 4: Environmental parameters for fruiting of some selected *Pleurotus* species

Species	**Temp. (°C)**	**Relative humidity (%)**	**CO_2 (ppm)**	**Light (lux)**
P. pulmonarius	18-24	85-90	400-800	1,000-1,500 (2,000)
P. cystidiosus	21-27	85-90	<2,000	500-1,000
P. djamor	20-30	85-90	500-1,500	750-1,500
P. eryngii	15-21	85-90	<2,000	500-1,000
P. euosmus	21-27	90-95	<1,000	750-1,500
P. ostreatus	10-21	85-90	<1,000	1,000-1,500
P. tuberregium	30-35	85-90	<2,000	750-1,500
P. sajor-caju	18-25	80-90	400-800	500-1,000
P. cornucopiae	20-30	85-90	< 1,000	750-1,500
P. f lo r id a	15-25	90-95	< 800	300
P. citrinopileatus	21-29	90-95	< 1,000	500-1,000

Data after OECD (2005); Kang (2004) and Stamets (1993)

Nutritional and individual value of *Pleurotus* species

Mushrooms have been an important food item concerning human health, nutrition and disease prevention (Chang, 1999). It is believed that mushrooms and foods have a common origin (Kaul, 2001). Dietary mushrooms provide a wide variety of medicinal properties and they are effective against certain life threatening diseases. The major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects. Oyster mushrooms (*Pleurotus* spp) are very effective in reducing total plasma cholesterol and triglyceride levels in humans (Nuhu *et al*. 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery related

disorders. For example, *P. florida* has antioxidant and antitumour activities (Nayana and Janardhanan, 2000; Manpreet *et al*. 2004). *P. sajor-caju* (now *P. pulmonarius*) has hypertensive reducing effects through its active ingredients which affect the renin-angiotensin system (Chang, 1999). *P. ostreatus* possesses antitumour activity (Yoshioka *et al*. 1985) and hypoglyceamic effects in experimental diabetic induced rats (Chorvathoba *et al*. 1993). Gunde and Cinerman (1995) have shown that *P. ostreatus* and other related species produce Lovastatin (3-hydroxy-3 methylglutaryl-coenzyme A reductase) a drug approved by the Food and Drug Administration, FDA, in the USA for treating excessive blood cholesterol. Lovastatin is concentrated in the mature gills and cap than the stipe.

White rot fungi like *Phanerochaete chrysosporium* and *P. ostreatus* have been reported to degrade different xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyl (PCBs), synthetic dyes, explosives and many others (Paszczynski and Crawford, 2000; Bogan *et al*. 1999). *P. ostreatus* contain enzyme complexes such as cellulose, cellobiose, hemicellulose lignase, laccase (Adamovic *et al*., 1998). Some of the cellulolytic, hemicellulolytic and ligninolytic enzymes include endo-1, 4-β-glucanase, exo-1, 4-β-glucanase, 1, 4-β-glucosidase, endo-1, 4-β-xylanase, 1, 4-β-xylosidase, endo-1, 4-β-mannanase and 1, 4-β- mannosidase, Mn – peroxidase and lignin peroxidase respectively (Petr and Jiri, 2003).

Chemical analyses of oyster mushrooms show that generally they contain total energy value of 320Kcal/100g, carbohydrates 57.6%; crude protein, 5.0%; lipids, 3%; fibre, 6%; ash dry wt 9.8% (Bhatti *et al*. 2007; Daba *et al*. 2008). Total carbohydrates include polysaccharides such as glucans, monosaccharides and disaccharides, sugars alcohols, glycogen and chitin (Hung and Nhi, 2012).

P. ostreatus cultivated in wawa sawdust in Ghana contained 49.93% total carbohydrates, 20.02 crude protein, 2% lipids, 15.81% crude fibre, 7.62% ash, 279.92Kcal/100g (or 1171.60KJ/100g), 4.5% nitrogen,; as well as Na (56.24mg/100g), Ca (43.06mg/100g) P (939.0mg/100g), K (3334mg/100g), Fe (42.65mg/100g) and Ascorbic Acid 99.8mg/100g (Obodai, 1992). *P. eous* cultivated elsewhere had the following nutritional content; crude protein 46%, total carbohydrates 50%, fat 1.2%, sugars 24%, crude fibre 12% (Cohen *et al*. 2002; Petr and Jiri, 2001; Ingale and Ramteke, 2010; Palmieri *et al*. 1997). Preliminary phytochemical screening of extracts of *P. eous* showed that they contain flavonoid compounds which are known to target prostaglandins involved in the late phase of acute inflammation and pain perception (Suseem and Saral, 2011); it is rich in minerals and contain appreciable amount of Ca (35.9mg), P (608.0mg), K (2620.0mg), Na (227.0mg), Mg (229.0mg), Fe (20.16mg), Mn (4.1mg)and Zn (3.1mg)/100gm dry mushroom (Ingale and Ramteke, 2010).

There is hardly any information on the pertinent literature on the nutritional content of *P. eous* cultivated in Ghana. This thesis aims at providing this novel information. These aforementioned nutritional properties of *Pleurotus* spp make them very good dietary and medicinal foods. Their consumption could have positive effects on the general human health because of a number of special nutritive and medicinal substances. Furthermore, because of their ability to degrade lignocellulose, they can be used in eliminating xenobiotic pollutants such as the pentachlorophenol (PCP), dioxin and polycyclic aromatic hydrocarbons (PAH's). This connotes that they can also be used for environmental bioremediation (Kubatova *et al.* 2001; OECD, 2005). The genus *Pleurotus* has complicated morphological variations of basidia-spores, cap, stipe and carpophore architecture resulting in taxonomic

confusion and difficulties in determining species boundaries (Venturella, 2000). The variations in Biological Efficiency, growth rate, nutrient composition and mineral contents etc. may be elucidated at the gene level. Recent molecular phylogenic studies have demonstrated that the ITS (Internal Transcribed Spacer) region of genomic DNA is very useful for assessing phylogenetic relationships at lower taxonomic levels. ITS of rDNA is considered as a variable region among the species and even among strains (Iraçabal et al. 1995; Zervakis et al. 1994 and Vilgalys et al. 1993. In this thesis the ITS region of the rDNA of *P. ostreatus* and *P. eous* were amplified by PCR techniques using universal primers with the view to ascertaining their taxonomic status and also serve as ancillary information in elucidating their growth performance, Biological Efficiency and the differences in elemental composition and nutrient status observed *in vivo*.

Spent mushroom compost (SMC) as bio-fertilizer and animal feed

After utilizing the compost for mushroom production, the spent mushroom substrate is the composted organic material remaining after a mushroom crop is harvested. Spent mushroom compost (SMC) is a nutrient-rich by-product of commercial mushroom production. However, it has the potential to cause environmental pollution when not managed appropriately. It is an excellent source of humus, although much of its nitrogen content would have been used up by the growing mushroom. However, the remaining SMC is a good source of nutrients such as 0.7% N, 0.3% K, in addition to all full range of trace elements as well as a useful soil conditioner (Mullen and McMahon, 2001). SMC used as an organic soil amendment or fertilizer could be prudent environment stewardship strategy in Africa. Compost products earmarked for agricultural crop production, horticultural plant production, gardening or land use reclamation should be applied correctly and in the proper

amount. Spent mushroom compost (SMC), although not a top soil could serve as an excellent compost useful to improve soil health and plant growth (Mullen and McMahon, 2001). Interestingly, SMC from the button mushroom (*Agaricus bisporus*) production is already in wide use in horticulture as compost of potting soil mixes; in agricultural or landscape trade to enrich soil, as a casing material in the cultivation of *Agaricus* spp in vermiculite as a growing medium, in wetlands for remediation of contaminated water, in stabilizing severely disturbed soils, in bioremediation of contaminated soils, as a bedding for animals, as an animal feed, and to control plant diseases (Danny, 2002). SMC has many other appropriate uses as spread on top of a newly seeded lawn (because it provides cover against birds eating the seeds and will also hold water in soil while seed germinate) (Beyer, 2003).

Reutilization of SMC after cultivation of *P. eous* on wheat straw has been carried in the form of serving as ingredients for the cultivation of three oyster mushroom species, *P. sajor-caju* (Malaysia), *P. florida* Strain P-1, *P. flabellatus* and as a fertilizer for growing *Spinacea oleracea*. All the test mushrooms showed highest yield and Biological Efficiency in test supplemented with 25% proportion of SMC (Siddhant and Singh, 2009). As a fertilizer, the spent compost was mixed at 1, 3, 5% (w/w) and added to the soil. SMC-containing sets showed early germination of *S. oleracea* than pure soil. The time of germination decreased with increasing proportion of SMC and recorded minimum in 5% (w/w) SMC. The yield (120g) of *S. oleracea* was higher in all treatments but was significant in 5% w/w soils. Supplementation of higher doses of SMC not only decreased time of germination but also increased yield performance (Siddhant and Singh, 2009).

Önal and Topcuóglu (undated) investigated the effect of SMC as an organic fertilizer for pepper grown in greenhouse soil in Turkey. Pepper plants were grown in pots containing different amounts of SMC i.e. 0, 15, 30 and 60 t/ha as dry weight basis. The effects of SMC application on dry matter and N, P, K, Ca, Mg, Fe, Zn, Cu, Ni, Cd, and Pb content of pepper were determined. SMC application caused statistically ($p<0.05$) important effects on dry matter yield and N, P, K, Fe and Zn contents in the pepper plant. SMC applications increased yield until 30 tons/ha of SMC application, but higher application rates of SMC depressed plant growth. All spent mushroom compost treatments, except control resulted in higher mineral content. However, no important changes in heavy metals were detected. All metal concentrations were below the phytotoxic maximum limits (Önal and Topcuóglu, undated). The best results for yield with regard to productivity were obtained at 30 tons/ha (Önal and Topcuóglu, undated). Clearly, this research showed that SMC could be applied to greenhouse soil at the agronomic rates without heavy metals and salinity defects. The phytonutritive capacity of SMC has often been demonstrated to be analogous to that of manure; the same level of productivity, both quantitatively and qualitatively, can be maintained by replacing manure with compost (Beyca *et al*. 1993).

Recently, Gonani *et al* (2011) studied the effect of SMC from *Agaricus bisporus* mushroom culture on growth of Cucumber (*Cucumis sativus* cv. Super dominos). Leached SMC to remove excess of minerals in rates 15, 25, 35 and 45% were used in amending a sandy-loam soil medium. Cucumber plant growth rate was evaluated based on fruit number and plant height. Results showed that addition of 15% and 25% of leached SMC to growing medium significantly ($p<0.05$) improved Cucumber plant growth. Spent mushroom compost processed to reduce soluble salts was used among other things to ascertain its effect on the

growth of maize in the utisoils in Port Harcourt in Nigeria. The results showed that organic waste compost application especially soil + SMC + poultry dropping at 40 t/ha improved soil physical properties, increased nitrogen content, phosphorus, potassium and micronutrients zinc, iron and copper in maize plants (Ogbonna *et al*. 2012). Vegetative growth parameters were better enhanced in maize stem, length, girth, number of leaves and chlorophyll content (Ogbonna *et al*. 2012). Several studies in Dublin, Scotland by Maher *et al*. (2000) have shown the benefits of using SMC to improve growth and yield of tomatoes, lettuce, potatoes, spring and winter wheat. Undoubtedly, spent mushroom compost in its raw form or leached can be used with beneficial effects in filed crop production. This technology is yet to be utilized on a wider scale in Africa owing to paucity in research results showing its efficacy. This thesis provides information on the use of SMC in the cultivation of tomato, pepper and cowpea using SMC from cultivation of *Pleurotus* in Ghana.

Interestingly, species of *Pleurotus* (including *P. ostreatus* and *P. eous*) have been found to produce a compound Pleurotin capable of destroying nematodes through release of toxin into soil (Bano and Rajarathnam, 1998). Thus spent compost may contain this compound which would destroy pests during cultivation of *Pleurotus* and thus become a potential biological agent for control of pests in agricultural soil. Various endogenous cytokinins such as zeatin and zeatin riboside have been extracted from fruit bodies of *Pleurotus* and bio-assayed (Jandaik and Goyal, (1995). These cytokinins which are active plant hormone substances have been thought to contribute to long storage life of the fruit bodies. Undoubtedly, the spent compost has many advantages and an investigation into its influence on crop productivity after commercial mushroom farmers harvested their fruiting bodies will provide vital information which could boost agricultural productivity in Ghana.

Currently, the Africa Rice Centre in Benin a member of CGIAR (Consultative Group on International Agricultural Research) and its partners are conducting research on innovative uses of rice straw and husk (which include bioenergy and biochar systems) for carbon sequestration and soil improvement. The West Africa Rice Development Institute is part of this programme and has supported this thesis to provide scientific data on the use of rice wastes for the cultivation of indigenous mushroom particularly *P. ostreatus* and *P. eous* strains in Ghana. Today, *Pleurotus* species are among the popular edible fungi cultivated in many tropical and subtropical countries including Ghana. The use of the spent mushroom compost (SMC) as bio-fertilizer would be an added economic advantage to improve plant nutrition and provide jobs and financial benefits to the rural dwellers.

OBJECTIVES

Overall Objective of this PhD studies was:

To establish the efficiency of *Oryza sativa* L. lignocellulose materials in the cultivation and growing of two ligninolytic oyster mushrooms (*Pleurotus eous* strain P-31 and *P. ostreatus* strain EM-1)

Specific Objectives of this project were:

a) To identify Mycoflora resident in the raw materials.
b) To study some aspects of the physiology of *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31 with the view to providing useful data for improved commercial production.
c) To establish the best growth conditions for the two mushroom species on unfermented and fermented rice wastes and its amendments.

d) To ascertain the efficacy of unfermented and fermented rice lignocellulose and its amendments on growth, yield performance and biological efficiency of the two species.
e) To estimate the proximate analyses of the product of cultivation and elemental composition of fruiting bodies
f) To establish the identity of the two *Pleurotus* spp. using PCR
g) Determine the role of spent mushroom compost (SMC) as organic / bio-fertilizer in the crop production of tomato (*Lycopersicon esculentum* Mill), pepper (*Capsicum annum* L) and cowpea (*Vigna unguiculata* Walp.).
h) To assess the role of SMC on the growth and development of cowpea seedlings and on the population of *Rhizobium* species and the commensurate nodulation of cowpea plant.

CHAPTER TWO

MATERIALS AND METHODS

1. MATERIALS

1.1. Agricultural Lignocellulose Materials

Dry rice (*Oryza sativa* L.) straw, bran and husks were collected from Dawenya and Aveyime-Battor Rice Farm in the Volta Region of Ghana and Ashaiman a suburb of Accra respectively.

1.2. Mushroom varieties used for the studies

Two species of oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer and *Pleurotus eous* (Berk.) Sacc., were obtained from the Mycology Unit of Food Research Institute (FRI) of the Council for Scientific and Industrial Research (CSIR) of Ghana.

1.3. Additives used as supplements to substrate

Calcium carbonate ($CaCO_3$) / lime and rice bran were purchased from Madina market and Ashaiman a suburb of Accra, respectively.

1.4. Materials used for bagging

Polypropylene (PPP) (heat resistant bags) and Polyvinyl chloride (PVC) pipe were purchased from Poly Products Limited, Accra. Rubber bands and non-absorbent cotton wool were obtained from the Local Makola Market.

1.5. Plants used for bio-fertilizer test

Cowpea (*Vigna unguiculata* Walp,), pepper (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum* Mill.) seeds were supplied by the seed bank of the Department of Botany and Department of Crop Science, College of Agriculture and Consumer Science, University of Ghana.

2.0. GENERAL METHODS

2.1.0. Maintenance of stock cultures on media

Stock cultures of both species of *P. ostreatus* and *P. eous* were maintained on slopes or slants of Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA) in McCartney tubes and on 9.0 mm Petri- dishes. All media used were sterilized at 1.05 kg/cm^3 pressure (121°C) for 15 minutes and were kept in a refrigerator at 8°C.

2.1.1. Method of Inoculation of cultures

An inoculum of 3 mm agar disks obtained from the growing edge of the culture was used throughout.

2.2.1. Spawn Preparation

Sorghum grains (10kg) were soaked overnight and strained using a basket and net. The grains were boiled for 1hr, strained and allowed to settle for about 30mins on a net. Two percent $CaCO_3$ (lime) was added to the grains and then mixed thoroughly until a uniform

mixture was obtained. The grains were put in spawn glass medicinal bottles and autoclaved for 1hr at 121°C at 1.05kg/cm^3 steam pressure after which they were allowed to cool at room temperature for 2hrs. The grains were then inoculated with 5mm agar disk of 7-day old cultures of either *Pleurotus eous* or *P. ostreatus* respectively. The spawn bottles were then incubated at 25°C for complete mycelia growth on the grains. Full mycelia growth through the grains was achieved within 14-21 days depending on the ambient climatic condition (28-32°C) and the vigour of the culture as well as the nature of sorghum grain.

2.2.2. Maintenance of spawns

Spawns used for this study were kept in an electric incubator until complete growth occurred (i.e. when the mycelia grew to cover the entire sorghum grains up to the bottom of the bottle). Fully complete spawns were kept in refrigerator at 8°C.

3.0. Composition of media

3.1.0. Preparation of Potato Dextrose Agar (PDA)

Irish potato (*Solanum tuberosum* L.) tubers were peeled and cut into smaller pieces. Two hundred grammes was weighed and boiled in 500ml distilled until it became softened. The water was strained with white muslin cloth and the supernatant was made up to 1000ml in a conical flask. Fifteen grammes of agar and 10g of dextrose were added and the entire mixture was warmed in a water bath to thoroughly melt the agar. It was then transferred into smaller medicinal bottles after which the medium was autoclaved at 121°C and 1.1Kg/cm^3 pressure for 15 minutes.

3.2.0. Preparation of Double Strength Potato Dextrose Agar

Exactly 400g of Irish potato was boiled in 500ml of distilled water until it became soft. The water was strained with white muslin cloth and the supernatant made up to 1000ml with distilled water in 1 litre conical flask; 30g of agar and 20g of dextrose were added and the entire mixture was warmed in a water bath to melt the agar properly. The medium was then transferred into smaller medicinal bottles after which it was autoclaved at 121°C and 1.1Kg/Cm^3 pressure for 15 minutes.

3.3.0. Preparation of Potato Dextrose Broth (PDB)

Exactly 200g of Irish potato was boiled in 500ml of distilled water until it became soft. The water was strained with white muslin cloth and the supernatant was poured into a conical flask and made up to 1 litre and 10g of dextrose were added. It was then transferred into smaller medicinal bottles after which it was autoclaved at 121°C and 1.1Kg/Cm^3 pressure for 15 minutes. No agar was added.

3.4.0. Preparation of Double Strength Potato Dextrose Broth

Exactly 400g of Irish potato was boiled in 500ml of distilled water until it became soft. The water was strained with white muslin cloth and the supernatant was poured into a 1000ml conical flask. 20g of dextrose was added and the entire mixture was warmed in a water bath. No agar was added. The mixture was then topped to the 1000ml mark with distilled water. It was then transferred into medicinal bottles after which they were autoclaved at 121°C and 1.1Kg/Cm^3 pressure for 15 minutes.

3.5.0. Malt Extract Agar (Oxoid CM167)

Malt extracts……………………………..30.0g

Mycological peptone……………………… 5.0g

Agar……………………………………….15.0g

Distilled water…………………………...1000ml

Final pH………………………………….5.4±0.2

3.6.0. Formulation of Cooke's Medium (Cooke, 1954)

Glucose…………………………………....10.0g

Peptone…………………………………….5.0g

KH_2PO_4..1.0g

$MgSO_4.7H_2O$………………………………..0.5g

Rose Bengal………………………………0.035g

Streptomycin………………………………0.35g

Agar……………………………………….15.0g

Distilled water……………………………1000ml

3.7.0. Formulation of Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Oxoid CM0727)

Peptone…………………………………….5.0g

Glucose……………………………………10.0g

Potassium dihydrogen sulphate (KH_2PO_4)…..1.0g

Magnesium sulphate ($MgSO_4.7H_2O$)………...0.5g

Dichloran…………………………………...0.002g

Rose Bengal………………………………...0.025g

Chloramphenicol…………………………….0.1g

Agar………………………………………15.0g

Distilled water…………………………….1000ml

pH………………………………..5.6±0.2 at 25°C

3.7.1. Yeast extract-manitol agar (YMA) after Addo, 1991

Yeast extract-manitol agar (YMA) was prepared as described by Addo, 1991 was used as the growing media for the *Rhizobium* species. The composition is as below:

Manitol…………………………………10.0g

K_2HPO_4…………………………………0.5g

$MgSO_4.7H_2O$……………………………0.2g

NaCl……………………………………...0.1g

Yeast extract (Oxoid)…………………....0.4g

Agar……………………………………...15.0g

Distilled water………………………...1000ml

The prepared media were autoclaved at 121ºC and 1.1Kg/cm^3 pressure for 15 minutes.

3.7.2. Congo Red Yeast extract-manitol agar (YMA) (Hahn, 1966)

This medium contained the same components as Yeast extract-manitol agar supplemented with 10ml of 0.0025ml (1/400) aqueous solution of Congo red sterilised separately and added aseptically to give a final Congo red concentration of 25gml^{-1} just before use.

3.8.0. Preparation of Compost

3.8.1. Preparation of rice straw compost without supplementation (after Mondal *et al*. 2010 and Obodai *et al*. 2011)

Dry paddy rice straw was chopped into small pieces about 2-5cm long. Exactly 10kg was weighed and put into two metal barrels. The tanks were then filled with 432 litres of tap water. It was soaked for 3hrs and then removed. It was strained using raphia baskets and then allowed to settle for about 45mins to drain excess water. Once the excess water was drained a sample was taken and squeezed in the hands, when no water exudes through the fingers and just a drop or dampness it assumed that the desired moisture has been achieved. The substrate was then gathered to form a heap. The fermentation process was allowed to go for 0, 4, 8 and 12days. The unfermented or uncomposted rice straw served as the zero (0) day substrate. The compost was turned using shovel every 4 days to obtain a uniform composting and also to avoid anaerobic fermentation in the middle portion of the heap of the compost. Each of the compost has a dimension of 48 x 76 x 69cm.

3.8.2. Preparation of rice straw compost supplemented with lime and rice bran

The same procedure as above was followed only that the straw was supplemented with 1% and 10% $CaCO_3$ and rice bran respectively at the onset of fermentation process. The unfermented rice straw served as the zero (0) day substrate. During the composting process the pile of rice straw was turned every 4 days to obtain a uniform composting and also to avoid anaerobic fermentation in the central portion of the compost. Before bagging 0.5% of

lime was added to each of the composted and uncomposted substrate. Each of the composting days had the same dimensions as above.

3.8.3. Preparation of rice straw compost which was supplemented with different proportions of rice bran at bagging

Dry rice straw of about 15kg was weighed and transferred into metal drums. The same procedure as above was followed. The rice straw was supplemented with 1 and 10% $CaCO_3$ and rice bran respectively before the composting process. At the end of the composting period, both fermented and unfermented rice straw were divided into 3 equal parts with each part weighing about 10kg wet weight. Each part was supplemented with different proportions of rice bran (5, 10 and 15%) and this was calculated based on the dry weight of straw. Dimension of the entire compost heap for each composting days was 55 x 76 x 91cm.

3.8.4. Preparation of compost that constituted of rice straw and Engelberg Mixture (rice husk and rice bran mixture)

Ten kilograms of dry rice straw was soaked for 3hrs and was then amended with 10kg of rice husk and bran in a ratio of 2:1. After the mixture was mixed thoroughly; 1 and 10% $CaCO_3$ and rice bran was added respectively. The entire mixture was mixed several times repeatedly until a uniform mixture was obtained. To ascertain the moisture content of the entire substrate; the squeezed technique method was employed. The resulting mixture was heaped and allowed to compost for 4, 8 and 12 days respectively. The unfermented or uncomposted substrate served as the zero (0) day substrate. During the composting process the pile of compost was turned every 4 days to obtain a uniform composting and also to

avoid anaerobic fermentation in the central portion of the compost. The compost dimension for each day was measured as 58 x 81 x 96cm.

3.8.5. Preparation of sawdust compost

Sawdust was mixed with 1 and 10% $CaCO_3$ and rice bran respectively. The entire mixture was moistened with tap water till moisture of about 70% was reached. A sample was taken and squeezed in the hands. The moisture content was taken to be about 70% when a drop of water oozes out through the fingers and dampness is felt in the hand. The entire mixture was allowed to compost for 4, 8 and 12 days respectively. The unfermented or uncomposted sawdust served as the zero (0) day substrate. During the course of composting process the heap of sawdust was turned every 4 days to ensure uniform fermentation at all depths of the compost and also to avoid anaerobic fermentation in the central portion of the pile of sawdust. Each of the composting days has dimensions of 38 x 66 x 58cm.

3.8.6. Preparation of Engelberg mixture compost with no additives

About 10kg of the mixture was weighed and was moistened with tap water until a moisture content of about 65-70% was attained. This moisture content was determined by performing the squeeze test (Buswell, 1984). The mixture was mixed thoroughly and was allowed to compost for 4, 8 and 12 days respectively. The compost was turned over every 4 days to ensure uniform fermentation at all depths of the compost and also to avoid anaerobic fermentation in the central portion of the pile compost. Each of the compost has a dimension 38 x 70 x 68cm.

3.8.7. Preparation of Engelberg mixture compost with additives such as lime and rice bran

About 15kg of the mixture was measured and 1 and 10% of $CaCO_3$ and rice bran were added respectively. The entire mixture was moistened with tap water until a moisture content of about 65-70% was attained. This moisture content was determined by performing the squeeze test (Buswell, 1984). The mixture was mixed thoroughly and was allowed to compost for 4, 8 and 12 days respectively. The compost was turned over every 4 days to ensure uniform fermentation at all depths of the compost and also to avoid anaerobic fermentation in the central portion of the pile compost. Each of the compost has a dimension 40 x 78 x 75cm.

3.8.8. Preparation of substrate for bagging

3.8.9. Bagging of compost substrate without supplementation

At the end of the composting process each of the compost (0, 4, 8 and 12 days) was mixed thoroughly for several times. The mixture was then moistened with some amount of water till the moisture content was about 70%. When this moisture is attained a sample was taken and squeezed in the hands, until no water exudes through the fingers and only some dampness is felt. This moisture content level was found to be optimum for oyster mushroom production (Khanna and Garcha, 1984). The substrate was packed into heat-resistance polypropylene bags of dimensions 32.5 x 9.7 x 8.7cm. Each bag was filled with 1kg of the substrate and then compacted and the mouth of the bag was pushed through a polyvinyl chloride (PVC) pipe of dimension 2.0 cm thick and 2.5 cm long, which was then pulled down and fastened with a rubber band. This served as a bottleneck into which cotton wool

was inserted. The purpose of the PVC pipe was to provide an opening for inoculation and for gaseous exchange. The bags were labelled appropriately before sent to oil drums (tanks) placed on metal stoves fueled with gas. The bags were steam sterilized for about 3hrs at a temperature of about 100°C.

3.9.0. Bagging of compost substrate supplemented with 1 and 10% $CaCO_3$ and rice bran respectively before composting

The same procedure was followed as above (3.8.9) except that before the fermentation process 1 and 10% $CaCO_3$ and rice bran were added to the substrate respectively. At bagging 0.5% $CaCO_3$ was added to both the fermented and unfermented compost substrates. The entire mixture was then mixed thoroughly and moistened with water until a moisture content of 70% was achieved. After which a sample was taken and squeezed in the hands and when no water oozes through the fingers only some slight wetness the substrate was then packed into the bags. Each bag was filled to weight of 1kg after which the opening of the bag was pushed through a PVC pipe and a rubber band was used to tie the overlapping polyethylene over the pipe to hold it upright and securely in place. The opening of the PVC pipe which served as the bottleneck was plugged with a cotton wool. The bags were steam sterilized for about 3hrs at a temperature of about 100°C.

3.9.1. Bagging of compost substrate supplemented with different proportions of rice bran

The matured compost of each treatment was divided into three equal parts. Each part was then supplemented with 5, 10, and 15% rice bran. These proportions were calculated based on the dry weight of the straw. The substrate was put in heat resistant transparent polyethylene sachets, with each bag containing 1kg of compressed substrate. The open end of each bag was passed through PVC pipe of dimension 2.0 cm thick and 2.5 cm long, which served as a bottleneck it which cotton wool, was inserted. Rubber band was used to tie the overlapping polyethylene over the pipe to hold it upright and securely in place.

3.9.2. Sterilization of bagged substrates

The substrate was compressed in 32.5 x 9.7 x 8.7cm heat resistant polypropylene bags. Each bag contained approximately 1kg of the substrate with a minimum of 10 replicates for each treatment. The bagged substrates were steam sterilized at a temperature of 100°C for 3hrs in oil drums placed on gas stove.

3.9.3. Inoculation of compost bags

After the bags were steam sterilized and allowed to cool, about 30-100 grains (3.5g) of spawn were inoculated into each bag of weight 1kg under sterile conditions and was then shaken to disperse the spawns (seeds) for uniform distribution of the grains in the bag.

3.9.4. Incubation of compost bags

The inoculated bags were transferred into an incubation room and were left for as long as mycelia will grow through the grains in the bag. The incubation period ranged from 30-70 days depending on the spawn vigour and climatic conditions. The ambient temperature of the incubation room was maintained at 25-31°C. During this period, the mycelial growth rate was measured every week until the entire content of the bag was filled with the mycelia. After the spawn run period (i.e. the period required for complete impregnation of the substrate). The bags were transferred into a cropping house for the formation of fruit bodies once the mycelia become thickened in the bags.

3.9.5. Cropping of fresh mushroom fruit bodies

The bags were transferred into the cropping house with the environmental conditions: light, moisture, air exchange and temperature altered to induce fruiting (Stamets, 2000). For instance the cropping house had a relative humidity ranged from 80-90% with temperature ranged from 23-30°C and was maintained by watering the cropping room floor with 2,500 litres twice daily (morning and evening). The cropping house was constructed with a bamboo and thatch mats to allow for good ventilation and in order to maintain the above-mentioned temperature range. Mushrooms were harvested upon attaining maturity when the fruiting bodies start curl up with wider gills opening or when the mushroom cap surface were flat to slightly up-rolled at the cap margins. All mushrooms within a given bag were harvested at the same time irrespective of the size or stage of maturity. This was done

because at the primordia stage all were counted together and therefore in order to account for the how many grown to maturity stage and also in order not affect each flush.

3.9.6. Assessment of total yield of fruiting bodies

Several parameters such as total number of pinheads formed, total number of fruiting bodies and fresh weight of fruiting bodies were taken. The weight was assessed using an electric balance and the Biological Efficiency (BE) was determined using the methods of Pathmashini *et al.*, (2008) and Patra and Pani (1995). The biological efficiency (BE) value was computed using the mathematical expression below:

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

3.9.7. Harvesting Technique

The first primordia appeared 2 - 4days after bag opening depending upon the types of substrate treatment, environmental conditions and spawn quality. The harvesting date also varied depending upon types of substrate treatment. Matured mushrooms were identified by curl margin of the cap and were harvested by twisting to uproot from the base. In cases where it was difficult to uproot the fruiting bodies from the base the mushrooms were harvested with a sharp scalpel and put into polypropylene bags. Each treatment was identified and weighed on an electric balance to determine the fresh weight. Generally,

mushroom matured 48 hours (2 days) after the appearing of primordia. The Biological Efficiency per yield was calculated using the expression above.

3.9.8 Chemical Analyses of compost Used as Substrate for Mushroom Cultivation

Chemical analyses were performed on rice straw and husk with appropriate methods. Quantitative estimation of crude protein, cellulose, hemicellulose, lignin and ash were carried out. Crude protein, lignin, hemicellulose and cellulose estimation were determined by Kjeldahl's and detergent fibre methods (NDF, ADF) respectively outlined by AOAC, (2005).

3.9.9 Determination of Neutral Detergent Fibre (NDF) (after Van Soest *et al*. 1991)

Neutral detergent fibre was determined by the method employed by Van Soest *et al*. (1991) with enzyme addition. About 0.5g (W_1) of powdered sample was weighed into a 600ml Berzelius beaker for refluxing; 50ml of cold neutral detergent solution was added. The solution was heated gently to avoid foaming. Refluxing was then done for 60 min after which the solution was filtered on a previously weighed Gooch crucible (W_0) using light suction (vacuum) it was washed several times with hot water (90-100°C); 3ml of amylase solution was added to the crucible for smooth filtration after which 30ml of boiling water was added to the entire content and was then allowed to stand for 10mins. The solution was washed several times with acetone and sucked with light suction and dried in an oven at 105°C for 8hrs. The sample was cooled in a desiccator and weighed to obtain yield of cell wall (W_2). The crucible was ashed at 510°C in a furnace (Vecstar-Furnace Model PS3, Sweden) for 3hrs and was removed and kept in a desiccator to cool after which it was

weighed (W_3). The loss in weight is the ash free cell wall and the %NDF was estimated using the formula below:

$$\%\ \mathrm{NDF} = \frac{\mathrm{W2} - \mathrm{W0}}{\mathrm{W1}}\ \mathrm{x}\ 100\%$$

$$\%\ \mathrm{NDF\ (DM)} = \frac{\%\ \mathrm{NDF}}{\mathrm{DM}}\ \mathrm{x}\ 100\%$$

Wo = weighed of Gooch crucible; W_1= air dry weight of powdered sample;

W_2= oven dry weight of sample; W_3= weight of sample after ignition; DM= dry matter

4.0.1 Determination of Acid Detergent Fibre (ADF) (after Van Soest *et al*. 1991)

About 0.5g (W_1) of each air dried powdered sample was weighed and sieved through 1.0mm mesh into a 600ml Berzelius beaker for refluxing. Fifty millitres of cold acid detergent solution was added. The solution was heated gently to avoid foaming. Refluxing was then done for 60 min after which the solution was filtered on a previously weighed Gooch crucible (W_2) using light suction (vacuum) washed several times with hot water (90-100°C). It was subsequently washed with acetone until a colourless solution was obtained and was sucked with light suction and was dried at 105°C overnight. The sample was put in a desiccator to cool and weighed (W_3).The contents in the crucible were then covered with cooled (20°C) 72% H_2SO_4. With a glass rod, the solution was stirred to break up lumps and was left for 3hrs and stirring at hourly interval as the acid drained away. After 3hrs the solution was filtered using the light suction and it was washed with hot water until free from acid. The contents were dried overnight and weighed after cooling in a desiccator (W_4). The

crucible with its content were ignited in a furnace at 510°C for 3hrs after which it was put in a desiccator to cool to about 105°C and weighed (W_5). Lignin content in the substrate was estimated as the difference between the W_5 and W_4.

The mathematical relations below were used to estimate hemicellulose and cellulose:

NB W_2= Empty crucible weight; W_1= air dry weight of powdered sample

W_3=oven dry weight of sample; W_4=initial weight of crucible and sample before ignition

W_5 = final weight of crucible and content after ignition

DM = dry matter

$$\% \text{ ADF} = \frac{\text{W3} - \text{W2}}{\text{W1}} \text{ x } 100\%$$

$$\% \text{ ADF} = \frac{\%\text{ADF}}{\%\text{DM}} \text{ x } 100\%$$

i.e. % Hemicellulose (DMB) = % NDF (DMB) - % ADF (DMB)

$$\% \text{ Cellulose} = \frac{\text{W3} - \text{W4}}{\text{W1}} \text{ x } 100\%$$

$$\% \text{ Cellulose(DMB)} = \frac{\% \text{ Cellulose}}{\%\text{DM}} \text{ x } 100\%$$

$$\% \text{ Lignin} = \frac{\text{W4} - \text{W5}}{\text{W1}} \text{ x } 100\%$$

$$\% \text{ Lignin(DMB)} = \frac{\% \text{ Lignin}}{\%\text{DM}} \text{ x } 100\%$$

4.0.2 Determination of moisture content

The moisture content was determined by the gravimetric method (Black, 1965). One gramme of a sample was measured separately into previously weighed moisture can. It was then dried in the oven at 105°C for 6hrs, cooled in a desiccator and re-weighed. The cooled sample was returned to the oven for further drying. Drying, cooling and weighing were repeated at 1hr intervals until no further reduction in the weights was obtained (constant weight obtained). The weight of moisture loss was determined and expressed as a percentage of the sample analyzed.

4.0.3 Determination of Crude protein (AOAC, 2005 and James (1995)

About 1g of dried ground sample was weighed (W) and transferred into a digestion tube through a 1.0mm screen mesh. About 3g of a catalyst mixture of $CuSO_4$ and K_2SO_4 was added to the sample. Subsequently, about 20ml of concentrated H_2SO_4 was added to the mixture and the tube was then placed in a digester at 380°C for 5hrs. After 5hrs the tube was removed from the digester block and was allowed to cool in a hood. At the completion of digestion when the solution was colourless or light blue; 30ml of distilled water was carefully added and subsequently 40ml of 32% NaOH solution was also added in a distillation set up (Kjeldahl apparatus). The entire mixture was distilled and collected into a conical flask containing 50ml of 4% boric acid solution. The distillation process was allowed for 6mins when about 100ml of the distillate was collected. The distillate (ammonium borate) was titrated against 0.1N H_2SO_4 and using 3 drops of a mixture indicator

(methyl red and bromo cresol green) was added to the solution. A colour change from steel blue to pink upon addition of few drops of the acid (0.1N H_2SO_4) indicated an end point. A reagent blank determination was also carried out. The crude fiber content was calculated using the formula below:

$$\% \text{ Crude protein} = \frac{\text{K x N x (V} - \text{B)}}{\text{W}} \text{ x } 100\% \text{ x F}$$

$$\% \text{ Crude protein} = \frac{0.01401 \text{ x N x (V} - \text{B)}}{\text{W}} \text{ x } 100\% \text{ x } 6.25$$

$$\% \text{ Crude protein (DMB)} = \frac{\% \text{ Crude protein}}{\% \text{ DM}} \text{ x } 100\%$$

% Crude protein = % Nitrogen x 6.25; DM= dry matter

Protein content (% dry weight) = (total nitrogen - chitinous nitrogen) x 6.25

V= titre volume of acid consumed: B= blank titre volume in titration

N= normality of H_2S0_4 used in titration: W= weight of sample taken.

K = constant = 0.01401: F = conversion factor of nitrogen in protein = 6.25

4.0.4 Determination of proximate composition of mushroom

Nutrient analyses of fruiting bodies were done using methods adopted by AOAC (2005). To determine the total nitrogen content in the fruiting bodies, samples were dried at 60°C and analysed by the Kjeldahl method (AOAC, 2005). The total (crude) protein was determined

from the total nitrogen content, using the correction factor 4.38 or 6.25 (Breene, 1990). Total carbohydrate content was estimated using the mathematical formula:

Total carbohydrate (%, DW) = 100% - protein content (%, DW) – lipid content (%, DW) – ash (%, DW).

Carbohydrate (%) = [100 – (moisture – total ash – fiber – protein – fat)] (Nilsen 2010).

Crude fibre, fat, and minerals were analysed by method outlined by AOAC (2005).

4.0.5 Determination of Fat content in the mushroom sample

About 2g of the sample was weighed (W_1) and transferred into a clean alundum thimble which was previously extracted with a porous filter paper at 60°C. The thimble with the sample was covered with defatted cotton and was placed in a soxhlet reflux flask which was previously kept in an oven at 105°C for 8 hrs. The flask was then kept in a desiccator to cool to about a 25°C and weighed (W_2). The thimble was mounted in a reflux flask containing 70ml of petroleum ether. When heated the solvent condenses into the reflux flask. The sample was covered until the flask was filed up and siphoned over carrying oil (fat) extract down to the boiling flask. The process was allowed to go on repeatedly for about 4hours before the defatted sample was removed and kept for crude analysis. The solvent was recovered and the flask with its oil extract was dried in the oven at 105°C for 30 minutes, cooled in a desiccator and re-weighed to obtain the weight (W_3) of the oil extract (fat). It was then expressed as a percentage of the sample analyzed. The percentage fat content was calculated using the mathematical relationship below:

$$\% \text{ Ether extract (DMB)} = \frac{\text{W3} - \text{W2}}{\text{W1}} \text{ x } 100\%$$

$$\% \text{ Ether extract (DMB)} = \frac{\% \text{ Ether extract}}{\% \text{ DM}} \text{ x } 100\%$$

% Fat = 100 (W_2-W_1) x 100; W_1= weight of sample;

W_2 = Initial weight of extraction flask and content

W_3 = Oven dry weight of flask + oil (fat) extract.

4.0.6 Determination of Crude fibre content in the mushroom sample

In crude fibre determination only de-fatted sample was used. 0.5g (W_1) of sample was weighed and transferred into 600ml Berzelius beaker through a 1.0mm screen mesh. 50ml of cold (25°C) 1.25% H_2SO_4 solution was added to the sample. The beaker was placed on a reflux condenser unit and heated at 100°C for exactly 30mins. A few drops of anti-reagent such as amyl alcohol was added at this stage to minimized frothing or foaming. After 30mins of boiling the H_2SO_4 solution was washed off with hot water repeatedly and was filtered immediately using light suction. Fifty millitres of 1.25% of NaOH was added to the washed sample and was refluxed for additional 30mins. After 30mins the NaOH solution was washed off with hot water several times and then filtered using vacuum suction through Gooch crucible. The crucible with the fibre content was dried in an oven at 105°C for 10hrs and the fibre content was weighed (W_2) to obtain the yield. Crucible content was ashed in a furnace at 510°C for 3hrs was then removed from the furnace and put in an oven at 105°C for 8hrs and then weighed (W_3). An estimation of crude fibre content was calculated as change in weight lost and was mathematically expressed as below:

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight on ignition}}{\text{Initial weight of sample}} \times 100$$

$$\text{i.e. } \% \text{ Crude fibre} = \frac{(W_2 - W_3)}{W_1} \times 100\%$$

$$\% \text{ Crude fibre} = \frac{\text{Crude fibre}}{\%\text{DM}} \times 100\%$$

W_1= weight of sample; W_2= oven dry weight of Gooch crucible and content (i.e. before ignition)

W_3= weight of Gooch crucible and content after ignition

4.0.7 Determination of Minerals or Heavy Metal Elements content in the mushroom sample

About 250mg of ground air dried sample was weighed into a beaker was then placed in ignition muffle furnace (Vectar-furnace, PS3-Sweden) for drying at 400°C for 24hrs. Five millitres of hydrochloric acid (HCl) was added to the sample and the solution was dried again; subsequently 5ml of nitric acid (HNO_3) was added. After evaporation, the sample was diluted to 50ml with water. Sodium (Na) and calcium (Ca) content of the ashed sample were determined by flame photometer and K, P, Mg, Cu, Zn, Mn, Fe, and Pb by Unicam 929 Atomic Absorption Spectrophotometer (AAS) (Model PinAAcle 900T).

4.0.8 Determination of Ash content (Van Soest *et al*. 1991)

Ash was determined by ignition in a muffle furnace; about 2.0g of the sample was weighed (W) into a porcelain crucible of known weight (W_1). It was then burnt into ashes in the muffle furnace at 130°C for 3hrs. It was then removed and put in a desiccator for cooling, it was re-weighed (W_2) and the weight of the ash was obtained and expressed as a percentage of the weight of sample analyzed. The ash content was then estimated using the formula below:

$$\text{Ash content} = \frac{(W2 - W1)}{W} \times 100\%$$

W_1 = Weight of empty crucible

W_2 = Weight of crucible + ashes

W = Weight of sample

4.0.9 Phenology of resident mycoflora

Conventional Decimal serial dilution technique was used to estimate population of residing fungi on all the substrates used for this study. About 1.0g of the sample was placed in a sterile 250ml Erlenmeyer flask containing 100ml of 0.1% peptone diluent distilled water. The mixture was shaken in a Gallenkamp (England) Orbital shaker for 5mins at 140rev/min. About 1ml of aliquot of the suspension in the flask was transferred into a sterile McCartney tube containing 9ml of 0.1% peptone and was serially diluted up to $1:10^4$; 1ml of an aliquot of each dilution in duplicate was poured into a sterile Petri dish containing 20ml of either

Cooke's or DRBC media. The plates were then incubated at 29±2°C up to 7days. After 7days fungal growth was determined by counting colonies and the fungal population was estimated as $\log_{10}$ CFC/g sample. Identification of mycoflora was done using morphological and culture characteristics such as colour, mycelia and spore structure and colony appearance as outlined by Charles and Kenneth (1945); Sigurd (1953); George and Harold (1960); Von-Arx (1970); Barnett and Barry (1972) and Robert and Ellen (1988). When appropriate, photographs were taken of the species under the microscope for authentication with the assistance of the principal supervisor.

4.1.0 Determination of pH and moisture content of substrates

This was done before and after sterilization of the substrate. Approximately 20g of each collected sample from all composting days was transferred into a 250ml Erlenmeyer flask containing 200ml of distilled water and was soaked for 3hrs. The sample was filtered using Buckner funnel with a filter paper and the supernatant was taken and transferred into a 250ml beaker. The pH was measured using TOA pH HM-60 (Ogawa Seiki Co. Ltd. Japan) laboratory meter. Dry weight of the sterilized treatments was determined by drying 20g of each treatment at 103°C for 4hrs in a hot oven (Gallenkamp oven, 300 plus series, England). The moisture contents of the treatments were calculated using the formula below:

Moisture Content = [(Initial weight – Dry weight)] / Initial weight x 100%

All weight measurements were done by the use of a Digital Computing Scale (Hana Electronics Company Limited, Korea).

4.1.1 Cultural Media and Chemicals

All chemicals and media used for this study were either Analar grade, Merck or Oxoid the distributed by Merck KGoA Darmstadt, Germany and Oxoid Limited, Basingstoke, Hampshire, England, United Kingdom respectively.

4.1.2 Assessment of Vegetative Growth

a) **Radial Growth of Mycelium**

Vegetative growth of the mycelium of the mushrooms was assessed by measuring growth of the fungus along two diameters drawn at right angles at the bottom of the Petri plates prior to inoculation. Measurements were made every three days until complete colonization of the Petri plates was obtained. The incubation periods and temperature are mentioned at the appropriate places in the text ranging from 7-12 days and 28-32°C.

b) **Assessment of Vegetative Growth of mushroom in Liquid Medium**

Growth in liquid culture medium was assessed by determining the oven dry weight method of the harvested mycelium at the end of the incubation period. The unamended Potato Dextrose Broth served as control whereas the remaining media which were amended with either filtrate from antagonistic fungi served as the treated basal medium. Thirty millitres of the prepared basal medium and was poured into 250ml Erlenmeyer flasks and was inoculated with a 3mm agar disc with three replicates for each treatment. The incubation periods and temperature are mentioned at the appropriate places in the text.

4.1.3 Extraction of DNA from dry mushroom sample

Total genomic DNA was isolated and purified using E.Z.N.A. ™ SP Plant DNA Mini Kit. 0.3g of dried ground mushroom samples was weighed into a microfuge tube. 700µL of Buffer SP1 was added followed by 5µL of RNase A after which samples were incubated at 65°C for 15 minutes. Two hundred and ten micro litres of buffer SP2 was added and the samples were mixed vigorously by vortex followed by incubating samples on ice for 5 minute and centrifuged at 14000 rpm for 10 minutes. The supernatant obtained was carefully aspirated into an Omega® Homogenizer Column placed in 2 mL collection tube and was then centrifuged at 14000 rpm for 2 minutes. 500 µL of the clear lysate that resulted was transferred into to a 1.5 mL tube. Binding conditions of the sample was adjusted by pipetting 750 µL of buffer sp3/ ethanol mixture directly on to cleared lysate. 650µL of the resulting mixture was transferred into a Hiband® DNA Mini Column placed in a 2mL collection tube and centrifuged for 1 minute at 14000 rpm after which the flow through was discarded. This was repeated for the remaining mixture. The columns were placed into a new 2mL collection tube and 650µL of SPW Wash Buffer diluted with ethanol was added. This was centrifuged at 14000 rpm for 1 minute and the flow through discarded. This step was repeated with the sample volume of buffer SPW wash buffer. The empty column was centrifuged at 14000 rpm for 2 minutes. The Hiband® Mini column was then transferred into a sterile 1.5mL tube and 100µL of pre-warmed (65°C) elution buffer was added. This was then centrifuged at 14000 rpm for 1 minute to elute DNA.

4.1.4 Details of oligonucleotide primer sequences used for the PCR amplification

ITS1	TCCGTAGGTGAACCTTGCGG	White et al, 1990
ITS4	TCCTCCGCTTATTGATATGC	White et al, 1990

4.1.5 Polymerase chain reaction (PCR) amplification

PCR was performed with ITS 1 and ITS 4 primer to characterize the ITS region of mushroom samples. ITS amplification was carried out in a 25 µL PCR reaction composed of 1X GoTaq PCR master mix, 0.2 µM each of ITS 1 and ITS 4 primers and 1 µL of extracted DNA. The thermal cycling conditions were as follows; 94 for 5min followed by 35 cycles of 94 for 1 min, 55 for 1 min and 72 for 2 min, and a final extension at 72 for 6min. Products were run and visualized on a 1.5% agarose matrix stained with ethidium bromide.

4.1.6 Restriction digestion

Restriction enzymes were used to segregate the two mushroom species based on the generated restriction patterns of ITS 1 and ITS 4 amplicons. Restriction digestion was performed in a 20 µL volume composed of 1x reaction buffer, 1 µL of restriction endonuclease and 4ul of ITS 1 and ITS 4 PCR amplicons. Final volumes were made with deioned water.

4.1.7 Influence of Spent Mushroom Compost (SMC) used in amending soil on the growth and development of some economic crops

Three economic crops namely tomatoes (*Lycopersicon esculentum* Mill cv. Wosowoso variety), pepper (*Capsicum annum* L. Legon 18 variety) and cowpea (*Vigna unguiculata* Walp black eye variety) were used for this experiment. These three are the most cultivated in backyard gardens and commercial farms for sale on the local indigenous markets. The aim of this experiment was to study the effect of SMC on the growth of these three economic crops. A modified method of Önal and Topcuoğlu, undated; Çayci, 1998; Polat *et al*. 2009; Gonani *et al*. 2011. SMC in the following rates of 5, 10, 15, 20, 25 and 30% were added to a sandy-loam soil medium to obtain a total weight (w/w) of 6.5kg for each plastic bucket of dimensions 25cm x 22cm of height and diameter respectively. After thorough mixing of the combination the growing media were irrigated with 1litre tap water. Three weeks nursed seedlings of either pepper or tomato was transplanted 4cm whereas one cowpea seed was planted 4cm per bucket for all treatments. The seedlings in the buckets were arranged in a randomised complete-block design with five replications of all eight treatments under greenhouse condition. Soil medium only served as control treatment whereas soil medium amended with different rates of SMC served as treated growing media and 100% SMC served as only SMC. Seedlings were watered on every other day with equal volumes of tap water (500ml). The following parameters were taken and recorded; plant height, number of leaves, floral buds, flowers, axillary buds, leaf area, chlorophyll content of leaves, stem girth, number of fruits and weight of fruits. Plant height was measured using a meter rule, number of number of leaves, floral buds, flowers, axillary buds and fruits were counted. Tomato and pepper fruits and cowpea pods were weighed using a digital

Computing Scale (ACB plus Adams Equipment Company Limited, Milton Keynes, UK) after counting. Leaf area was determined using digital leaf area meter as well measuring the length and width dimensions of the leaf whereas stem girth was determined using micrometer instrument. Total chlorophyll content was determined using chlorophyll meter (Optic Sciences CCM-200 plus) by attaching the chlorophyll meter knob to the leaf. Chlorophyll a and b content was assessed using the method adopted by Bansal *et al*. (1999). The alues were quoted as CCI (chlorophyll content index).

4.1.8 Assessment of the effect of spent mushroom compost on total chlorophyll content

After 8 weeks of planting total chlorophyll content of the leaves were determined using a modification of the method adopted by Bansal *et al*. (1999). Exactly 100ml of 70% acetone extract using 2g of fresh cowpea leaves was prepared by grinding in a porcelain mortar with a pestle. The supernatant was filtered through Whatman No. 1 filter paper in Büchner funnel. The absorbance of the resultant filtrate was read on a Shimadzu, Osaka, Japan Model Spectrophotometer at 663nm and 645nm; 80% acetone served as blank. Chlorophyll concentration was calculated as follows:

Chlorophyll a = (12.7 ×Absorption at 663nm) – (2.69 ×Absorption at 645nm) mg/l.

Chlorophyll b = (22.2 × Absorption at 645nm) – (4.67 ×Absorption at 663nm) mg/l.

Total Chlorophyll = (20.2 × Absorption at 645nm) + (8.02 ×Absorption at 663nm) mg/l.

4.1.9 Influence of spent mushroom compost, SMC on nodulation of cowpea, radicle development and nitrogen assimilation

Sandy-loam soil was amended with SMC with the following rates 5, 10, 15, 20, 25 and 30% whereas 0 and 100% served as soil (control treatment) and SMC only respectively. After the growing medium was thoroughly mixed it was irrigated with 1litre tap water. One cowpea seed was planted 4cm per bucket 25cm x 22cm of height and diameter respectively for all treatments. At the end of 8 weeks of planting the number of Nodule Index (Rebah *et al*. 2002) was used to assess influence of SMC on nodulation of cowpea. The Nodule Index is calculated by the formula: **Nodule Index = A x B x C ≤ 18**

A = Nodule Size; **B** = Nodule Colour; **C**= No. of Nodule

Nodule size……..Value of A	**No. of Nodule……………..Value C**
Small……………….…….1	Few………………....………..1
Medium………………..……2	Several………………………..…2
Large………………..……3	Many…….…………………........3

Nodule Colour………………Value B

White……………………………...1 Pink to Red…………………………..2

Root nodules were counted and weighed using digital electric weighing balance. Using a sharp scalpel the nodule was carefully cut open and the colour of the nodules was determined by visual observation of the colour on the cut surface and using the method

adopted by Rebah *et al*. 2002 the Nodule Index was calculated. Nodules diameter was measured using a millimeter rule. Radicle development and nitrogen assimilation was assessed by counting the number of nodules, pods and determining the dry weight accumulation of the roots using oven dry weight method.

4.2.0 Method of Isolation and identification of *Rhizobium* strains in nodules

The root systems of cowpea were washed carefully under gentle running water. Nodules on the tap root of each plant for each treatment were cautiously detached for the isolation of the bacterium. The nodules were then put in sterile distilled water containing a little clean acid-washed sand in McCartney tubes and the tubes vigorously shaken to remove gross surface contamination. The nodules were subsequently surface-sterilised for five minutes in 0.1% mercuric chloride (3% W/V hydrogen peroxide) and repeatedly rinsed in 6 changes of sterile distilled water. They were further put in 70% ethanol for 3 minutes and finally rinsed with sterile distilled water. The nodules were then crushed with a sterile glass rod in a few drops of sterile distilled water in sterile McCartney tubes. Six serial dilutions of 1/10, 1/100, 1/1000, 1/10000, 1/100000 and 1/1000000 (10^{-1} to 10^{-6} dilutions) of the suspension were prepared. Dilution of 10^{-4} to 10^{-6} were streaked on Petri plates of Congo red yeast-manitol agar medium and incubated at 30ºC for five days. At the end of the incubation period, isolated colonies of the *Rhizobium* species were sub-cultured on YMA slants in McCartney tubes. The tubes with pure cultures were filled with sterile liquid paraffin to completely submerge the slants and stored in the refrigerator 4ºC for further used.

4.2.1 Determination of physic-chemical and heavy metal composition of soil and SMC before use as a bio-fertilizer

In assessing the amount of nitrogen in the soil and SMC and the subsequent assimilation of these nutrients by the cowpea plants the method described by Hesse (1972) was adopted. One gram of air-dry soil sample (W) was placed in 300ml Kjeldahl flask and moistened with a few drops of distilled water. Three grammes of a catalyst mixture of $CuSO_4$ and K_2SO_4 was added to the sample. Subsequently, about 10ml of concentrated H_2SO_4 was added to the mixture and the tube was then placed in a digester at 380°C for 2.5hrs until digest was clarified. The digest was allowed to cool and the volume made up to 100ml with distilled water in a volumetric flask. Amount of 5ml each diluted digest were pipetted into a distillation set up (Kjeldahl apparatus) and 50% of sodium hydroxide was added. The entire mixture was distilled and the distillate collected in 4ml of 2% Boric acid-indicator solution (20g Boric acid crystals was dissolved in 900ml hot water). The mixture was allowed to cool and a mixed indicator solution prepared by dissolving 0.1g bromocresol green and 0.07g methyl red in 100ml 95% ethanol was added to the solution until a colour change from steel blue (green) to pink. The distillate was then titrated against 0.1N H_2SO_4. The whole process was repeated using 2.0g sugar cane in place of soil to correct for any nitrogen compound present in the reagents (a reagent blank determination). Total nitrogen content in soil sample was determined from the equation below:

$$\% \text{ Nitrogen} = \frac{\text{Meq.Of acid x Meg of N x Vol.of extract}}{\text{Weight of sample x Volume of diluted digest}} \text{ x } 100$$

Where; Meq. (One-thousandth of an equivalent)

Meq. of acid = Normality of acid x Titre volume

Normality of acid = 0.01

Meq. of Normality = 0.014

Vol. of extract = 50ml

4.2.2 EXPERIMENTAL PRECAUTIONS

Glassware were cleaned and dried thoroughly before autoclaving or sterilizing for use.

Dried filter papers were kept in desiccators immediately they were removed from the oven to prevent the reabsorption of moisture from the environment.

The filter papers were kept in an oven at a temperature of 70ºC in order to avoid burning of the filter papers and the mycelia.

Petri dishes used in these experiments were packed in canisters for sterilization in an oven at a temperature of 151ºC for a period of 24 hours.

Erlenmeyer flasks used in the experiments were covered with non-absorbent cotton wool plug.

Inoculation needles (pins), cork-borer and forceps were kept in ethanol and after that, were sterilized by flaming them over ethanol glass burners until they appeared red hot.

All working areas were wiped with 70% absolute alcohol before use.

The opening edge of medicinal bottles and Erlenmeyer flasks containing PDA and PDB respectively were flamed sterilized before each inoculation was done.

The laminar flow cabinet was switched on for about 15 minutes before use.

The inoculation chamber was also sterilized using 70% absolute alcohol to wipe the inside.

In Atomic Absorption Spectrometer (AAS) analysis it was ensured that there was uniformity in the flame while standards, blanks and repeats were done.

CHAPTER THREE

EXPERIMENTAL PROCEDURE

EXPERIMENT A. Fungal population and phenology profiles of different agricultural lignocellulose waste used in the cultivation of *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31

Composting is a process of microbial activity and chemical reactions within a substrate used in the cultivation of mushrooms. There are indicators which show the substrate may contain undesirable resident microorganisms. The compost would eventually turn into a desirable stable medium for growth of the mushroom and not the competing microorganisms which may be antagonistic. At different stages of composting, different groups of organisms dominate (Hayes, 1977). A rich mixture of organic materials is converted into a stable medium which is suitable for growth of a particular mushroom but not for the competing microorganisms (Oei, 1991). During the composting process, the phenology (appearance and disappearance) of microorganisms including bacteria, actinomycetes, fungi, protozoa etc. is different at different stages. The initial microflora may be mesophilic and utilize the soluble organic carbohydrates and nitrogen. This is followed by the increased growth of more heat-tolerant organisms and the release of carbon dioxide, ammonia and a considerable amount of heat. At a latter stage of composting, the temperature is higher and thermophilic organisms become dominant. Thermophilic microorganisms are present throughout the compost and are mainly responsible for the second stage of fermentation following spawning.

The series of experiments in this section were aimed at providing information on the fungal population and succession in the raw and fermenting compost of rice straw, rice husk, bran and their amendments over a period of 12 days. The appearance and disappearance of fungal species was followed using the decimal serial dilution technique (Materials and General Methods Section). The population of fungi was calculated as $\log_{10}$ CFU/g sample and fungi encountered on media (Cooke's medium and DRBC) were recorded. Results are presented in Figs 3 &4; 7&8; 11&12; and 15&16

EXPERIMENT B. Influence of pH on radial and vegetative growth of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on potato dextrose agar at 30±2˚C for 10 days.

The optimum pH and temperature for cultivation of mushrooms differ from one climatic condition to another and is influenced also by other relevant eco-physiological conditions. In Ghana the optimal pH for maximum yield of *P. ostreatus* ranged between pH 5.5 to 6.5 (Narh *et al.* 2011) but this may vary with different strains of *P. ostreatus*. The optimum pH for growth of *P. eous* in Ghana has otherwise not been determined.

In Ghana the optimum growth of *P. ostreatus* was found to be at 30˚C (Obodai *et al*. 2010) whereas Uddin *et al* (2011) showed that *P. eous* (Berk) Sacc. prefers temperature range from 21-35˚C. There is no information in the pertinent literature on the best temperature for growth of *P. eous* in Ghana. In this section B of Chapter 3 pH range of 5.6 to 9.0 was used to ascertain the growth of mycelium of *P. ostreatus* EM-1 *P. eous* P-31 at 28-32˚C in Ghana. The method of preparation of media and measurement of radial growth on agar medium has been described in the Materials and General Methods Section.

In Experiment B of the Results Section (Chapter 4) the influence of pH on radial growth of *P. ostreatus* EM-1 and *P. eous* P-31 in liquid and solid medium was investigated. Throughout the study of the yield and Biological Efficiency of the differently formulated substrates for cultivation of these two *Pleurotus* species pH of substrate varied from pH 5.6-7.8 (Tables 5 to 8). Results obtained are presented in Tables 5 to 8.

EXPERIMENT C. Radial growth of *P. ostreatus* EM-1 and *P. eous* P-31 on Potato Dextrose Agar amended with the cultural filtrate of three resident contaminating fungi at 28 - 30°C for 12 days

Antibiosis is the suppression of growth of one microorganism by the culture filtrate of another organism under the prevailing conditions. The phenomenon of antibiosis can be found in mushroom composts contaminated by competing saprophytic fungi. The Experiments in Chapter A showed that there were several mycoflora resident in the substrate during the composting period. They were predominated by *Aspergillus flavus, Penicillium citrinum* and *Trichoderma harzianum*. In this Chapter, the influence of these cultural metabolites contaminating saprophytic fungi on the growth of the mycelium of *Pleurotus* species on agar was tested *in vitro*.

The basal medium (Potato Dextrose Agar) was amended with varying concentrations (1:1-1:10 v/v dilutions) of the culture filtrate of *A. flavus*, *P. citrinum* and *T. harzianum* and the medium inoculated at the centre of the plate at the transect of two diameter at right angles drawn at the bottom of the petri dishes. One set of plates (in triplicates) ranging from 1:1-1:10v/v dilutions were inoculated with 3mm discs of either *P. ostreatus* EM-1 or *P. eous* P-31. Growth of culture along two diameters was measured at 3 days intervals for 12days. Results obtained are presented in Tables 9 to 14.

EXPERIMENT D. Vegetative growth of *P. ostreatus* EM-1 and *P. eous* P-31 in liquid culture (Potato Dextrose Broth) amended with different concentrations of the culture filtrates of three resident contaminating fungi at 28 - 30˚C for 10 days

There are instances in which growth of a fungus on agar was different from what existed in liquid medium owing to differences in aeration and osmotic differences created by the nature of the medium. The antibiosis of the test fungi against radial growth of the two *Pleurotus* species varied with the type of fungus whose metabolites were used as antagonist. Would the antagonism between the three contaminating fungal species namely *A. flavus, P. citrinum* and *T. harzianum* differ in a submerged liquid medium?

The experimental set up was exactly the same as in Chapter E except that no agar was added to the amended Potato Dextrose Broth (1:1-1:10v/v dilutions) and 30ml of each amended PDB of the varying dilutions of the cultural filtrates of the test fungi (*A. flavus, P. citrinum* and *T. harzianum*) was poured into 250ml Erlenmeyer flasks as outlined in the Materials and General Methods Section. The flasks were plugged with cotton wool before sterilization and then inoculated with 3mm discs of the two *Pleurotu*s mushrooms after cooling. The dry matter accumulation over 12days at 28-30˚C was assessed by the oven dry weight method (Materials and General Methods Section). Results obtained are summarised in Tables 15 to 20.

EXPERIMENT E. Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on differently formulated substrates composted for varying periods of 0-12days at 28±2°C

The method of formulation and composting of 7 differently formulated rice straw based substrates and wawa sawdust are spelt out in the Materials and Methods Section. Each formulated substrate was studied in a separate Experiment. There were 7 Experiments. The surface mycelial density, total no. of fruit bodies, total no. of pinheads, total yield and Biological Efficiency, dimension of stipe and cap of the fruit body were recorded as well as correlation between these and BE Tables 21 to 70 and Figs 19 to 37 show results obtained.

EXPERIMENT 1

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw only composted for different periods

The rice straw only as a growth substrate was tried to ascertain its efficiency in supporting vegetative growth and yield of the two species of *Pleurotus*. The unamended rice straw 10kg was steeped in 432 litre of tap water in a plastic barrel for 3h (see Materials and General Methods section). The straw was removed strained and allowed to settle for 30mins to remove excess water and stacked into a heap to undergo fermentation. The dimension of the heaped compost was 48 x 76 x 69cm and was turned every 4 days for aeration and uniform fermentation. At the end of the appropriate composting periods of 0 day (uncomposted), 4, 8 and 12 days respectively. The rice straw was bagged into 10 replicates and steam-sterilised for 3h (Materials and General Methods section) cooled at room temperature (28-32°C) and

were inoculated with 3.5g of sterilised sorghum grain spawn of either *P. ostreatus* EM-1 or *P. eous* P-31 (Materials and General Methods). The bags (containing substrate composted for different periods) were incubated at 28-32°C for 34 days in an incubation chamber. The following data were collected:

i) The spawn run period (number of days from inoculation to complete colonization of the substrate by mycelium).

ii) Mycelial growth rates at different composting periods and pH of substrate.

iii) Total yield and Biological Efficiency / Flush.

iv) Correlation of stipe length and pileus width of the mushrooms to their Biological Efficiencies. Results obtained are present in Tables 21 to 29.

EXPERIMENT 2

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods

The procedure in Experiment 1 was repeated. This time, the rice straw was amended with 1% $CaCO_3$ and 10% rice bran and composted for 0, 4, 8 and 12 days before inoculation with 3.5g of sterilised sorghum grain spawn of the two *Pleurotus* species. The methods are spelt out in the Materials and General Methods Section. Again there were 10 replicates for each treatment and the bags containing amended substrates composted for different period 0 (uncomposted), 4, 8 and 12 days were incubated at 28-32°C for 34 days in an incubation chamber. The following data were collected:

i) The spawn run period (number of days from inoculation to complete colonization of the substrate by mycelium).

ii) Mycelial growth rates at different composting periods and pH of substrate.

iii) Total yield and Biological Efficiency / Flush.

iv) Correlation of stipe length and pileus width of the mushrooms to their Biological Efficiencies.

Results obtained are present in Tables 30 to 38.

EXPERIMENT 3

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31on rice straw amended with 1% $CaCO_3$ and 10% rice bran.

Procedures adopted for Experiment 2 were repeated. It was exactly the same as Experiment 2 except that after composting the substrate for 0, 4, 8 and 12 days the batch of composted substrates was divided into three equal batches; one batch was supplemented with 5% rice bran, another 10% rice bran and the third with 15% rice bran to augment the nitrogen source before bagging. There were 10 replicates for each treatment. The bags were inoculated with the spawn on sterile sorghum grains and incubated at 28-32°C for 34 days. To bring the pH of the substrate within the range for optimum growth of *Pleurotus* sp., 0.5% of $CaCO_3$ (lime) was added. It was conjectured that this formulation would improve the growth and Biological Conversion Efficiency of the mushroom beyond what was obtained in Experiment 2. The following data were collected:

i) The spawn run period (number of days from inoculation to complete colonization of the substrate by mycelium).

ii) Mycelial growth rates at different composting periods and pH of substrate.

iii) Total yield and Biological Efficiency / Flush.

v) Correlation of stipe length and pileus width of the mushrooms to their Biological Efficiencies. .

Results obtained are present in Tables 39 to 45.

EXPERIMENT 4

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw and rice husk amended with 1% $CaCO_3$ and 10% rice bran

In this experiment, the formulation and procedure was exactly as in Experiment 3 except that rice straw was mixed with rice husk in the ratio of 1:1w/w in addition to 1% $CaCO_3$ (lime) and 10% rice bran before composting for 0, 4, 8 and 12days. Thereafter, the only difference between Experiment 3 and 4 was the introduction of rice husk into the formulation of the substrate and at bagging 0.5% $CaCO_3$ (lime) was added to bring the pH of the substrate within the optimum range for *Pleurotus* spp growth and fructification. Would this treatment and formulation result in a better performance of the two *Pleurotus* species?

The following data were collected:

i) The spawn run period (number of days from inoculation to complete colonization of the substrate by mycelium).

ii) Mycelial growth rates at different composting periods and pH of substrate.

iii) Total yield and Biological Efficiency / Flush.

Correlation of stipe length and pileus width of the mushrooms to their Biological Efficiencies. Results obtained are present in Tables 46 to 52.

EXPERIMENT 5

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on Engelberg Mixture (rice husk and rice bran 2:1w/w)

The Engelberg mixture is a combination of rice husk and rice bran in the ratio 2:1 w/w. There was no supplementation of the substrate. The preparation of this mixture is spelled out in the Materials and Methods Section. The substrate was composted in the manner described in the Materials and Methods section and thereafter sealed with spawn on the sterile sorghum seed in polypropylene bags the set up was left at 28±2˚C to grow and fructify for 34 days. The following data were collected to compare with the other treatment described in Experiments 1 - 4:

i) The spawn run period (no of days from inoculation to complete colonization of substrate by the mycelium.)

ii) Mycelial growth rates at different composting periods and pH of substrates

iii) Total yield and Biological Efficiency/ Flush.

Results obtained are summarized in Tables 53 to 58.

EXPERIMENT 6

Growth and Biological Efficiency of *P. ostreatus* strain EM1 and *P. eous* strain P-31 on Engelberg mixture amended with 1% $CaCO_3$

The rationale of this Experiment was to ascertain the effect of Engelberg substrate supplementation on the yield and other growth parameters of the two *Pleurotus* species.

The method adopted was same as in Experiment 5 with the exception of the added supplements (1% $CaCO_3$ and 10% rice bran).

The incubation and growth condition were the same as the previous five experiments. Data collected were as follows:

i. The spawn run period (no. of days from inoculation to complete colonization of substrates by the mycelium.
ii. Mycelial growth rates at different composting periods and pH of substrate.
iii. Total yield and Biological Efficiency /Flush.

Results obtained are summarized in Tables 59 to 64.

EXPERIMENT 7

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran

'Wawa' (*Triplochiton scleroxylon*) sawdust and its amendments have been used for a considerable length of time for the cultivation of *Pleurotus* species in Ghana by the Mushroom Farmers Cultivation Association and there is documented evidence of its efficiency although it has to be composted for over 28 days for optimal utilization by the

mushrooms (Obodai, 1992; Obodai *et al*. 2010). The objective of this Experiment was to compare the conventional substrate used for the cultivation of *Pleurotus* species with the new formulation used in Experiment 2. The essential difference in this instance was the replacement of rice straw with wawa sawdust (see Expt. 2). The substrate was composted for a shorter period of 0, 4, 8 and 12 days before seeding with spawn of the test oyster mushrooms. The incubation and growth condition were the same as the previous five experiments. Data collected were as follows:

i. The spawn run period (no. of days from inoculation to complete colonization of substrates by the mycelium.
ii. Mycelial growth rates at different composting periods and pH of substrate.
iii. Total yield and Biological Efficiency /Flush.
iv. Correlation of stipe length and pileus width to their Biological Efficiencies.

Results obtained are summarized in Table 65 to 71.

EXPERIMENT F. Chemical, nutritional and mineral composition of the different lignocellulose agro-wastes and the fruiting bodies of *P. ostreatus* strain EM-1 and *P. eous* strain P-31.

Proximate analyses of different substrates and the fruiting bodies and their mineral contents obtained with the best substrate combinations and composting period are presented in Tables 78 for *P. ostreatus* EM-1 and Table 79 for *P. eous*. Total mineral content (Ca, Cu, Fe, K, Mg, Mn, Na, P, Pb and Zn) obtained in the fruiting bodies growing on the best substrates composition and composting period are summarized in Table 77 for *P. ostreatus* and Table 76 for *P. eous*. The method of determination of these chemical parameters of the substrates and fruit bodies is spelled out in the Material and General Method Section.

EXPERIMENT G: Preliminary molecular characterisation of *P. ostreatus* EM-1 and *P. eous* P-31 using PCR Amplification and restriction digestion techniques

There were clear differences in the performance of the two *Pleurotus* species used in the bioconversion on different formulation and supplementation of the compost from rice lignocellulose in the studies reported in this thesis. However, species identification within the genus *Pleurotus* is difficult because of the morphological and possible environmental effects (OECD, 2005). Mating compatibility studies have demonstrated the existence of discrete intersterility groups in *Pleurotus* to distinguish species from the other.

In the concluding Experiment of this thesis an attempt was made to ascertain and distinguish between the two *Pleurotus* species using PCR Amplification and Restrictive Digestion Techniques. The method employed is spelled out in the Materials and General Methods Section. Results obtained are shown in Figs 41a and 41b.

EXPERIMENT H. Studies on mycoflora and some physicochemical, elemental and nutrient content of soil and Spent Mushroom Compost

The nutrient status of any soil and fertilizer for improvement of growth and yield of crops in the field is key to its usefulness in amended soil for cultivation of crops. In this experiment the presence and quantities of Zn, Cu, Mn, Pb, Ca, Fe, Mg, Na, P, K and N were determined Table 81. The methods used are spelled out in the Materials and General Methods Section. The pH of the substrates was also determined with the view to ascertaining suitability for the cultivation of crops as well as mushroom as it is known that spent mushroom compost can be used further for cultivation of other mushrooms. Rhizosphere mycoflora influence growth

and development of field economic crops like pepper, tomato, onion, cowpea etc. The role of resident mycoflora was also determined on Cooke's medium and DRBC using the conventional decimal serial dilution technique. Results obtained are presented in Tables 81, 82 and Fig 42 & 43.

EXPERIMENT I. Influence of the Spent Mushroom Compost, SMC of *P. eous* strain P-31 used as bio-fertilizer on the growth and development of tomato (*Lycopersicon esculentum* Mill.) seedling under greenhouse conditions

There are references in the pertinent literature on the use of Spent Mushroom Compost, (SMC) to improve yield of some economic crops including broccoli (*Brassica leracea* L. var *italica*) and kale (*Brassica oleracea* L. var. *acephalae* D.c. cv. Temei) (Peksen and Uzun, 2008), black pepper (*Piper nigrum*) (Önal and Topcuoglu, Undated), Cucumber (*Cucumis sativa* cv. Super dominos) (Gonani *et al*. 2011) and maize (*Zea mays* L.) (Ogbonna *et al*. 2012). Maher *et al*. (2000) have previously written about management of spent mushroom compost in crop production of tomato (*Lycopersicon esculentum*), lettuce (*Cucumis sativa*), and potatoes (*Solanum tuberosum*) to mention but a few. They reiterated that an education awareness campaign should be conducted amongst farmers, in areas removed and near to mushroom production, to introduce them to the benefits of SMC and ways to effectively utilize this material. The economic value of SMC to crop improvement when used as bio-fertilizers has not been tried in Africa not excepting Ghana. SMC have been discarded as refuse and this study was to show value of SMC from the cultivation of *Pleurotus* on crop improvement using tomato, pepper and cowpea as test economic crops.

In this Chapter, SMC from *Pleurotus* cultivation on rice straw, husk and bran was tested for its ability to improve germination, growth and yield of tomato (*Lycopersicon esculentum* Mill.) The methods used in the formulation of the soil: compost mixtures in varying proportions (0, 5, 10, 15, 20, 25, 30 and 100% (SMC only) w/w basis) have been spelled out in the Materials and General Methods Section. The Complete Randomised Block Design with five replicates was designed to obtain the following results: % Germination; Average plant height; average leaf area, No. of leaves, chlorophyll content, no. of floral buds; no. of flowers, no. of fruits, average weight of fruits, stem girth of the seedlings; dry weight of shoots and dry weight of roots. Results obtained are presented in Table 83 and Figs 44A-D (Appendix 1).

EXPERIMENT J. Influence of the Spent Mushroom Compost, SMC used as bio-fertilizer on the growth and development of pepper (*Capsicum annum* L.) seedling under greenhouse conditions

The Experimental set up was exactly as what obtained in Experiment G except that pepper was used as test plant. Results in Experiment G show that low proportion mixture of soil: SMC improve growth and yield of tomato. Would the SMC also serve as a good bio-fertilizer for pepper?

The same criteria were used in assessing the efficacy of SMC in improving plant productivity was used i.e. Percentage germination of seeds, average plant, average leaf area, average no. of leaves produced, chlorophyll content of leaves, no. of floral buds, no. of flowers formed, no. of fruit setting, weight of fruit produced, stem girth of the seedlings, dry matter accumulation by shoot and root. The plants were kept in the greenhouse at 31±2°C for

91 days before assessing the criteria listed above. Results obtained are presented in Table 84, Figs 45A-D and Plates 17 & 18.

EXPERIMENT K. Influence of the Spent Mushroom Compost, (SMC) used as bio-fertilizer on the growth and development of Cowpea (*Vigna unguiculata* Walp.) seedling under greenhouse conditions

The economic crops used in the previous two Experiments G and H were vegetables (tomato and pepper) in demand in Ghana. Their productivity was ($p<0.05$) significantly improved by the spent mushroom compost. In this Experiment I a popular legume, Cowpea (*Vigna unguiculata* walp.) was the test plant. The method of preparation of mixture of soil: SMC was the same as in Experiment G and H and the Randomised Block Design with five replicates was employed. Results obtained are summarised in Table 85 and Figs 46A-D (Appendix 3).

EXPERIMENT L. Assessment of cowpea seed germination and Nodule Index of nodules formed in soil amended with varying proportions (%) of spent mushroom compost SMC, under greenhouse conditions

The presence of nodulating bacteria in the soil is the key to successful nitrogen fixation in the nodules of legumes in the field. The role of rhizobium in the nodulation and successful fruit setting of cowpea is well-known in the literature.

In this Experiment the cowpea seedlings growing in the varying composition of soil: SMC mixtures in pots (soil only, 0%, 5, 10, 15, 20, 25, 30% SMC and only (100% SMC) were assessed using the criteria of: mean no. of nodules / plants, mean weight of nodules / plant, mean diameter of nodules / plant, mean radicle length / plant and intensity of the colour of the leghaemoglobin in the nodules (see Materials and General Methods). Results obtained are shown in Tables 87 & 88.

CHAPTER FOUR

RESULTS

EXPERIMENT A.

Fungal population and phenology profiles of the different agricultural lignocellulose wastes used in the cultivation of *Pleurotus ostreatus* strain EM-1 and *P. eous* strain P-31.

i) Rice straw only

The initial fungal population in the unfermented rice straw only (2.5 $\log_{10}$ CFU/g) was the same irrespective of whether samples were taken from the top (5-10cm), middle (15-20cm) or bottom (25-30cm) of the pile. However population of fungi increased by 2-3 log cycle after 4-8 days composting and thereafter remained stable at least in species plated on Cooke's medium (Fig. 3). There was variation in the mycoflora population plated on DRBC medium as the initial population recorded (4.25 $\log_{10}$ CFU/g) was about 1.8 log cycles lower than what was recorded on Cooke's medium (Fig. 4); but remained the same at the top, middle or bottom of the pile (Fig. 4). Population of fungi increased by less by 1.0 log cycle after 4-8 days and approximated what was obtained on Cooke's medium (Fig. 3). The use of two media enables a wide range of fungal species to be encountered and isolated from the compost (Figs 3 and 4). There was higher species diversity after 4-8 days composting. Six *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. niger, A. panamensis, A. penicilloides*) predominated followed by *Penicillium* (*P. glabrum, P. citrinum*) (Fig. 5). Fungi belonging to nine genera (*Aspergillus, Fusarium, Cladosporium, Curvularia,*

Rhizopus, Rhodotorula, and *Trichoderma*) not excepting *Mycelia sterilia* were isolated from the compost in 12 days (Figs 5 and 6). The most frequently encountered fungal species were *A. flavus, Rhodotorula* sp., *A. niger*, *Trichoderma harzianum* and *P. glabrum*.

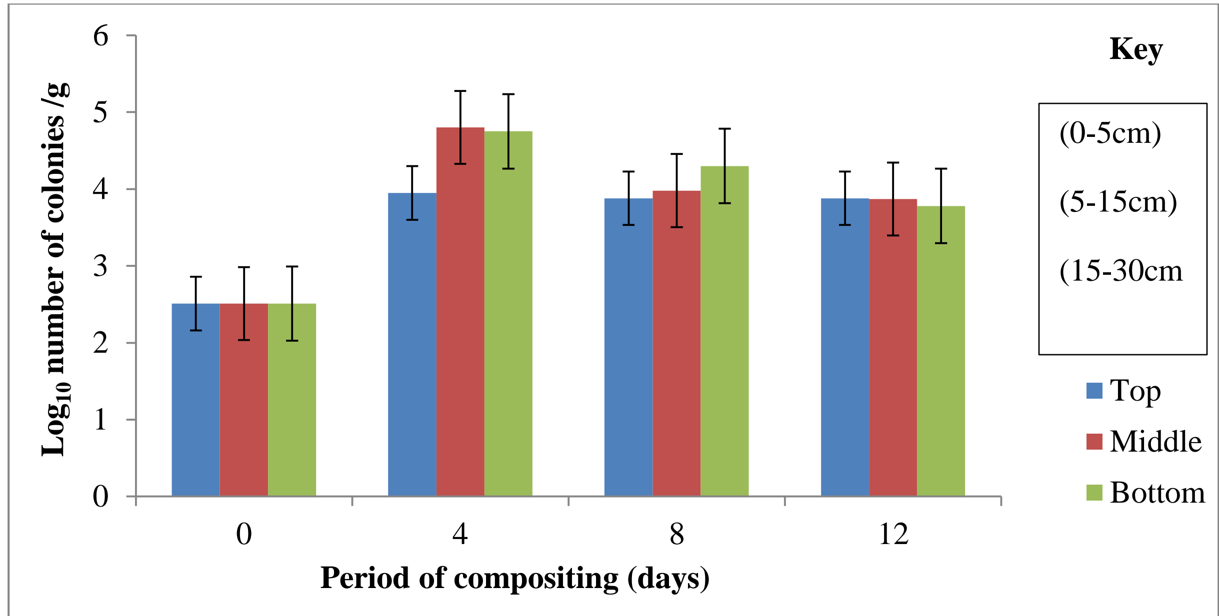


Fig 3: Profile of fungal population resident in rice straw substrate composted for varying periods at 28±2°C and plated on Cooke's medium.

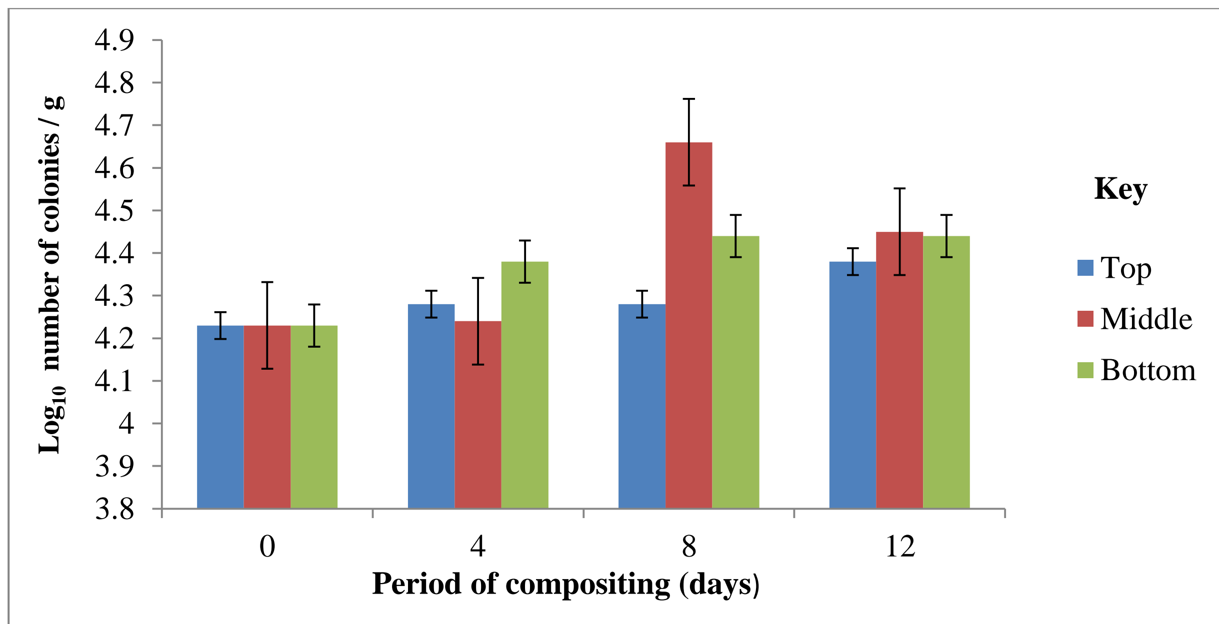


Fig 4: Profile of fungal population resident in rice straw substrate composted for varying periods at 28±2°C and plated on DRBC basal medium.

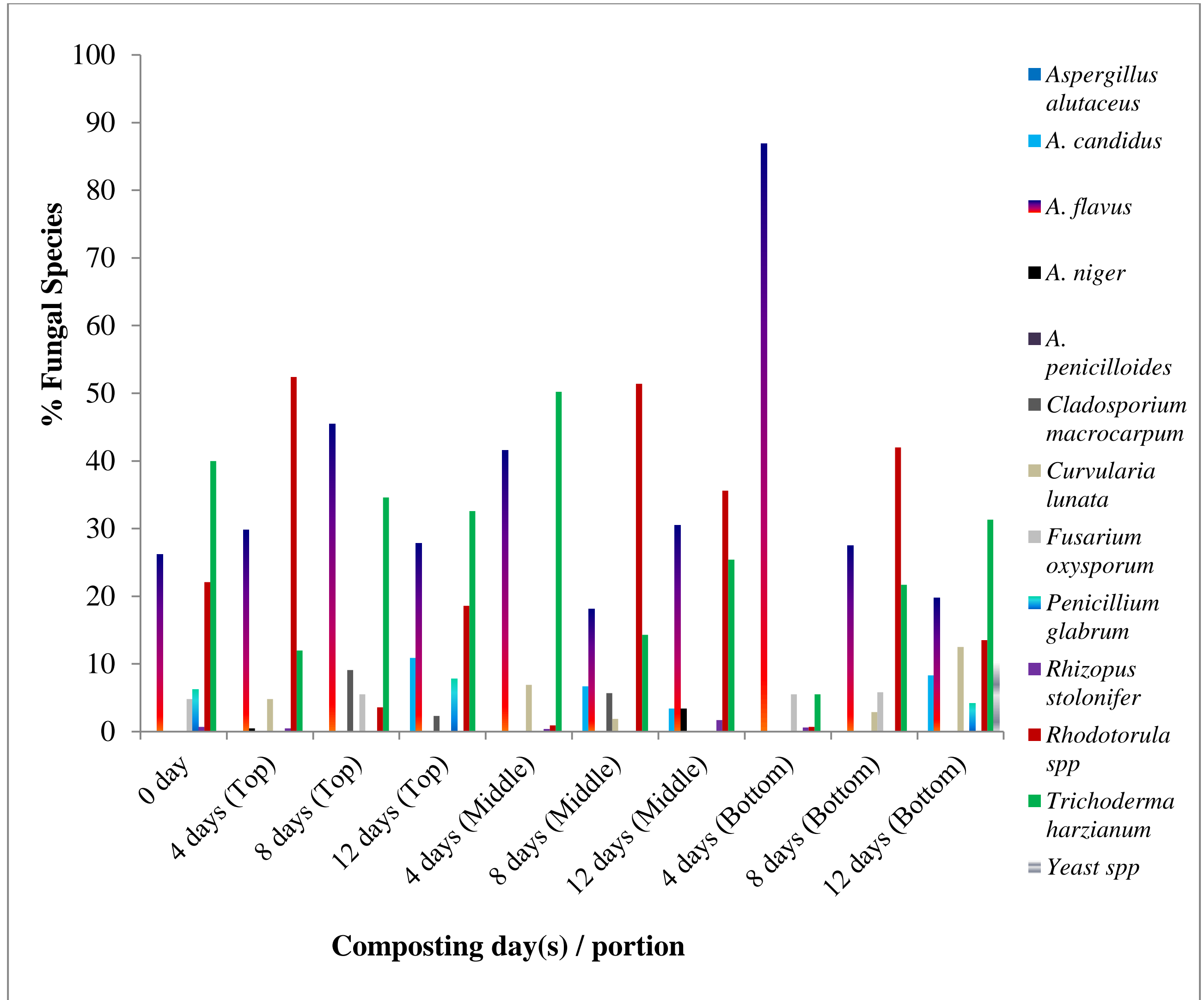


Fig 5: Percentage occurrence of some fungi resident in different fermented and unfermented rice straw only for the indicated periods and raised in Cooke's medium at 28±2°C for 7 days

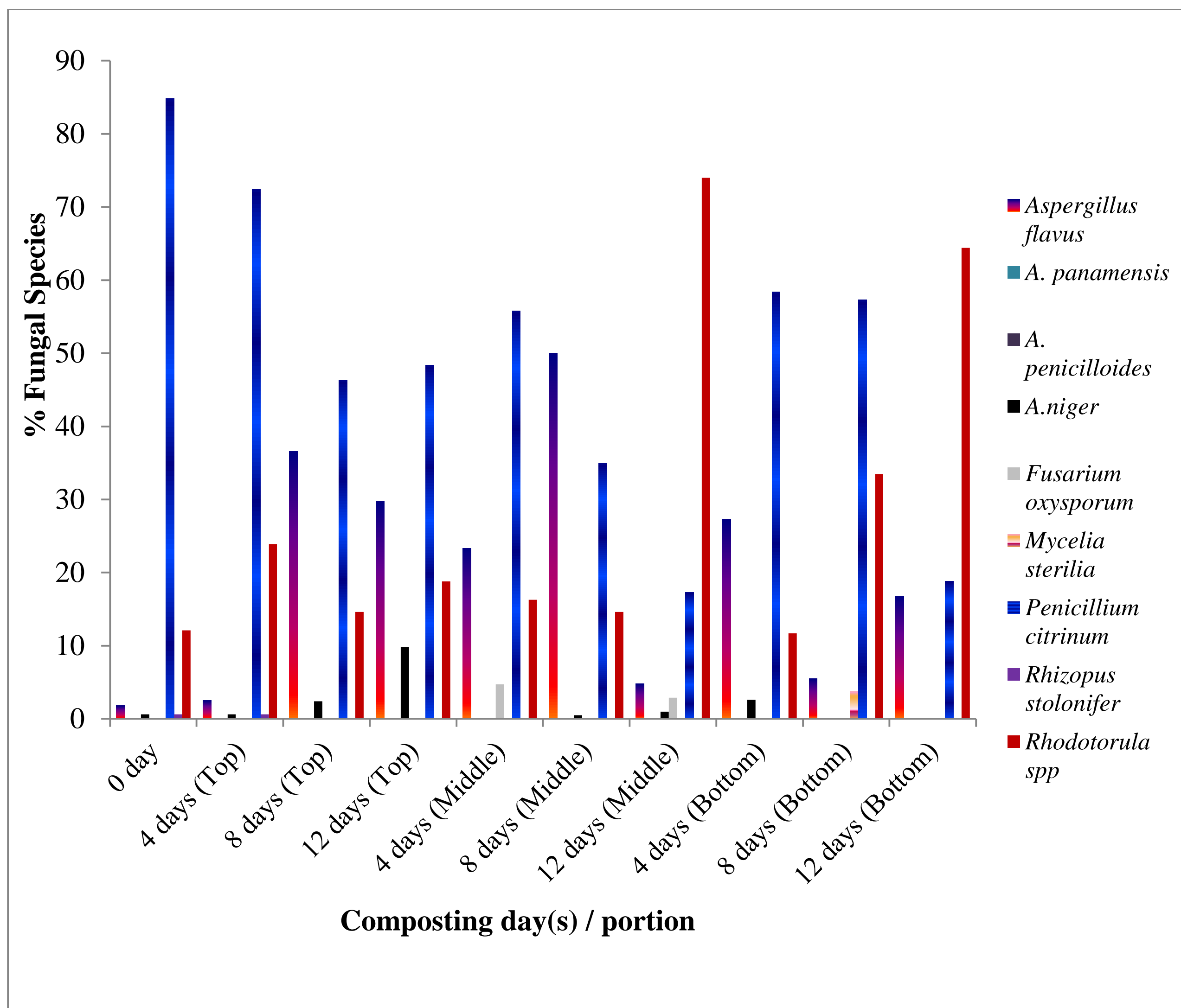


Fig 6: Percentage occurrence of some fungi resident in different unfermented and fermented rice straw only for the indicated periods and raised in DRBC medium at 28±2°C for 7 days

ii) Rice straw amended with 1% $CaCO_3$

Liming of compost only marginally increased (by 1.2 log cycles) the initial population of fungi in the rice compost (Figs 7&8). Composting for 4-8 days increased the population of fungi by 0.5-1.2 log cycles on both media used (Cook's and DRBC). There were no significant differences ($p>0.05$) between population isolated from the different depths in amended compost (Figs 7&8). The mycoflora in the rice straw amended with 1% $CaCO_3$ was predominated by six (6) *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. niger, A. panamensis*, *A. penicilloides*) constituting approximately 35-68% of the total population (Figs 9 and 10). This was followed by *Penicillium* (*P. glabrum, P. citrinum*). Generally, fungal species belonging to 11 genera (*Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizoctonia, Rhodotorula, Trichoderma, Neosartorya, Yeasts*) not excepting *Mycelia sterilia* were isolated on the two media. The most frequently encountered fungi were *A. flavus, A. niger, Trichoderma harzianum* and *Rhodotorula* (Figs 9&10). No clear cut trend in species occurrence and population of fungi were observed at the different depths as in the previous compost of rice straw only.

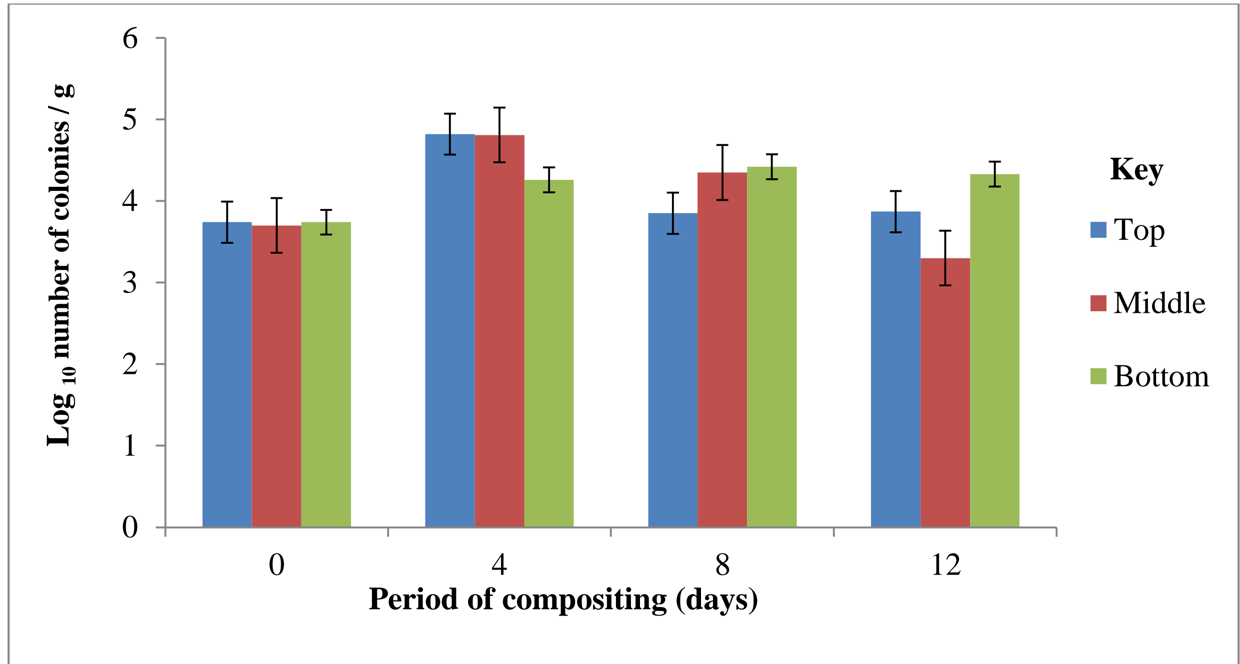


Fig. 7: Profile of fungal population resident in rice straw amended with 1% $CaCO_3$ and composted with varying periods at 28±2˚C and raised on Cooke's medium.

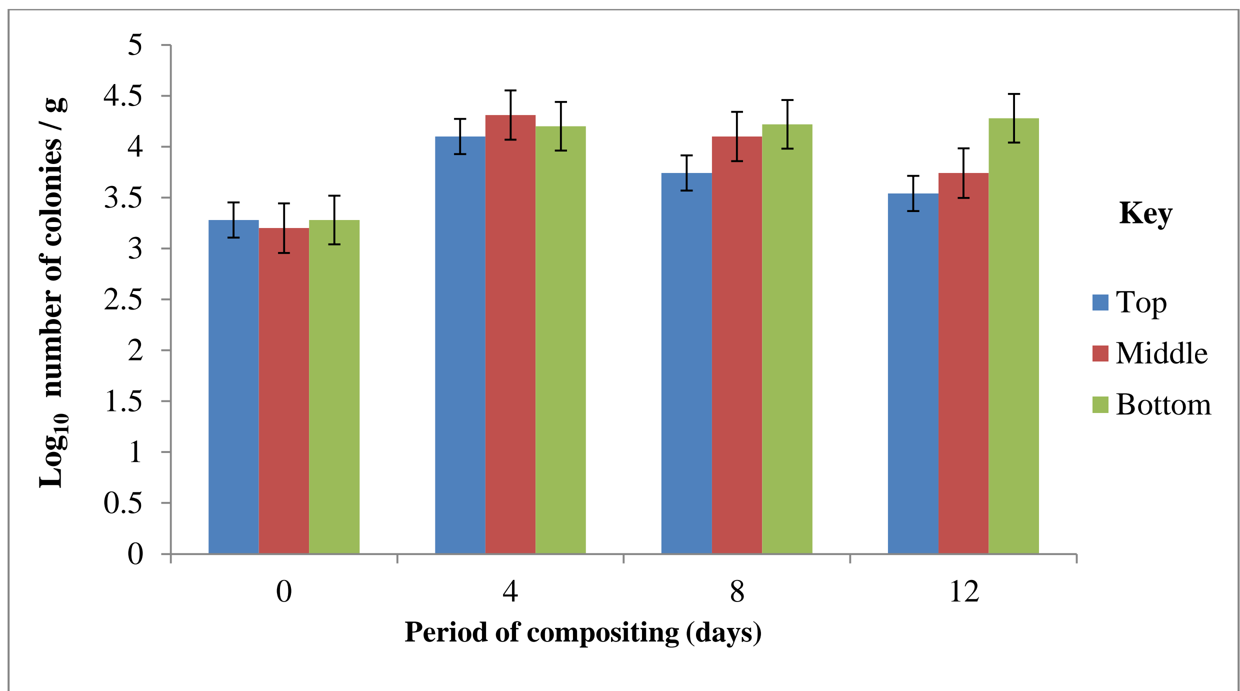


Fig. 8: Fungal population profile resident in rice straw substrate amended with 1% $CaCO_3$ composted for the indicated periods at 28±2˚C and plated on DRBC medium.

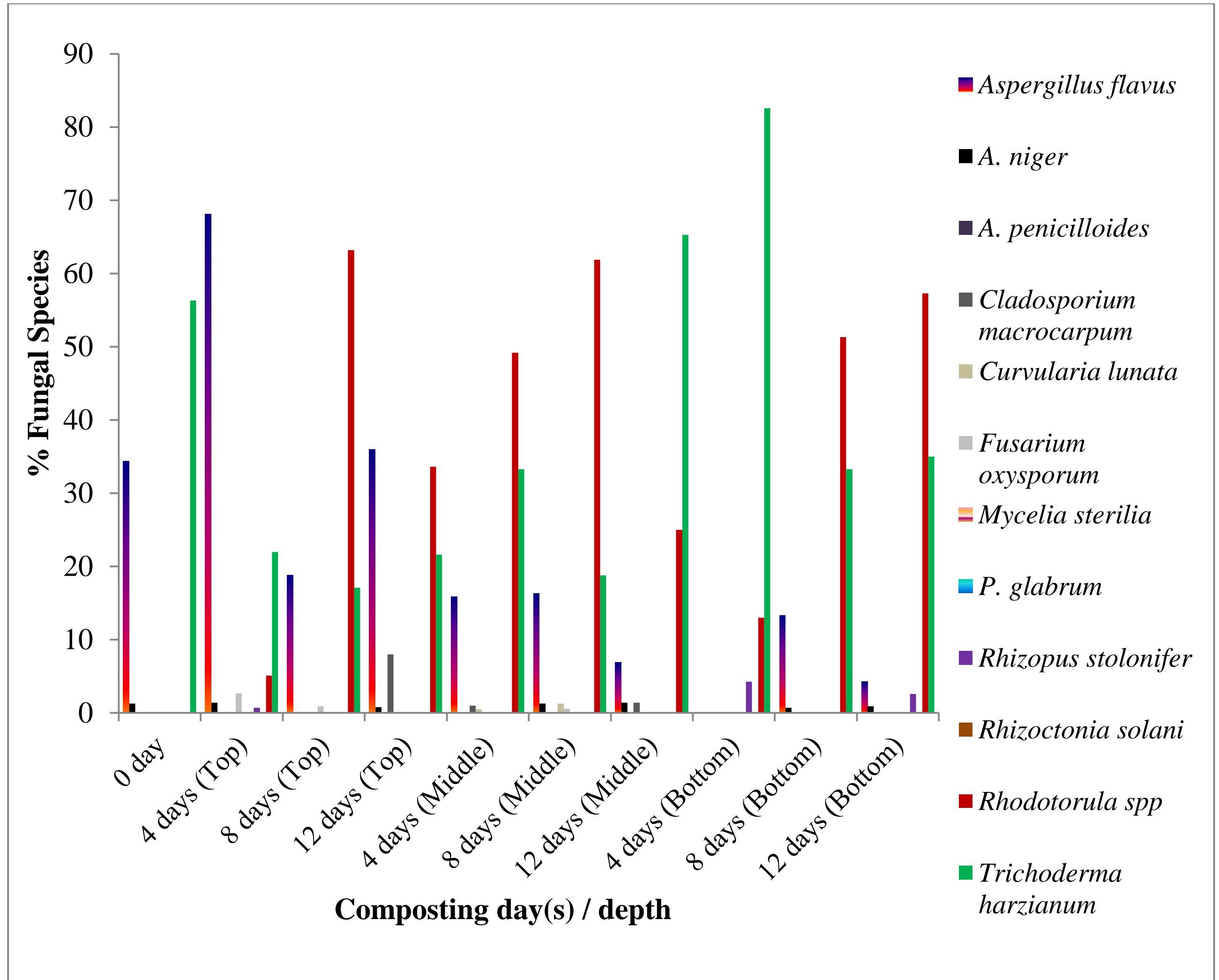


Fig 9: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw amended with 1% $CaCO_3$) and culture in Cooke's medium at 28-30˚C for 7 days

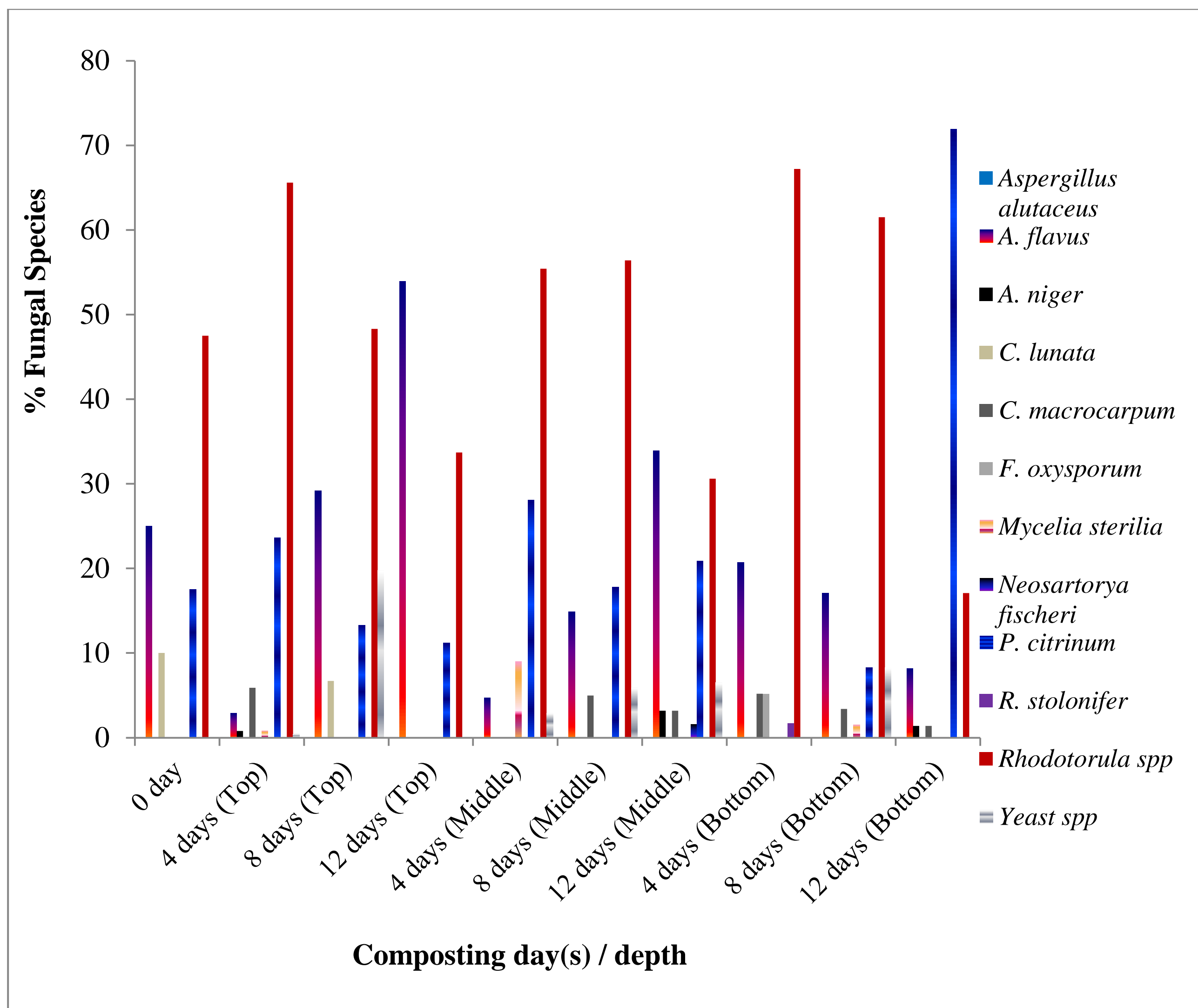


Fig 10: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw amended with 1% $CaCO_3$) and culture in DRBC mycological medium at 28-30˚C for 7 days

iii) Rice straw amended with 1% $CaCO_3$ and 10% rice bran

Amending the rice compost with 1% $CaCO_3$ and 10% rice bran marginally increased resident fungal population in the uncomposted samples by > one log cycle and there was no significant ($p>0.05$) difference in the initial population at all depths examined (Figs 11 and 12). Similar trend was obtained in the mycoflora population tested on Cooke's medium and DRBC.

During composting for up to 12 days the fungal population changed marginally (> one log cycle). Presumably the highest was after 4-8 days of composting (Figs 11&12). The marginal differences of population might be due to the turning of the compost. Again the mycoflora resident on this amended rice straw compost (with 1% $CaCO_3$ and 10% rice bran) was predominated by eight (8) *Aspergillus* species (*A. flavus, A. fumigatus, A. panamensis, A. niger, A. penicilloides, A. sulphureus, A. candidus, A. alutaceus*). This was followed *Penicillium* species (*P. glabrum, P. citrinum*). Fungal species belonging to twelve (12) genera (*Aspergillus, Cladosporium, Curvularia, Fusarium, Byssochlamys, Geotrichum, Penicillium, Rhizopus, Rhizoctonia, Rhodotorula,* and *Trichoderma*) not excepting Yeasts were encountered using the two isolating media. *Geotrichum* and *A. sulphureus* were being recorded for the first time in the rice straw amendment treatment. The lack of clear cut differences in population distribution and fungal occurrence in the three layers of the compost tested may be attributed to the turning of the heap at 4 days intervals. Again *A. flavus*, *A. niger, Rhodotorula sp., A. fumigatus* and *T. harzianum* predominated and some thermophilic species (*Byssochlamys* and *Geotrichum* were encountered for the first time.

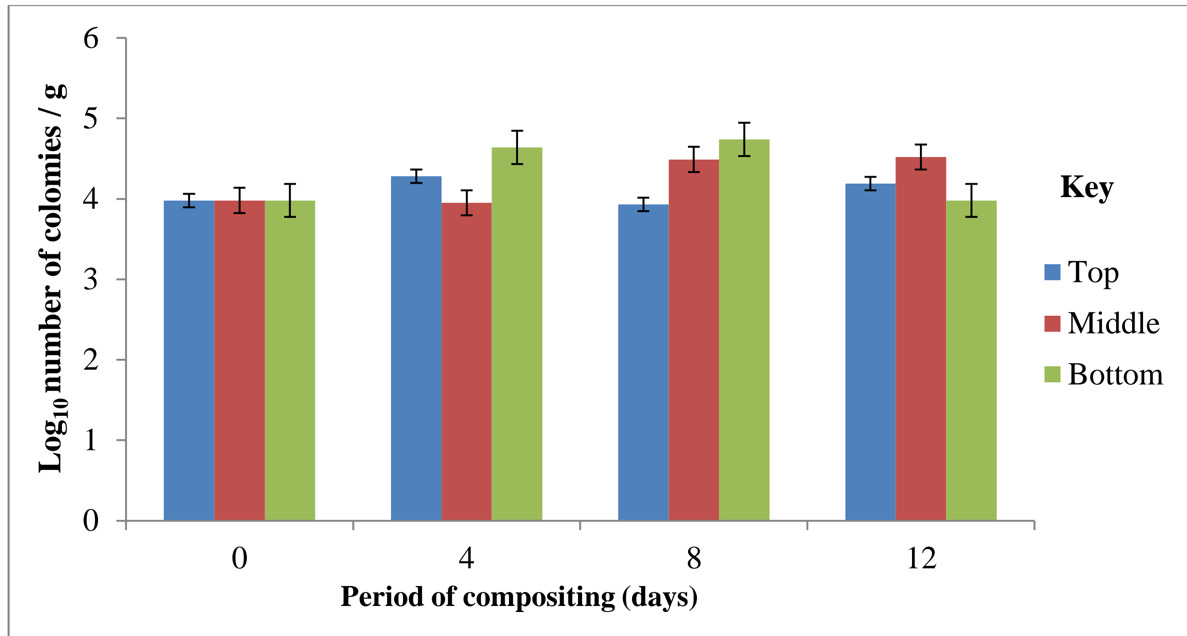


Fig 11: Fungal population profile in rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for the indicated periods at 28-30°C and plated in Cooke's medium for 7 days

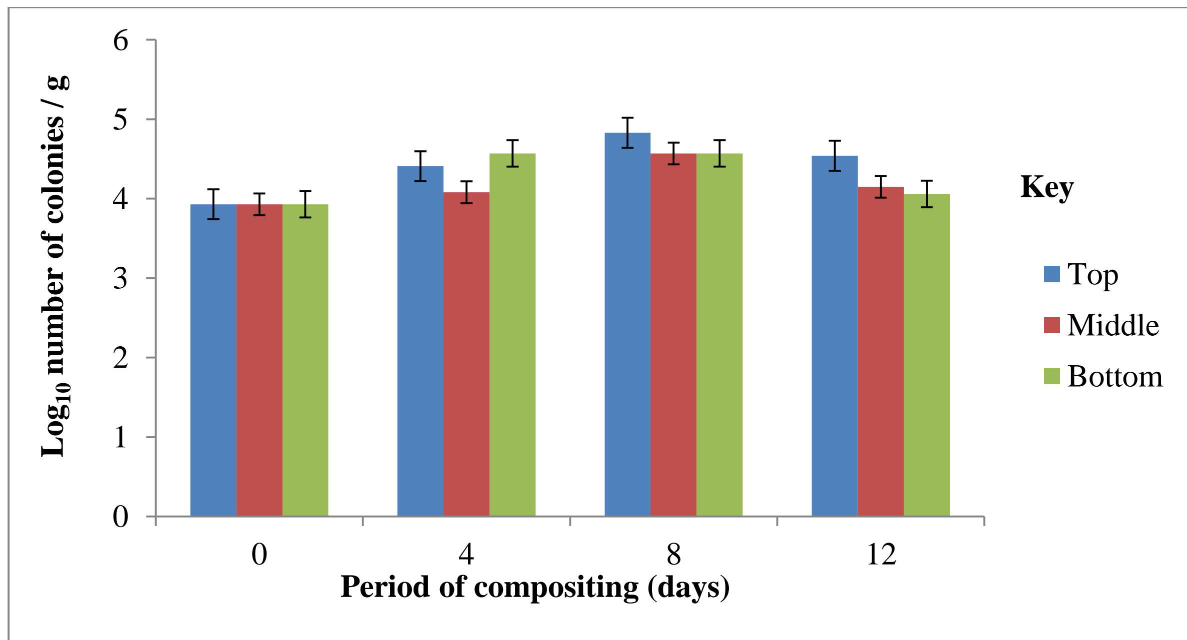


Fig 12: Fungal population profile in rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for the indicated periods at 28-30°C and plated in DRBC medium for 7 days

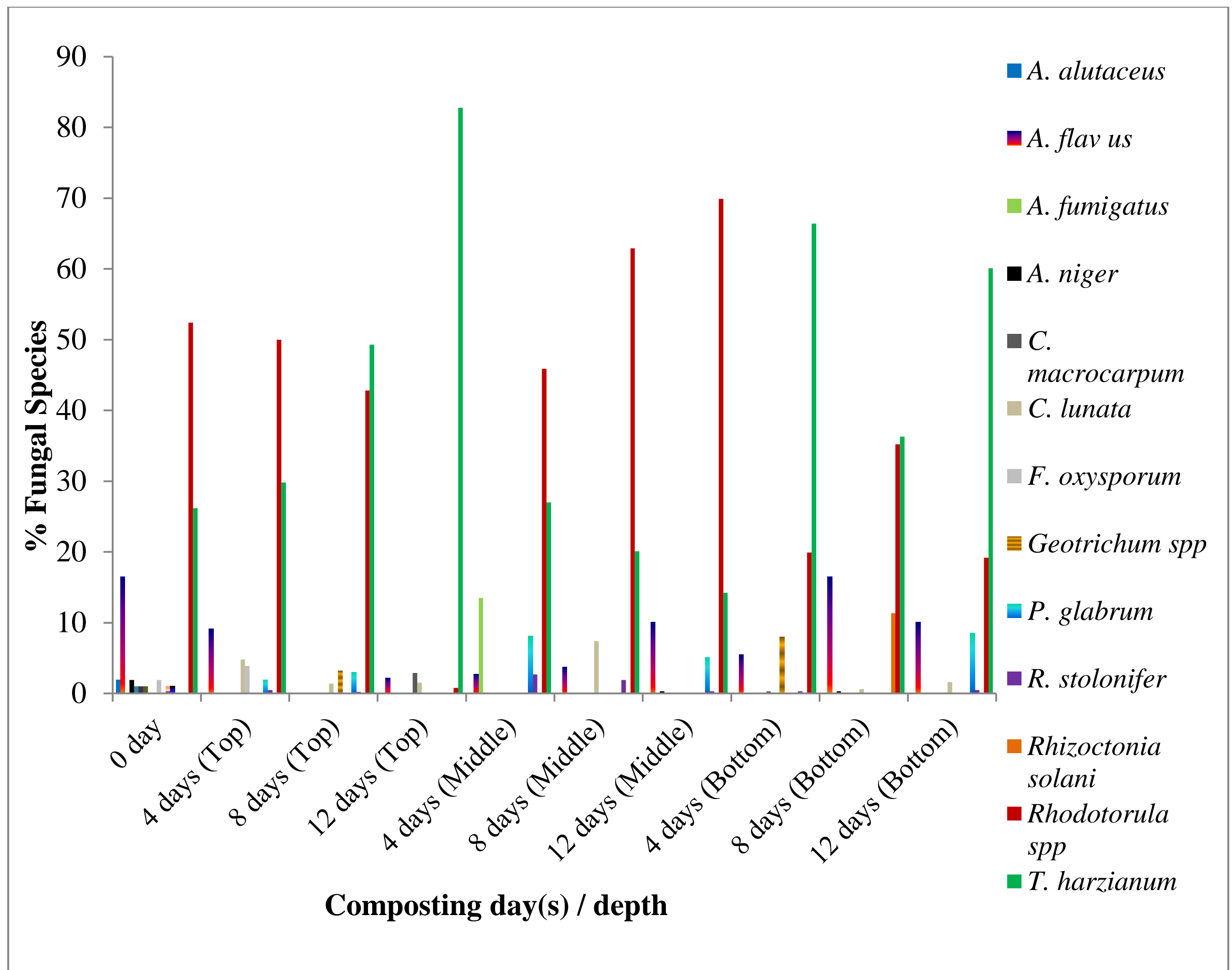


Fig 13: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw amended with 1% $CaCO_3$ and 10% rice bran) and culture in Cooke's medium at 28-30°C for 7 days

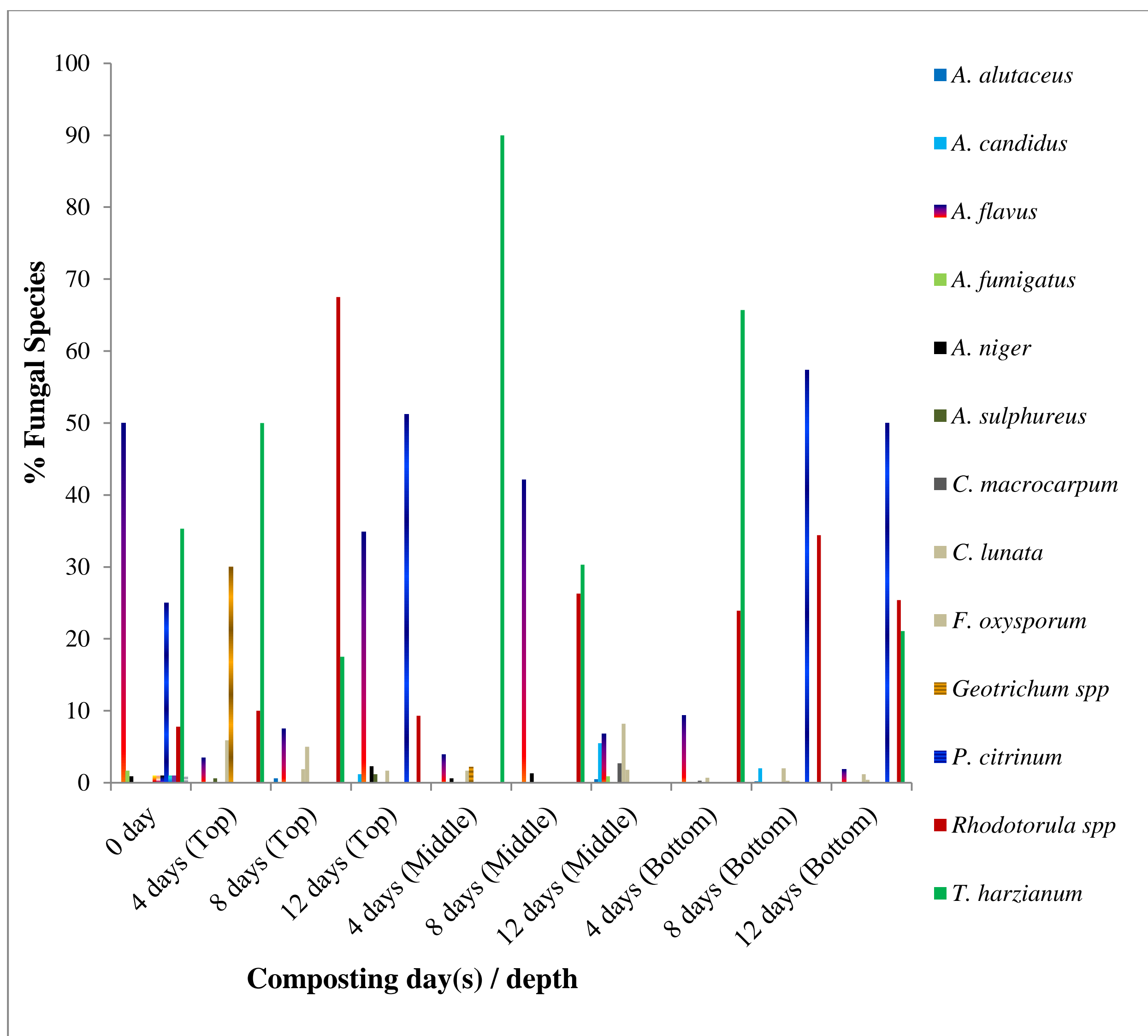


Fig 14: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw amended with 1% $CaCO_3$ and 10% rice bran) and culture in DRBC medium at 28-30°C for 7 days

iv) Rice straw amended with Rice husk and bran mixture (1:1 w/w)

The population of fungi resident in the mixture did not vary significantly at the different levels of the compost heap (top, middle and bottom) tested for the presence of fungi on the two media owing to the turning of the mixture at 4 days intervals (Figs 11&12). However, the fungal profile was different in this mixture (Figs 15&16). Ten (10) *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. fumigatus, A. sulphureus, A. oryzae, A. ustus, A. niger, A. terreus* and *A. versicolor*) predominated and were encountered at all the depths sampled and plated on the two isolating media (Figs 17&18). This was followed by *Penicillium* (*P. italicum* and *P. citrinum*). Nine (9) other genera of fungi were encountered (*Aspergillus, Cladosporium, Fusarium, Penicillium, Rhizopus, Rhodotorula, Rhizoctonia, Trichoderma* and Yeasts). The most frequently encountered species were *A. fumigatus* (<60%), *Rhodotorula* (<45-80%), *A. flavus* (12-30%), *T. harzianum* (>30%) and *Rhizoctonia solani* (>44%) (Figs 17&18). Plates 1-3a-b show some species of fungi belonging to different genera isolated from the compost.

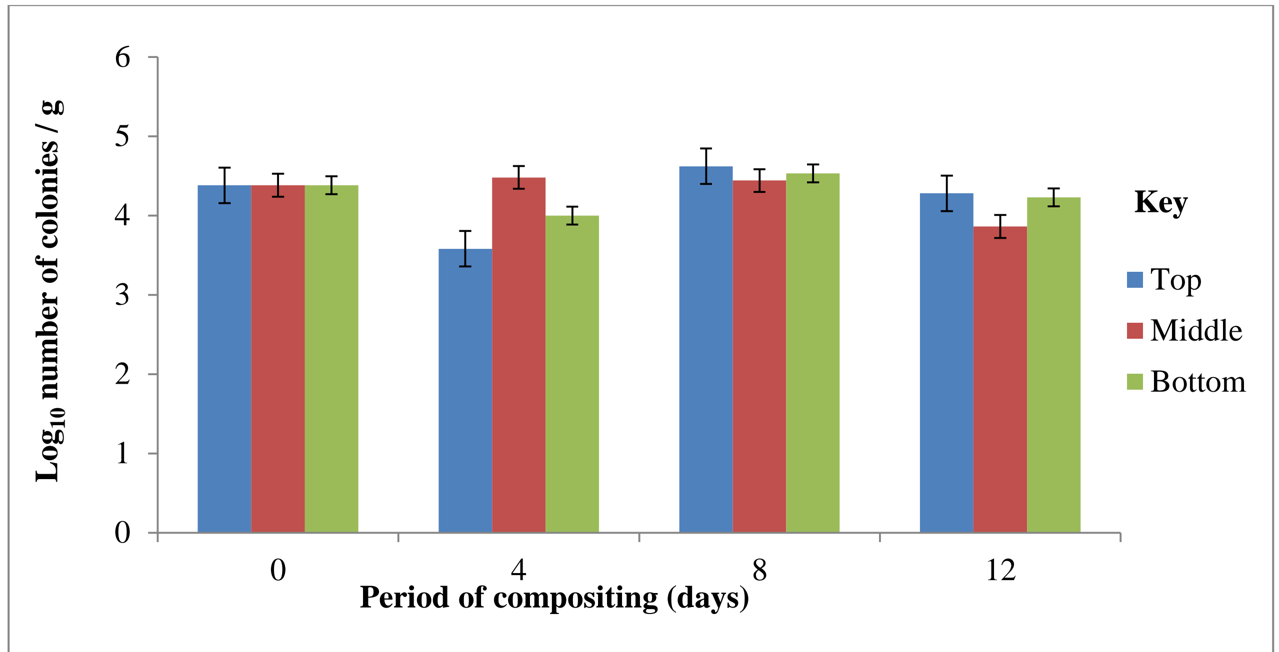


Fig 15: Fungal population profile in rice straw amended with rice husk and 1% $CaCO_3$ and 10% rice bran and composted for the indicated periods at 28-30°C and plated in Cooke's medium for 7 days

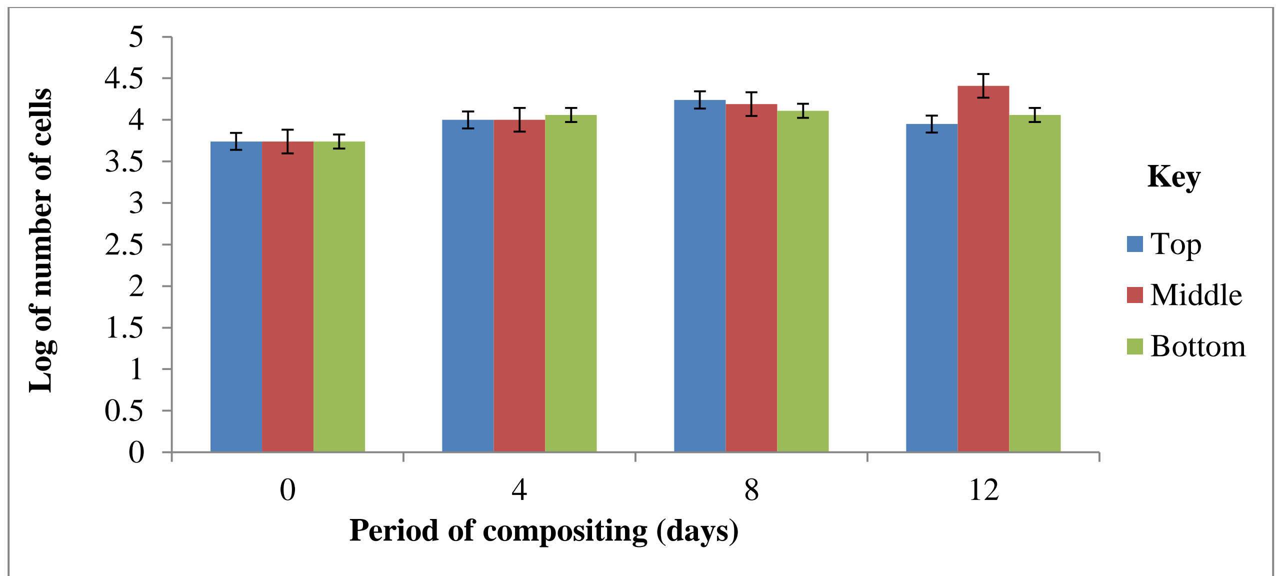


Fig 16: Fungal population profile in rice straw amended with rice husk and 1% $CaCO_3$ and 10% rice bran and composted for the indicated periods at 28-30°C and plated in DRBC mycological medium for 7 days

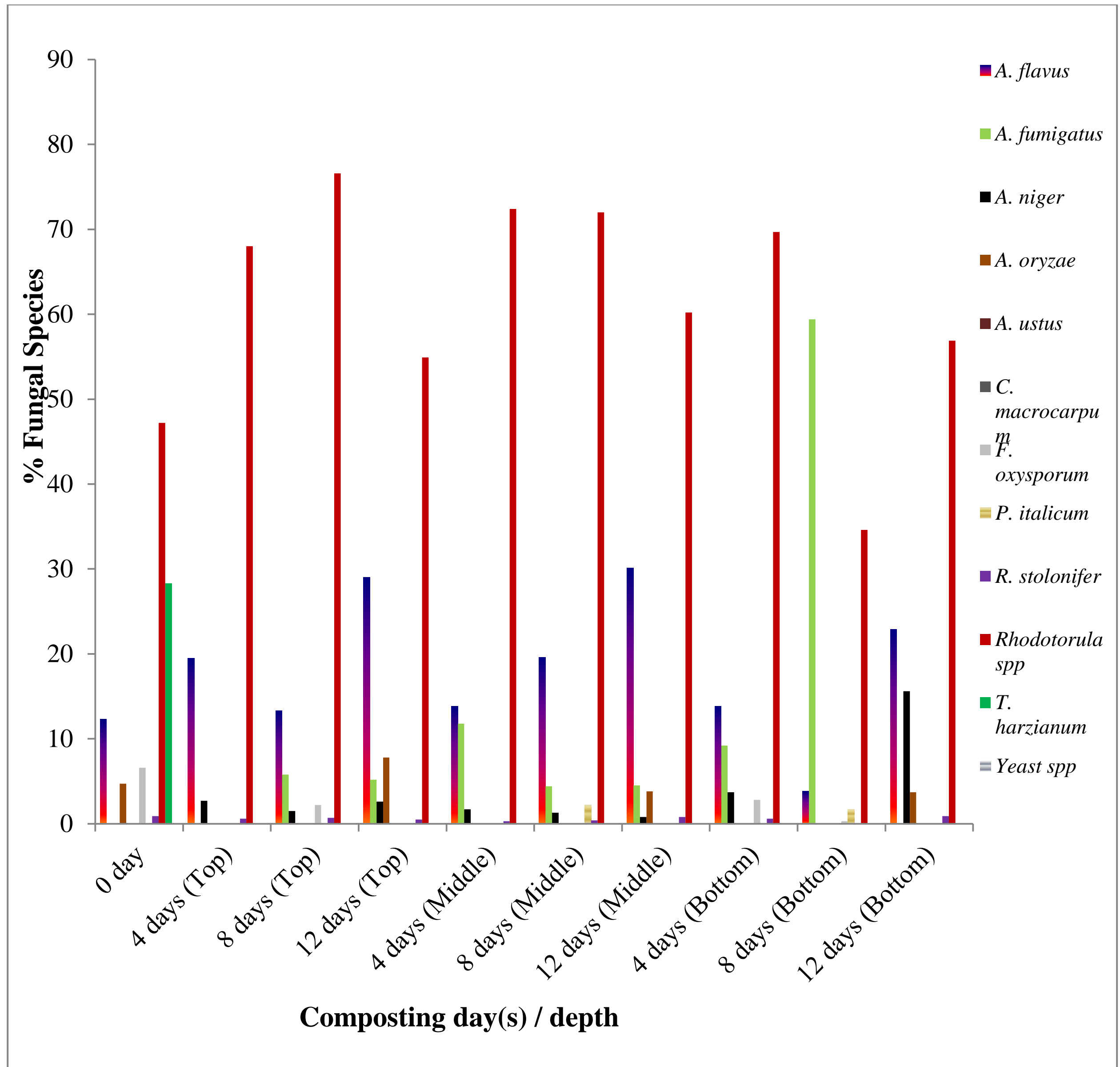


Fig 17: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw and rice husk mixture amended with 1% $CaCO_3$ and 10% rice bran) and culture in Cooke's medium at 28-30°C for 7 days

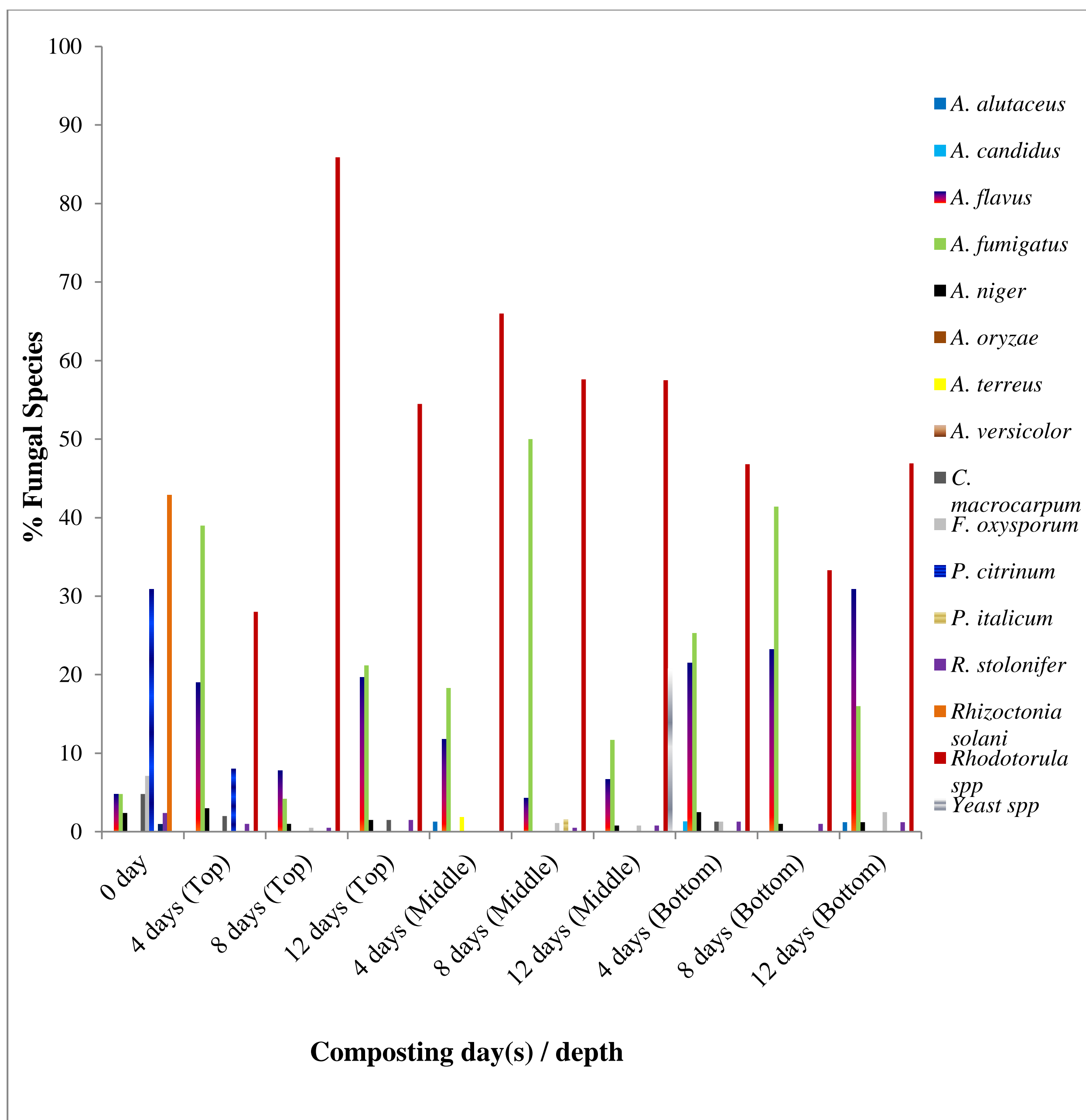
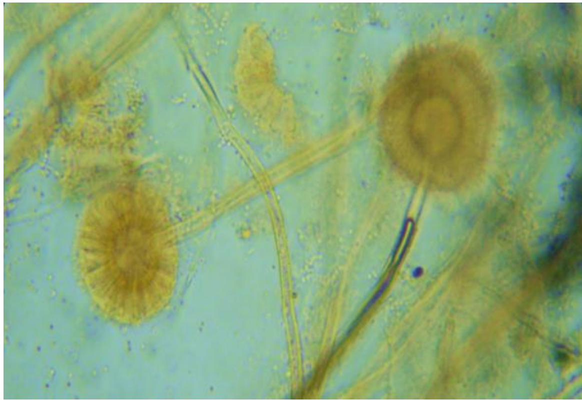
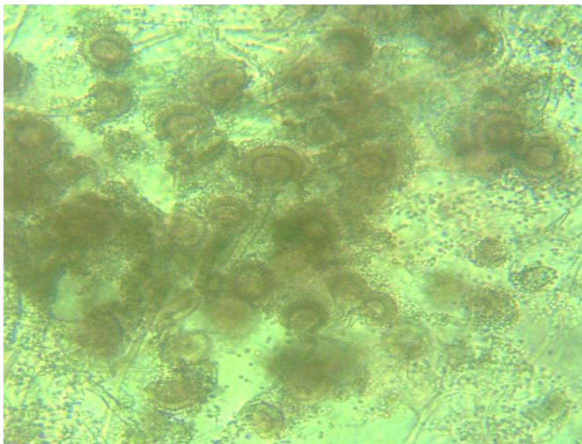
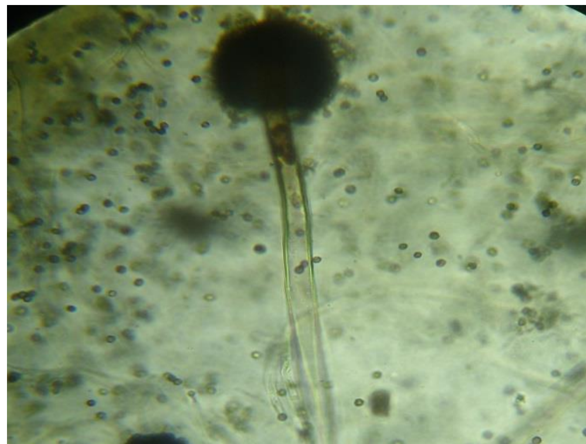


Fig 18: Percentage occurrence of some fungi resident in different unfermented (0 day) and fermented (4-12 days) at different depths of the compost heap (rice straw and rice husk mixture amended with 1% $CaCO_3$ and 10% rice bran) and culture in DRBC mycological medium at 28-30°C for 7 days



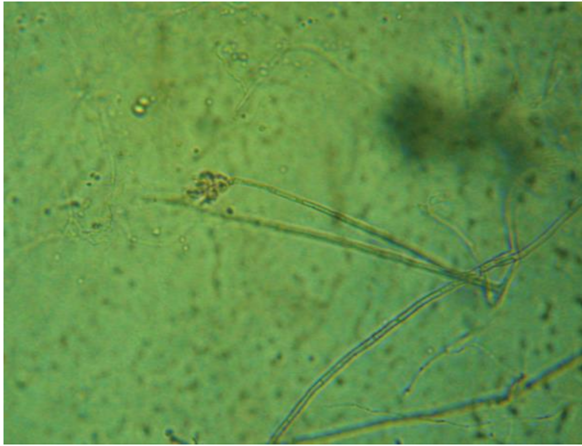
Aspergillus alutaceus ***A. flavus***



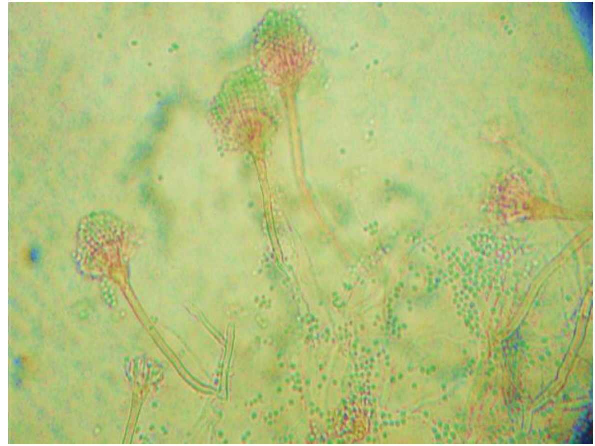


A. fumigatus ***A. niger***

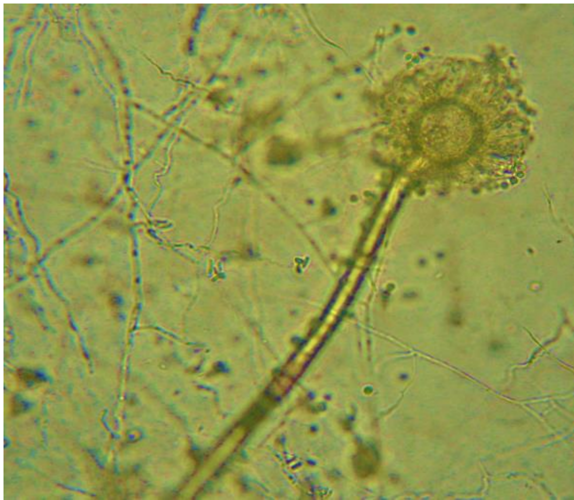
Plate 1a: Photographs of representative *Aspergillus* species isolated from samples of rice lignocellulose waste and composts used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31 in these studies. (Mag. x400)



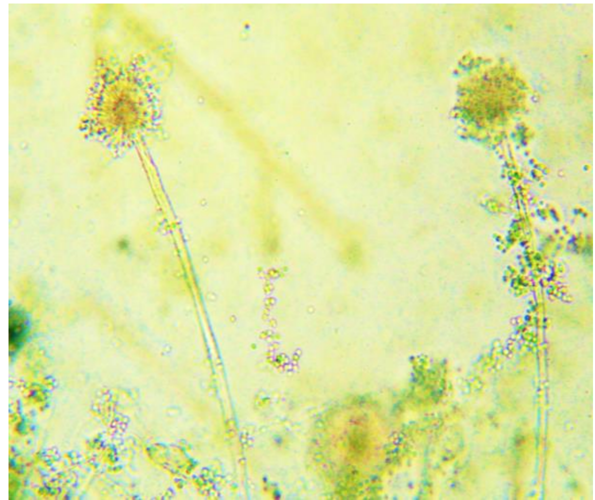
A. penicilloides



A. terreus

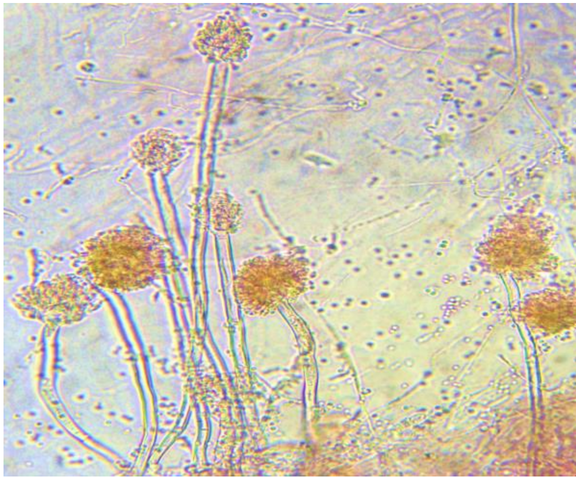


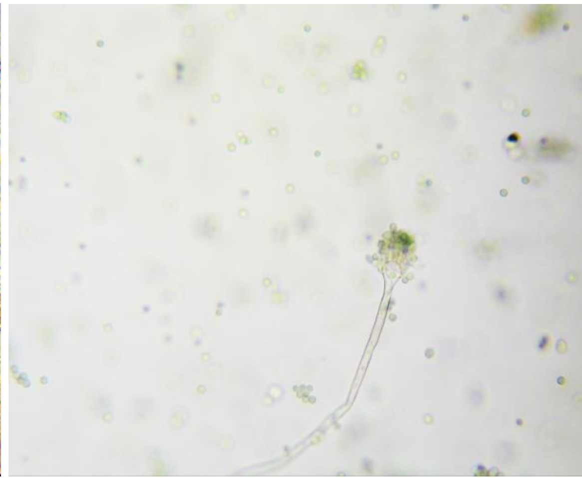
A. tamarii



A. oryzae

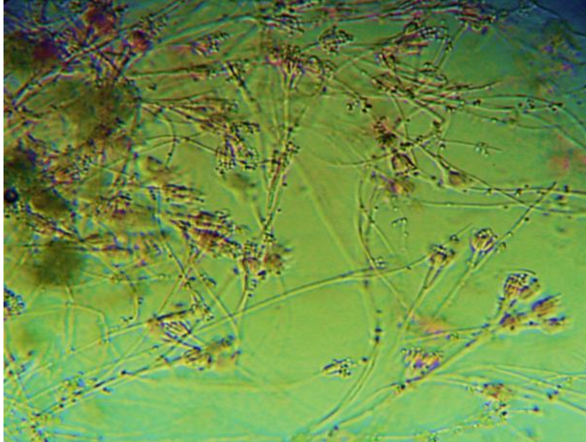
Plate 2b: Photographs of representative *Aspergillus* species isolated from samples of rice lignocellulose waste and composts used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31 in these studies (Cont'd). (Mag. x400)



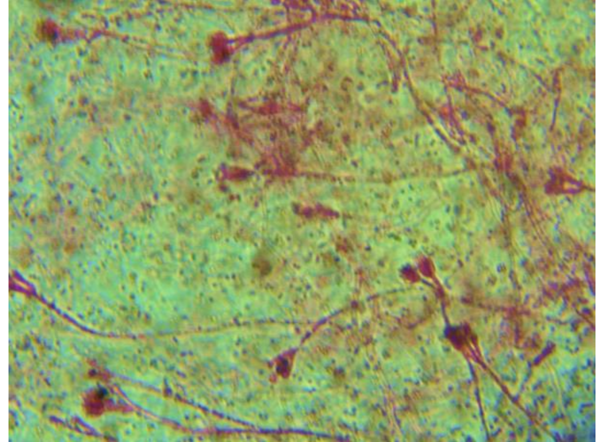


A. ustus ***A. versicolor***

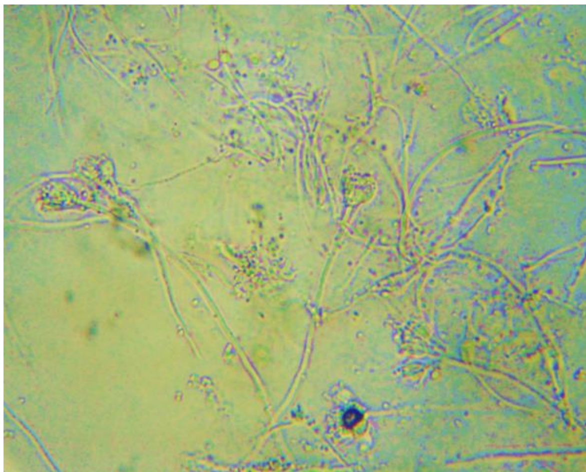
Plate 1c: Photographs of representative *Aspergillus* species isolated from samples of rice lignocellulose waste and composts used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31 (Cont'd). (Mag. x400)



Penicillium citrinum

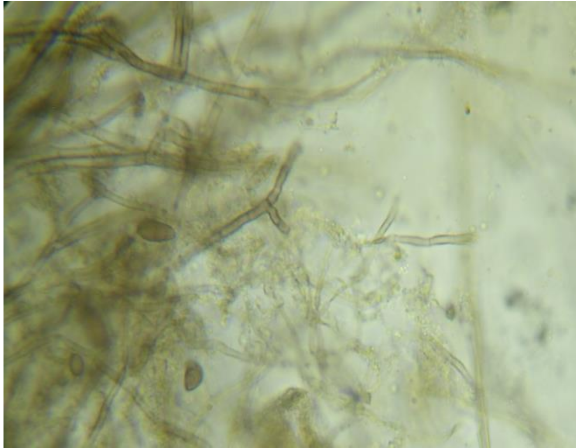


P. glabrum

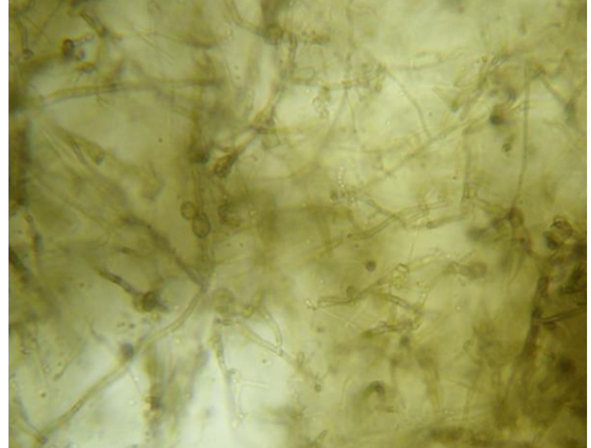


P. italicum

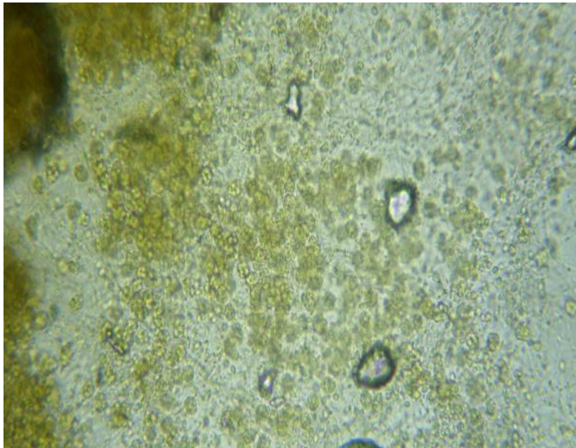
Plate 2: Photographs of representative *Penicillium* species isolated from samples of rice lignocellulose waste and composts used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31 in these studies.



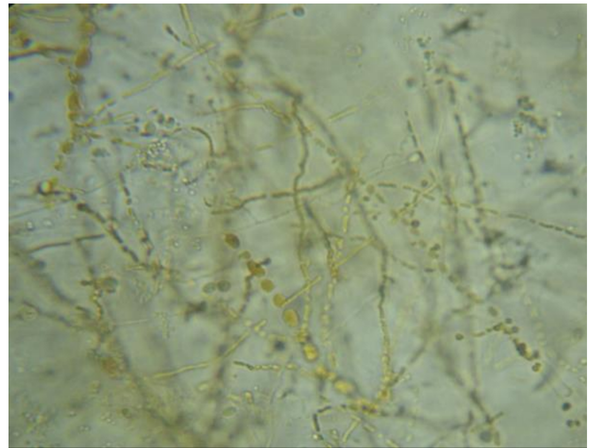
Cladosporium macrocarpum



***Cephalosporium* sp.**



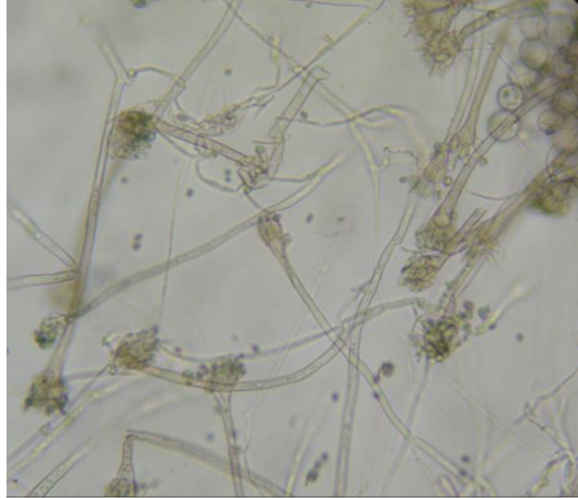
Byssochlamys fulva



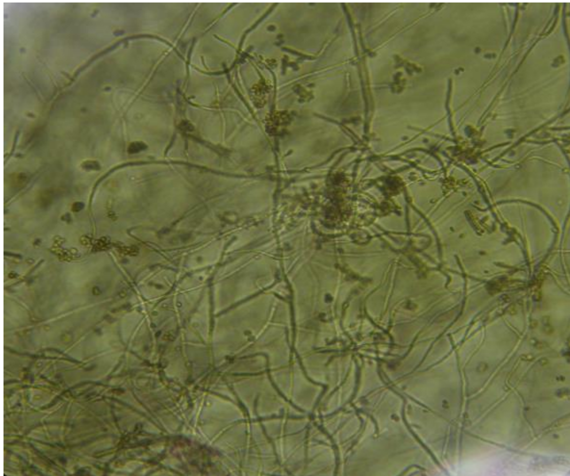
***Geotrichum* sp.**

Plate 3a: Photographs of representative of other genera of fungi encountered in the samples of rice lignocellulose waste and compost used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31. (Mag. x400)

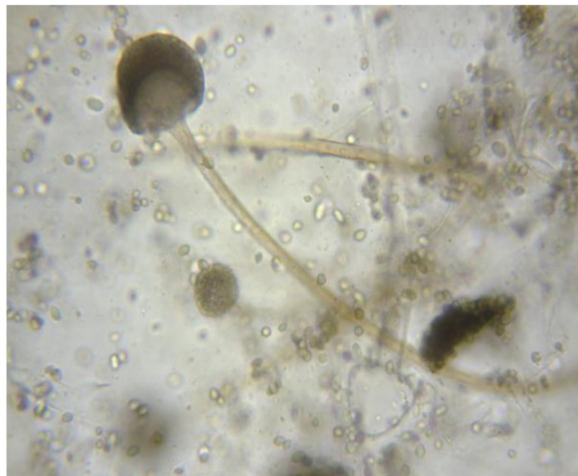
Fusarium oxysporum



Neosartorya fischeri



Mycelia sterilia



Rhizopus stolonifer

Plate 3b: Photographs of representative of other genera of fungi encountered in the samples of rice lignocellulose waste and compost used for the cultivation of *Pleurotus ostreatus* EM-1 and *P. eous* P-31. (Mag. x400)

EXPERIMENT B.

Influence of pH on radial growth of *P. ostreatus* EM-1 and *P. eous* P-31 in liquid medium (PDB) at 30±2°C for 10 days

Data obtained in the present study for both liquid and solid medium (Potato Dextrose Broth and Potato Dextrose Agar) show that pH 8.0 and 9.0 were unsuitable for radial growth and vegetative growth of *P. ostreatus* and *P. eous*. The best pH for the growth of both species was found to be pH 5.8-6.6 at 30±2°C under the Ghanaian tropic conditions (Tables 5 to 8). Previous studies have shown that the optimum pH for growth and maximum yield of *P. ostreatus* was pH 5.5 - 6.5 (Narh *et al.*2011). Obodai *et al.* (2011) also recorded the best growth of *P. ostreatus* in bags of moisture content 60-80% and pH 6.94-7.69. Presumably the optimum pH of *P. ostreatus* may vary with strain and substrate used. On the other the optimum pH for growth of *P. eous* P-31 is being recorded for the first time in Ghana as pH 5.8-6.6 at 30±2°C. The pH recorded is within the optimum range of pH 6.0-8.0 respectively and 60 -70% moisture reported by Stamets (2000) for *Pleurotus ostreatus* cultivation.

Results obtained are presented in Tables 5 to 8

Radial growth of *Pleurotus* species on agar (PDA)

The best growth of *P. ostreatus* EM-1 on PDA was attained at pH 5.4 – 7.0; pH of 8.0 and 9.0 were unsuitable and did not support growth on agar (Table 5, Plates 4&5).

The same results were obtained for *P. eous* P-3. No growth occurred in medium of pH 8.0 and 9.0. But pH ranging from 5.4 – 7.0 supported reasonable growth (Table 6).

Vegetative growth in Potato Dextrose Broth

The best pH for growth in liquid medium for *P. ostreatus* was between pH 5.8 – 6.6. Growth at pH 7.7 was good but was depressed of at pH 8.7 - 9.3. There was a slight drift in pH to the basic side during growth (Table 8). The best growth of *P. eous* was in medium of pH 5.8 - 6.6. There was no statistical difference ($p>0.05$) between dry weight attained at pH 6.6 and 5.8 (Table 7). There was a drift in pH during autoclaving and growth in the medium primarily the basic side (Table 7). pH 5.8 – 6.6 will therefore be suitable for growth of both *P. ostreatus* EM-1 and *P. eous* P-31 under the Ghanaian tropic conditions. Plates 4 and 5 show that radial growth of *P. eous* strain P-31 and *P. ostreatus* strain EM-1 on agar medium of different pH's.

Table 5: Effect of pH variation on radial growth of *P. ostreatus* strain EM-1 grown on Potato Dextrose Agar incubated at 30±2°C for 12 days

pH of medium	**Replicates**	**Diameter of colonies (mm) after indicated days**			
		3	**6**	**9**	**12**
Control	1	41.0	57.0	76.0	85.0
(only PDA)	2	35.0	49.0	75.0	85.0
pH 5.4	3	36.0	52.0	72.0	85.0
	Mean ± SE	37.3±1.85	52.7±2.33	74.3±7.20	85.0±0.0
pH 6.0	1	31.0	44.0	66.0	85.0
	2	36.0	48.0	78.0	85.0
	3	35.0	48.0	76.0	85.0
	Mean ± SE	34.0±1.52	46.7±1.33	73.3±3.71	85.0±0.0
pH 7.0	1	31.0	48.0	56.0	85.0
	2	28.0	38.0	56.0	85.0
	3	22.0	35.0	55.0	85.0
	Mean ± SE	27.0±2.65	40.3±3.92	55.7±0.33	85.0±0.0
pH 8.0	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
pH 9.0	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 6: Effect of pH variation on radial growth of *P. eous* strain P-31 grown on Potato Dextrose Agar incubated at 30±2°C for 12 days

pH of medium	Replicates	Diameter of colonies (mm) after indicated days			
		3	**6**	**9**	**12**
Control (only PDA) pH 5.4	1	51.0	76.0	85.0	85.0
	2	53.0	77.0	85.0	85.0
	3	50.0	79.0	85.0	85.0
	Mean ± SE	51.3±0.88	77.3±0.88	85.0±0.0	85.0±0.0
pH 6.0	1	53.0	72.0	85.0	85.0
	2	53.0	77.0	85.0	85.0
	3	50.0	77.0	85.0	85.0
	Mean ± SE	52.0±1.0	75.3±1.67	85.0±0.0	85.0±0.0
pH 7.0	1	46.0	69.0	85.0	85.0
	2	48.0	70.0	85.0	85.0
	3	48.0	73.0	85.0	85.0
	Mean ± SE	47.3±0.67	70.7±1.20	85.0±0.0	85.0±0.0
pH 8.0	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
pH 9.0	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 7: Effect of pH on the vegetative growth of *P. eous* P-31 on PDB incubated at 30 ± 2ºC for 10 days

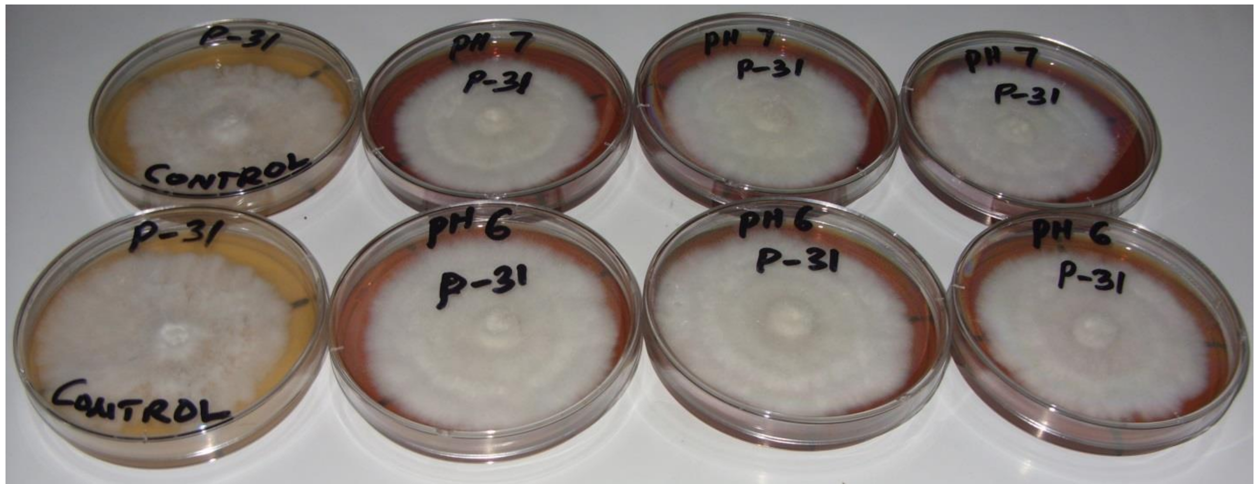
pH of Medium	pH of Medium		Dry weight of mycelium (mg)	Mean dry wt. of mycelium (mg) ±S.E(mg)
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.8	5.6	310.0*	290.0±15.3
	5.8	5.7	260.0	
	5.8	5.6	300.0	
pH 6	6.6	6.1	310.0	293.3±16.7
	6.6	6.0	260.0	
	6.6	6.1	310.0	
pH 7	7.7	7.3	260.0	273.3±8.8
	7.7	7.5	270.0	
	7.7	7.4	290.0	
pH 8	8.7	8.2	90.0	110.0±11.5
	8.7	8.1	130.0	
	8.7	8.2	110.0	
pH 9	9.3	8.8	110.0	96.7±6.7
	9.3	8.9	90.0	
	9.3	8.9	90.0	

Buffered with Mcilvaine Buffer *Figures rounded up to the nearest whole number

Table 8: Effect of pH on the vegetative growth of *P. ostreatus* EM-1 in synthetic PDB incubated at 30 ± 2ºC for 10 days.

pH of medium beofre autoclaving	pH of Medium		Dry weight of mycelium (mg	Mean dry wt. of mycelium (mg)±S.E(mg)
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.8	5.6	240.0*	250.0±5.8
	5.8	5.7	260.0	
	5.8	5.7	250.0	
pH 6.0	6.6	6.1	290.0	273.3±8.8
	6.6	6.0	270.0	
	6.6	6.1	260.0	
pH 7.0	7.7	7.3	220.0	243.3±18.6
	7.7	7.4	230.0	
	7.7	7.4	280.0	
pH 8.0	8.7	8.2	90.0	83.3±6.7
	8.7	8.3	70.0	
	8.7	8.1	90.0	
pH 9.0	9.3	8.8	70.0	73.3±8.8
	9.3	8.9	60.0	
	9.3	8.9	90.0	

Buffered with McIlvains Buffer *Figures rounded up to the nearest whole number



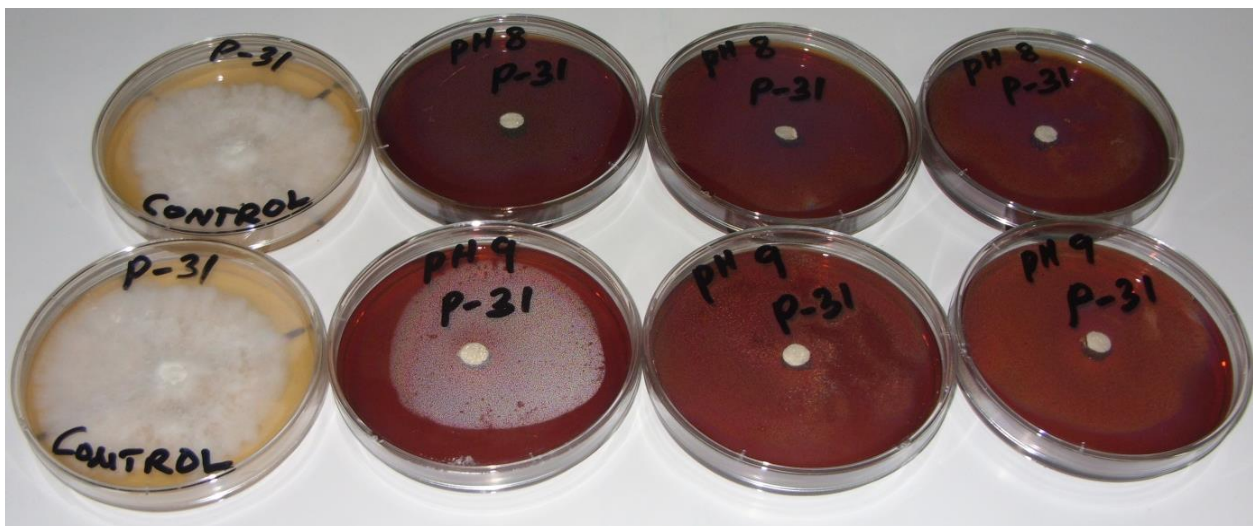
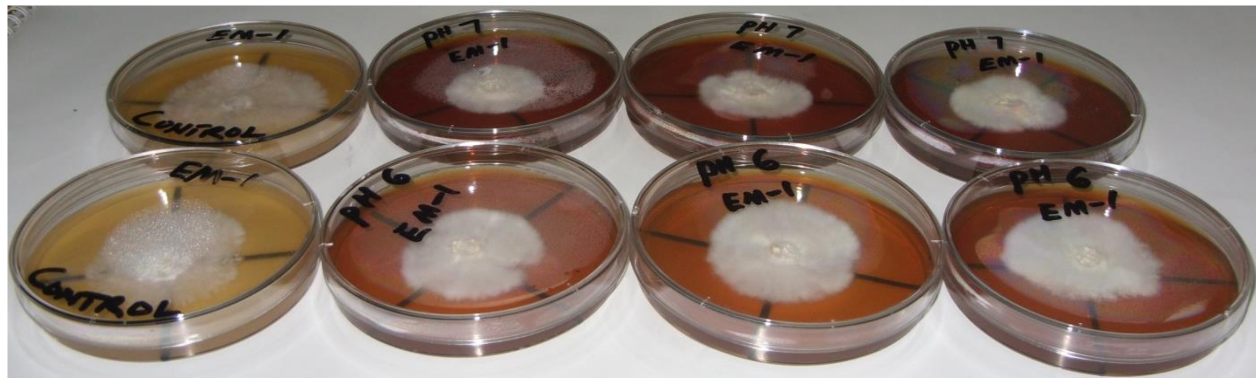


Plate 4: Influence of pH on radial growth of *P. eous* strain P-31 in PDA medium with different pH's.

Top: Left Radial Growth in control PDA (pH of 5.5) at (top) pH of 7.0 and at (bottom) pH 6.0 respectively.

Bottom: Radial growth of *P. eous* strain P-31 at (top) pH 8.0 and (bottom) pH 9.0 (Mag. x0.50)



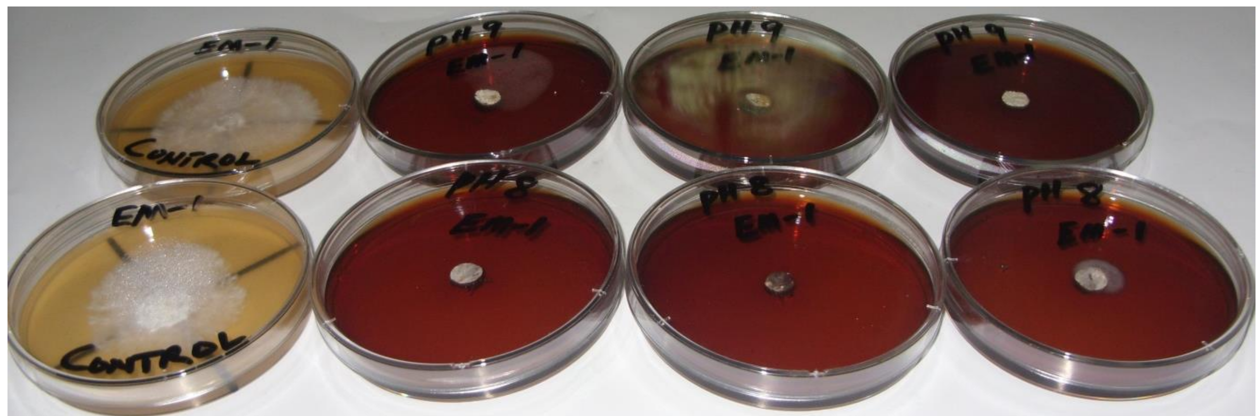


Plate 5: Influence of pH of medium on radial growth of *P. ostreatus* strain EM-1 at 32˚C for 6days.

Top: Radial Growth in the control (left) and on medium of pH 7.0 (top) and pH of 6.0 (bottom).

Bottom: Radial growth in the control (left) and in medium of pH of 9.0 (top) and pH of 8.0 (bottom) (x0.50)

EXPERIMENT C

Radial growth of *P. ostreatus* EM-1 and *P. eous* P-31 on Potato Dextrose Agar amended with cultural filtrates of three resident contaminating fungi in compost at 28-30°C for 12 days.

i. ***Aspergillus flavus* culture filtrate**

The culture filtrate used in amending the PDB at concentration of 1:1-1:5 v/v completely depressed radial growth of *P. ostreatus* EM-1 for 12 days (Table 9). Only cultural filtrate dilution of *A. flavus* (1:10v/v) permitted some growth recording 20±5mm in 12days (Table 9).

The culture filtrate of *A. flavus* also at dilutions of 1:1-1:5v/v completely depressed growth of *P. eous* P-31. Although dilution of 1:10v/v permitted growth on agar, it was only about one-quarter of what was obtained in the filtrate-free control medium (Potato Dextrose Agar only) (Table 10).

ii. *Penicillium citrinum* culture filtrate

Potato Dextrose Agar amended with 1:1 and 1:2v/v dilutions of the culture filtrate of *P. citrinum* did not permit radial growth of *Pleurotus ostreatus* EM-1 on agar. The remaining dilutions 1:5 and 1:10v/v dilutions permitted radial growth but the diameter never approximated that of the control plates. (Table 11)

In the plates containing varying dilutions (1:1-1:10v/v) of the culture filtrate of *P. citrinum* radial growth of *P. eous* was permitted but was inferior to the control. The higher the filtrate

dilution, the better the growth recorded for *P. eous* culture, such that at 1:10v/v dilution, the radial growth was akin to what existed in the control i.e. 85.0mm in 12days (Table 12)

iii. *Trichoderma harzianum* culture filtrate

The culture filtrate of fungus was very potent. Growth occurred only in the filtrate-free control Petri plates inoculated with *P. ostreatus* and *P. eous.* In the medium containing 1:1-1:10 v/v dilutions of the filtrate, no growth of the mushrooms were recorded (Tables 13 and 14.).

Table 9: Effect of varying concentrations of culture filtrates of *Aspergillus flavus* on radial growth of *P. ostreatus* EM-1 grown on Potato Dextrose Agar incubated at 30±2°C for 12 days

Dilutions (v/v)	**Replicates**	**Diameter of colonies (mm)**				**Mean Diameter ± SE**
Period of incubation(days)		**3**	**6**	**9**	**12**	
Control (PDA)	1	72	80	85	85	81±0.3
	2	61	77	81	8.5	76±0.5
	3	63	80	83	85	78±0.5
1:10	1	17	17	19	22	19±0.1
	2	16	16	17	19	17±0.1
	3	15	15	17	20	17±0.7
1:5	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
1:2	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
1:1	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-

No growth (-) occurred

Table 10: Effect of varying concentrations of culture filtrates of *Aspergillus flavus* on radial growth of *P. eous* P-31 grown on Potato Dextrose Agar incubated at 30 ±2^{0}C for 12 days

Dilutions (v/v)	Replicates	Diameter of colonies (mm) after indicated days			
		3	**6**	**9**	**12**
Control (only PDA)	1	24.0	52.0	72.0	85.0
	2	24.0	51.0	70.0	85.0
	3	23.0	54.0	77.0	85.0
	Mean ± SE	23.7±0.3	52.4±0.9	73.0±2.1	85.0±0.0
1:10	1	19.0	20.0	22.0	22.0
	2	11.0	13.0	15.0	15.0
	3	17.0	21.0	21.0	21.0
	Mean ± SE	15.7±2.4	18.0±2.5	19.3±2.2	19.3±2.2
1:5	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:2	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 11: Effect of varying concentrations of culture filtrates of *Penicillium citrinum* on radial growth of *P. ostreatus* EM-1 grown on Potato Dextrose Agar incubated at 30 ±2°C for 12 days

Dilutions (v/v)	**Replicates**	**Diameter of colonies (mm) indicated days**			
		3	**6**	**9**	**12**
Control (only PDA)	1	60.0	82.0	85.0	85.0
	2	69.0	77.0	85.0	85.0
	3	45.0	76.0	85.0	85.0
	Mean ± SE	58.0±7.0	78.3±1.9	85.0±0.0	85.0±0.0
1:10	1	21.0	26.0	29.0	29.0
	2	21.0	31.0	34.0	34.0
	3	19.0	21.0	24.0	24.0
	Mean ± SE	20.3±0.7	26.0±2.9	26.0±2.9	26.0±2.9
1:5	1	15.0	18.0	18.0	18.0
	2	13.0	15.0	15.0	15.0
	3	13.0	15.0	15.0	15.0
	Mean ± SE	13.7±0.7	16.0±1.0	16.0±1.0	16.0±1.0
1:2	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 12: Effect of varying concentrations of culture filtrates of *Penicillium citrinum* on radial growth of *P. eous* P-31 grown on Potato Dextrose Agar incubated at 30 $\pm 2^0$C for 12 days

Dilutions (v/v)	Replicates	Diameter of colonies (mm) after indicated days			
		3	**6**	**9**	**12**
Control (only PDA)	1	41.0	73.0	85.0	85.0
	2	33.0	57.0	85.0	85.0
	3	40.0	74.0	85.0	85.0
	Mean ± SE	38.0±2.5	68.0±5.5	85.0±0.0	85.0±0.0
1:10	1	16.0	42.0	82.0	85.0
	2	22.0	29.0	64.0	85.0
	3	23.0	60.0	75.0	85.0
	Mean ± SE	20.3±2.2	43.7±8.9	73.7±5.2	85.0±0.0
1:5	1	16.0	32.0	66.0	66.0
	2	20.0	41.0	70.0	70.0
	3	19.0	29.0	69.0	69.0
	Mean ± SE	18.3±1.2	34.0±3.6	68.0±1.2	68.3±1.2
1:2	1	18.0	35.0	35.0	35.0
	2	20.0	23.0	23.0	23.0
	3	16.0	20.0	20.0	20.0
	Mean ± SE	18.0±1.2	26.0±4.6	26.0±4.6	26.0±4.6
1:1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 13: Effect of varying concentrations of culture filtrates of *Trichoderma harzianum* on radial growth of *P. ostreatus* EM-1 grown on Potato Dextrose Agar incubated at 30±2°C for 12 days

Dilutions (v/v)	**Replicates**	**Diameter of colonies (mm) after indicated days**			
		3	**6**	**9**	**12**
Control (only PDA)	1	72.0	82.0	85.0	85.0
	2	50.0	77.0	83.0	85.0
	3	66.0	80.0	84.0	85.0
	Mean ± SE	62.7±6.6	79.7±1.5	84.0±0.6	85.0±0.0
1:10	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:5	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:2	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

Table 14: Effect of varying concentrations of culture filtrates of *Trichoderma harzianum* on radial growth of *P. eous* P-31 grown on Potato Dextrose Agar incubated at 30±2°C for 12 days

Dilutions (v/v)	Replicates	Diameter of colonies (mm) after indicated days			
		3	**6**	**9**	**12**
Control (only PDA)	1	23.0	53.0	76.0	85.0
	2	23.0	52.0	76.0	85.0
	3	25.0	50.0	79.0	85.0
	Mean ± SE	23.7±0.7	51.7±0.9	77.0±1.0	85.0±0.0
1:10	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:5	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:2	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
1:1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-

No growth (-) occurred

EXPERIMENT D

Vegetative growth of *P. ostreatus* EM-1 and *P. eous* P-31 in liquid culture (Potato Dextrose Broth) amended with different concentrations of the culture filtrates of three resident contaminating fungi in the compost at 28-30°C for 10days

a) *A. flavus* culture filtrate

Tables 15 to 20 and Plates 6 and 7 show results obtained. There was a commensurate depression of growth of both *Pleurotus* species with increasing concentration of culture filtrate of *A. flavus*. The higher the concentration the greater the decrease in vegetative growth. For example, in the Potato Dextrose Broth (unamended), mycelium dry weight of *P. ostreatus* after 10days was 161.1±6.9mg as compared to 40.0±5.8mg in the 1:1v/v amendment. The vegetative growth increased with increased dilution of filtrate of *A. flavus* up to 1:10v/v but never approximated the control (Table 15).

The same trend was obtained with the culture of *P. eous.* However, the depressive effect of the culture filtrates at 1:1v/v dilution was less severe. For example, vegetative growth of *P. eous* in the unamended Potato Dextrose Broth was 213.3±14.5mg after 10days as compared to 123.3±18.6mg in the filtrate dilution of 1:1v/v. Growth of *P. eous* in the 1:10v/v dilution of *A. flavus* culture filtrate nearly approximated that of the control (203.3±3.3mg) (Table 16).

b) *Penicillium citrinum* culture filtrate

Tables 17 and 18.show results obtained. There was a commensurate depression of growth of both *Pleurotus* species with increasing concentration of *P. citrinum* culture filtrate such that at 1:1v/v dilution, only 30.0±5.8mg of *P. ostreatus* was obtained as compared to 303.3±1.8mg in the control (Table 17). The inhibitory effect was gradually removed as filtrate dilution increased such that at 1:10v/v dilution growth of *P. ostreatus* (226.7±7.6mg) nearly approximated that of the control (303.3±1.8mg) (Table 17).

The same trend was obtained with the culture of *P. eous*. However, the effect was less severe. While the mushroom yielded a mycelial dry weight of 353.3±6.9mg in the filtrate-free medium (PDB), growth in the 1:1v/v dilution of *P. citrinum* produced 63.3±8.8mg (Table 18). However, vegetative growth of *P. eous* in the 1:10v/v dilution of the culture filtrate of *P. citrinum* yielded 163.3±6..0mg after 10days nearly half of what obtained in the filtrate-free medium.

c) *Trichoderma harzianum* culture filtrate

The culture filtrate of *T. harzianum* was also potent in its effect on the vegetative growth of *P. ostreatus* EM-1. Although growth in the amended control PDB was 140.0±15.3mg in 10days, vegetative growth in the medium amended with 1:1v/v dilution of the culture filtrate of *T. harzianum* produced only 20.0±5.8mg of *P. ostreatus* (i.e. one-seventh of the control). Further dilutions 1:2-1:10v/v increased vegetative growth but never approximated that of the control (Table 19.).

The trend obtained from *P. ostreatus* was repeated in the case of *P. eous*. The least vegetative growth of the mycelium was (60.0±11.5mg) and was obtained in the PDB

amended with 1:1v/v dilution of *T. harzianum* culture filtrate as compared to 226.7±32.8mg in the filtrate-free control medium (i.e. one-quarter of the control). Further dilution of the filtrate up to 1:10v/v improved growth but approximated the control (Table 20).

Table 15: Effect of varying dilutions of culture filtrates of *A. flavus* on the vegetative growth of *P. ostreatus* EM-1 on Potato Dextrose Broth incubated at 30 ± 2ºC for 10 days.

Dilutions ratio (v/v)	pH of Medium		Dry weight of mycelium (mg)	Mean dry wt. of mycelium (mg)±S.E
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.4	5.7	195.0*	161.1±16.9
	5.4	5.7	150.0	
	5.4	5.6	140.0	
1:10	5.4	5.8	100.0	76.7±12.0
	5.4	5.7	60.0	
	5.4	5.6	70.0	
1:5	5.2	5.6	80.0	66.7±6.7
	5.2	5.4	60.0	
	5.2	5.5	60.0	
1:2	5.0	5.2	50.0	66.7±12.0
	5.0	5.4	90.0	
	5.0	5.3	60.0	
1:1	4.8	5.2	50.0	40.0±5.8
	4.8	5.1	30.0	
	4.8	5.0	40.0	

*Figures rounded up to the nearest whole number

Table 16: Effect of varying dilutions of culture filtrates of *A. flavus* on the vegetative growth of *P. eous* on Potato Dextrose Broth incubated at 30 ± 2℃ for 10 days.

Dilutions ratio (v/v)	**pH of Medium**		**Dry weight of mycelium (mg)**	**Mean dry wt. of mycelium (mg) ±S.E**
	Initial (after autoclaving)	**Final**		
Control	5.4	5.5	210*	213.3±14.5
	5.4	5.5	190	
	5.4	5.5	240	
1:10	5.4	4.9	130	203.3±43.3
	5.4	4.7	280	
	5.4	4.8	200	
1:5	5.2	5.6	160	143.3±12.0
	5.2	5.6	120	
	5.2	5.6	150	
1:2	5.0	6.2	160	136.7±14.5
	5.0	6.2	140	
	5.0	6.2	110	
1:1	4.8	3.7	100	123.3±18.6
	4.8	3.7	160	
	4.8	3.7	110	

*Figures rounded up to the nearest whole number

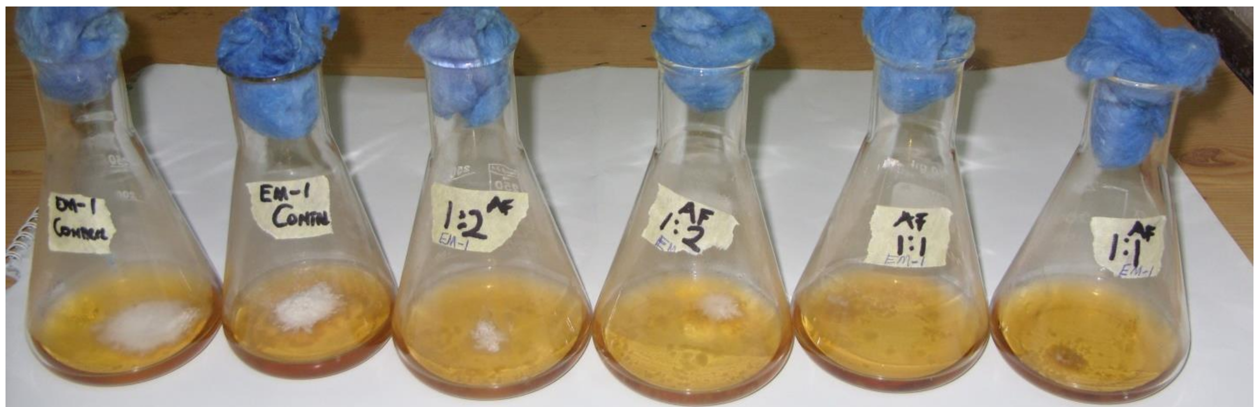


Plate 6: Influence of culture filtrate of *A. flavus* on vegetative growth of *P. ostreatus* strain EM-1 in liquid culture of Potato Dextrose Broth amended with varying concentrations (1:1-1:10v/v) of the culture filtrate at 28-30°C for 10 days. (Note the absence of growth in flasks containing higher concentrations of the metabolites (1:1 and 1:2 v/v dilutions) (Mag. x 0.5)

Table 17: Effect of varying dilutions of culture filtrates of *P. citrinum* on the vegetative growth of *P. ostreatus* on Potato Dextrose Broth incubated at 30 ± 2ºC for 10 days.

Dilutions ratio (v/v)	pH of Medium		Dry weight of mycelium (mg)	Mean dry wt. of mycelium (mg)±SE
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.6	5.9	340.0*	303.3±31.8
	5.6	5.9	300.0	
	5.6	5.8	240.0	
1:10	5.5	5.8	210.0	226.7±27.6
	5.5	5.8	280.0	
	5.5	5.9	190.0	
1:5	5.4	5.7	130.0	120.0±5.8
	5.4	5.8	120.0	
	5.4	5.8	110.0	
1:2	5.2	5.6	90.0	80.0±5.8
	5.2	5.6	70.0	
	5.2	5.6	80.0	
1:1	5.1	5.4	20.0	30.0±5.8
	5.1	5.3	30.0	
	5.1	5.5	40.0	

*Figures rounded up to the nearest whole number

Table 18: Effect of varying dilutions of culture filtrates of *P. citrinum* on the vegetative growth of *P. eous* on Potato Dextrose Broth incubated at 30 ± 2ºC for 10 days.

Dilutions ratio (v/v)	pH of Medium		Dry weight of mycelium (mg)	Mean dry wt. of mycelium (mg)±S.E
	Initial (after autoclaving)	Final		
	5.6	5.5	320.0*	
Control (Only PDB)	5.6	5.5	380.0	320.0±45.8
	5.6	5.5	350.0	
	5.3	6.0	210.0	
1:10	5.3	6.0	160.0	163.3±26.0
	5.3	6.0	120.0	
	5.6	6.1	120.0	
1:5	5.6	6.1	150.0	143.3±12.0
	5.6	6.1	160.0	
	5.3	6.2	120.0	
1:2	5.3	6.3	160.0	120.0±23.1
	5.3	6.3	80.0	
	5.7	5.3	50.0	
1:1	5.7	5.5	80.0	63.3±8.8
	5.7	5.5	60.0	

*Figures rounded up to the nearest whole number

Table 19: Effect of varying dilutions of culture filtrates of *T. harzianum* on the vegetative of *P. ostreatus* on Potato Dextrose Broth incubated at 30 ± 2ºC for 10 days.

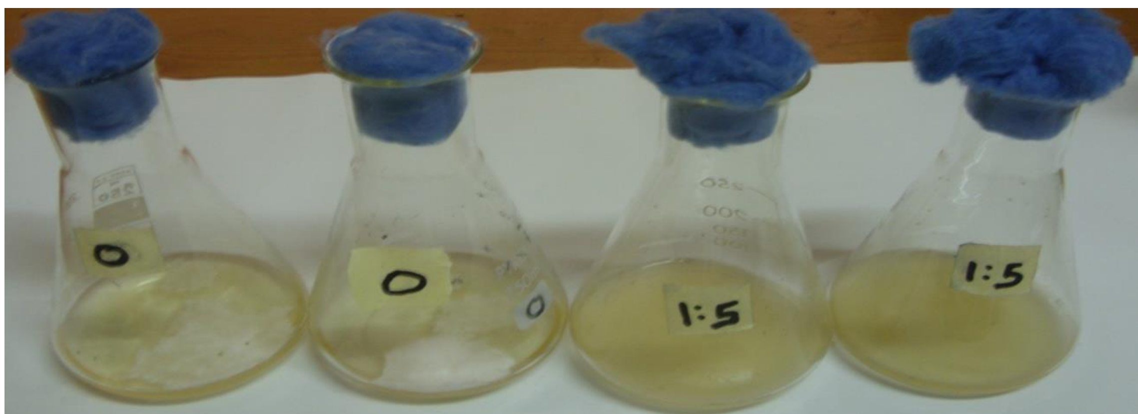
Dilutions ratio (v/v)	pH of Medium		Dry weight of mycelium (mg)	Mean dry wt. of mycelium (mg)±S.E
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.6	5.9	120.0*	140.0±15.3
	5.6	5.8	170.0	
	5.6	5.9	130.0	
1:10	5.3	5.6	80.0	86.7±6.7
	5.3	5.8	100.0	
	5.3	5.6	80.0	
1:5	5.6	5.7	80.0	66.7±6.7
	5.6	5.8	60.0	
	5.6	5.4	60.0	
1:2	5.3	5.5	30.0	26.7±3.3
	5.3	5.8	20.0	
	5.3	5.6	30.0	
1:1	5.7	5.2	30.0	20.0±5.8
	5.7	5.7	10.0	
	5.7	5.5	20.0	

*Figures rounded up to the nearest whole number

Table 20: Effect of varying dilutions of culture filtrates of *T. harzianum* on the vegetative growth of *P. eous* on Potato Dextrose Broth incubated at 30 ± 2ºC for 10 days.

Dilutions ratio (v/v)	pH of Medium		Dry weight of mycelium (mg	Mean dry wt. of mycelium (mg)±S.E
	Initial (after autoclaving)	Final		
Control (Only PDB)	5.6	5.9	180.0	226.7±32.8
	5.6	5.8	290.0	
	5.6	5.6	210.0	
1:10	5.4	5.6	130.0	166.7±27.3
	5.4	5.5	220.0	
	5.4	5.5	150.0	
1:5	5.3	5.4	100.0	133.3±20.3
	5.3	5.3	130.0	
	5.3	5.3	170.0	
1:2	6.3	5.1	90.0	113.3±14.5
	6.3	5.3	140.0	
	6.3	5.1	110.0	
1:1	6.6	5.2	60.0	60.0±11.5
	6.6	5.2	80.0	
	6.6	5.3	40.0	

*Figures rounded up to the nearest whole number



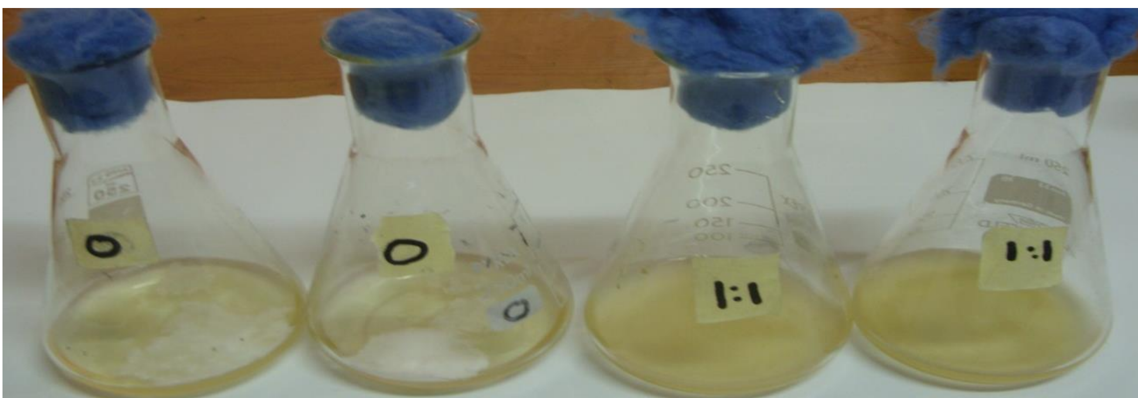


Plate 7: Influence of culture filtrate of *T. harzianum* of varying concentrations (control, 0-1:10v/v dilution) on vegetative growth of *P. eous* strain P-31 at 28-30°C for 10days (Mag. x 0.5)

EXPERIMENT E:

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on differently formulated substrates composted for varying periods at 28±2°C

EXPERIMENT 1

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw only composted for different periods

Results of this experiment are shown in Tables 21 and 22 (mycelial density; Average mycelia growth (cm/week); Spawn run period (weeks); pH of compost for *P. ostreatus* and *P. eous* respectively.

i) Surface mycelial density on rice straw only

The surface mycelial density for *P. eous* was denser than that of *P. ostreatus* in the unfermented compost. *P. ostreatus* in the 4-8 days composted rice straw grew through the bag and was uniformly white (Plate 8). Interestingly, the spawn run period for *P. ostreatus* on the same substrate with comparatively similar pH before and after sterilization was nearly two times (8weeks) that of *P. eous* (3 weeks); furthermore average mycelial growth rate (cm/week) was slower for *P. ostreatus* (4.7±1.1 to 7.8±0.7 cm/week) in contrast to *P. eous* with an average mycelial growth of 7.5±0.3 cm/week to 9.5±0.9 cm/week (Tables 21 and 22).

ii) Total No. of fruiting bodies

The total number of fruit bodies after 3 flushes on raw rice straw is shown in Table 23 (*P. ostreatus*) and Table 24 (*P. eous*). The total production of fruiting bodies in the unsupplemented rice straw was significantly ($p>0.05$) different from the 4days composted substrate. The substrate composted for 4 and 8 days before spawning produced same number of fruit bodies for *P. ostreatus* (Table 23). There was no advantage in composting the substrate for 12 days before spawning with the cultures so far as fruiting was concerned. However, on the same substrate given similar treatments, *P. eous* performed better (47-56 total fruit bodies in 34 days, as compared to only 15-19 total fruit bodies during the same period for *P. ostreatus* (Tables 23 and 24); a 32-33 difference performance.

iii) Total no. of pinheads per flush on raw rice straw compost

The same trend was recorded in the total no. of pinheads formed in the respective substrates seeded with either *P. ostreatus* (21-30) or *P. eous* (61-75). Another important observation was that not all the pinheads produced in the substrates matured into fruiting bodies although the success rate was much higher for *P. eous* than that of *P. ostreatus* (Tables 25 and 26).

iv) Total yield and Biological Efficiency on raw straw compost

There was a positive relation between period of composting of the rice straw, the total yield and Biological Efficiency (Tables 27 and 28). The highest yield (154.7g) and Biological

Efficiency (53.3%) for *P. ostreatus* strain EM-1 was attained in substrate composted for 4 days before spawning with the culture (Table 27), followed by the 8 days composted rice straw substrate (total yield 43.7g; BE: 49.6%) (Table 27). The best yield of *P. eous* P-31 was obtained after 4-8 days with BE of 75.6-74.4% (Table 28). However, yield on unfermented rice straw was comparatively good (161.5g; BE: 55.7%). Plates 9&10 show the fruit bodies of *P. ostreatus* in the uncomposted and 4 days old composted rice straw only while Plates 11a-c depict the fruit bodies of *P. eous* in raw unamended rice straw composted for varying periods.

v) Record of stipe and pileus length and average weight and the diameter of the *Pleurotus* species cultivated in the unamended raw rice straw composted for varying periods prior to spawning with mycelium and incubated at 28±2°C for 12 days

Table 29 summarises the results obtained. The best average weight of mushroom (18.7g), average width of pileus (73.6mm) and average length of the stipe (59.6mm) for *P. eous* was obtained in the culture growing in the unfermented raw rice straw (Table 29). In contrast the best of the listed parameters were recorded in the compost fermented for 4-12 days prior to seeding with spawn of *P. ostreatus* EM-1(Table 29). Therefore, growth of *P. eous* was best on the unfermented substrate in contrast with *P. ostreatus* which performed better on fermented compost. Changes in growth rate with respect to stipe length (mm) and pileus width (mm) in relation to BE showed that there was a high correlation and the regression line fits to the data. The highest coefficient of determination R^2=0.921 (stipe length of *P. eous*) R^2=0.875 (pileus width of *P. eous*); R^2=0.945 (stipe length *P. ostreatus*) R^2=0.944 pileus width *P. ostreatus*) were obtained in rice straw only (Figs 19.and 20).

Table 21: Mycelial growth rate and density of *P. ostreatus* EM-1 on rice straw only composted for indicated periods of at 28±2°C before spawning

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/weeks)	Spawn run period (weeks)	pH of compost	
				Initial	After sterilization
0	++	5.4 ± 0.3	8	7.5	6.9
4	+++	6.3± 0.5	8	7.3	7.2
8	+++	7.8 ± 0.7	8	7.1	7.4
12	+++	4.7 ± 1.1	8	7.1	7.3

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grew through the bag and was uniformly white
++ Mycelium totally grew through the bag but was not uniformly white.

Table 22: Mycelial growth rate and density of *P. eous* P-31 on rice straw only composted for indicated periods of at 28±2°C before spawning

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/week)±SE	Spawn run period (week)	pH of compost	
Treatment				**Initial (before)**	**After sterilization**
0	+++	8.8 ± 1.8	3	7.5	7.1
4	+++	9.5 ± 0.9	3	7.3	7.0
8	+++	9.6 ± 0.1	3	7.1	7.4
12	+++	7.5 ± 0.3	3	7.1	7.7

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grew through the bag and was uniformly white

Table 23: Record of fruiting bodies formation per flush of *P. ostreatus* EM-1growing on rice straw only composted for the indicated periods before spawning

Period of composting / day(s)	Total no. of fruit bodies			Total No. of fruit bodies
	1st Flush	2nd Flush	3rd Flush	
0	8	4	3	15
4	7	6	4	17
8	11	3	5	19
12	8	6	3	17

Table 24: Record of fruiting bodies formation per flush of *P. eous* P-31 growing on rice straw only composted for the indicated periods before spawning

Period of composting / day(s)	Total no. of fruit bodies			Total No. of fruit bodies
	1st Flush	2nd Flush	3rd Flush	
0	29	10	8	47
4	37	9	10	56
8	31	10	11	52
12	21	10	7	38

Table 25: Record of pinhead formation per flush of *P. ostreatus* EM-1produced in rice straw only composted for the indicated periods before spawning and incubation at 28±2°C for 34days

Period of composting	No. of pinheads			Total No. of pinheads
	1st Flush	2nd Flush	3rd Flush	
0	12	6	6	24
4	9	7	5	21
8	17	5	8	30
12	11	13	4	28

Table 26: Record of pinhead formation per flush of *P. eous* P-31produced in rice straw only composted for the indicated periods before spawning and incubation at 28±2°C for 34 days

Period of composting	No. of pinheads			Total No. of pinheads
	1st Flush	2nd Flush	3rd Flush	
0	43	13	17	61
4	44	14	17	75
8	38	16	18	72
12	30	16	16	62

Table 27: Total yield and Biological Efficiency (BE) of *P. ostreatus* EM-1 grown on rice straw only composted for the indicated periods prior to spawning and incubation at 28±2°C for 34 days

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	61.8±6.4	24.2±5.9	20.6±3.7	106.6[a]	36.8
4	82.1±9.7	49.3±12.9	23.3±6.1	154.7[b]	53.3
8	85.3±6.9	27.9±6.7	30.5±4.0	143.7[c]	49.6
12	57.2±8.7	49.7±6.4	17.8±3.5	124.7[d]	43.0

The letters indicate significant differences to 95% ($p<0.05$) by the one way ANOVA Test. Values in the same column followed by a different letter do differ significantly from each other. All values are means of five replicates

Table 28: Total yield and Biological Efficiency (BE) of *P. eous* P-31 grown on rice straw only composted for the indicated period prior to spawning and incubation at 28±2°C

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	111.6 ± 5.6	27.3 ± 4.1	22.6 ± 5.4	161.5[a]	55.7
4	126. 4 ± 3.5	55.8 ± 3.8	37.1 ± 3.8	219.3[b]	75.6
8	127.9 ± 3.9	55.6 ± 9.1	38.0 ± 5.8	221.5[b]	76.4
12	88.2 ± 7.1	68.4 ± 8.5	28.9 ± 2.7	185.5[c]	64.0

The letters indicate significant differences to 95% ($p<0.05$) by the one way ANOVA Test.

Values in the same column followed by a different letter do differ significantly from each other. All values are means of five replicates

Table 29: Record of weight of mushroom, stipe and pileus length of *Pleurotus eous* strain P-31 and *P. ostreatus* strain EM-1 grown on rice straw only after different periods of composting at 28±2°C for up to 12 days and cultivation of mushroom for 42 day

Period of composting (day(s))	Average weight (g) of mushroom ± SE		Average width (mm) of pileus ± SE		Average length (mm) of stipe ± SE	
	P-31	***EM-1***	***P-31***	***EM-1***	***P-31***	***EM-1***
0	18.7 ± 2.4[a]	9.5 ± 2.2[a]	73.6 ± 5.4[b]	63.0 ± 3.5[d]	59.6 ± 3.5[ab]	46.2 ± 5.3[a]
4	15.5 ± 1.7[b]	12.4 ± 1.6[b]	66.5 ± 5.7[c]	64.4 ± 5.0[d]	57.9 ± 3.2[ab]	49.8 ± 4.8[a]
8	13.3 ± 1.9[b]	12.8 ± 1.7[b]	57.0 ± 6.6[f]	64.5 ± 2.7[d]	50.0 ± 3.9[c]	69.4 ± 3.5[b]
12	12.1 ± 1.5[b]	13.7 ± 2.1[b]	54.1 ± 6.3[f]	67.7 ± 2.0[d]	51.0 ± 2.9[c]	72.6 ± 3.1[b]

Only 0 day compost was statistically significant with respect to average weight for P-31 whereas there was no statistical difference (P=0.05) between data obtained for average stipe and cap length for P-31 and EM-1 respectively as well as average for EM-1

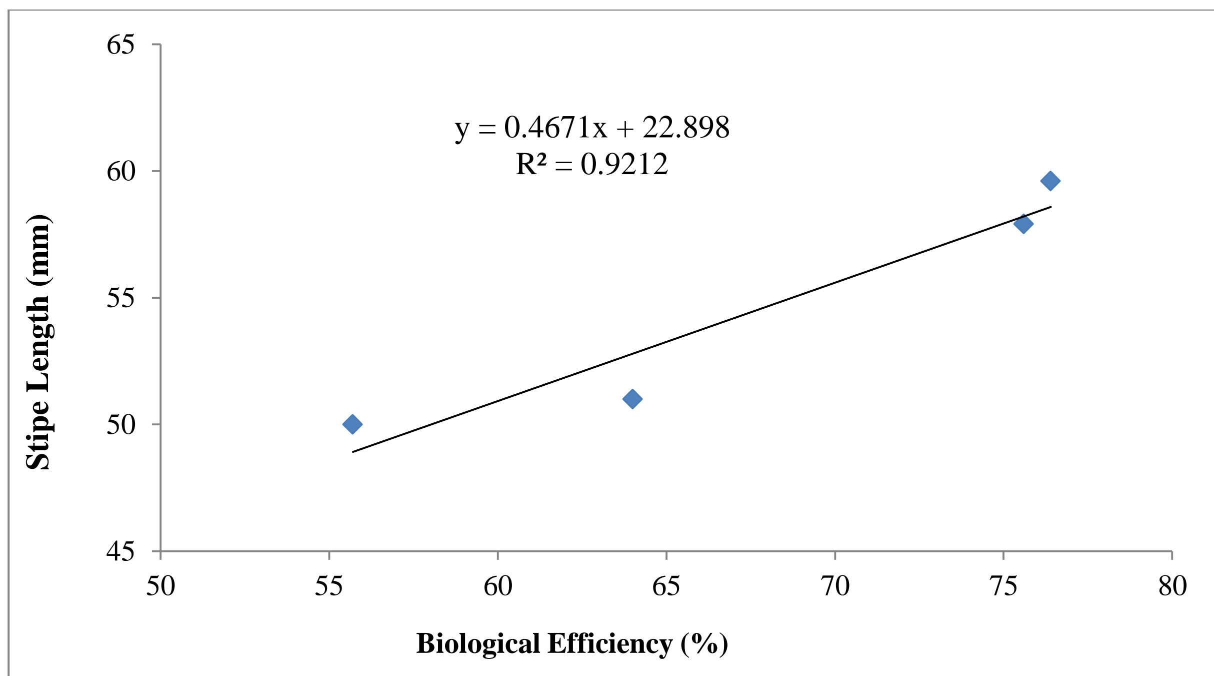


Fig 19: Correlation between Biological Efficiency and Stipe Length (mm) of *P. eous* grown on rice straw only

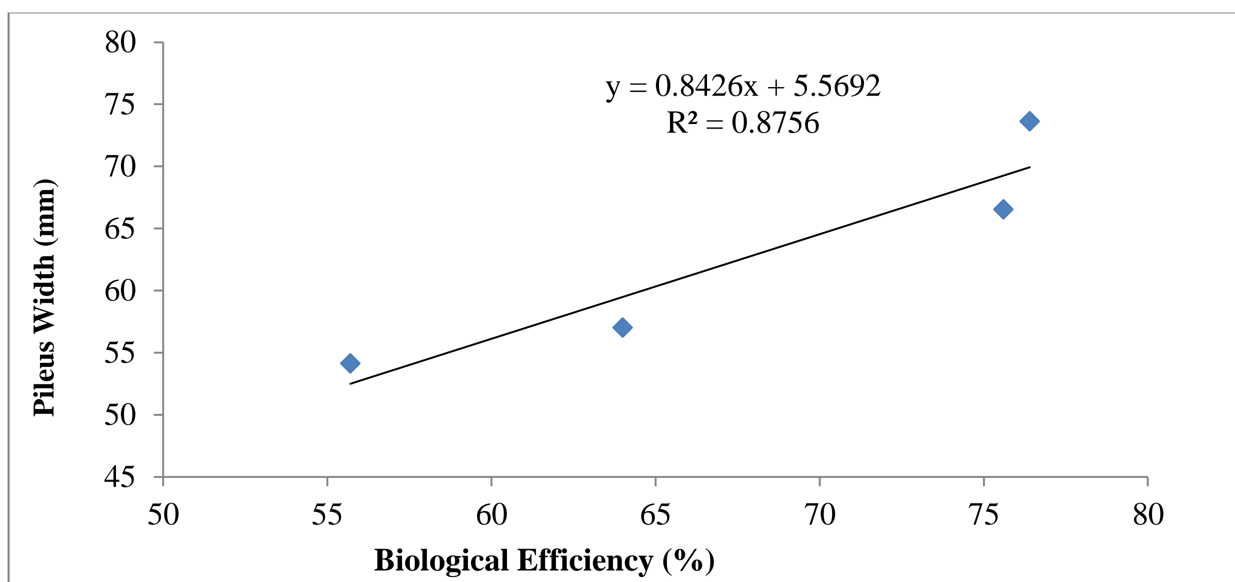


Fig 20: Correlation between Biological Efficiency and Pileus width (mm) of *P. eous* grown on rice straw only

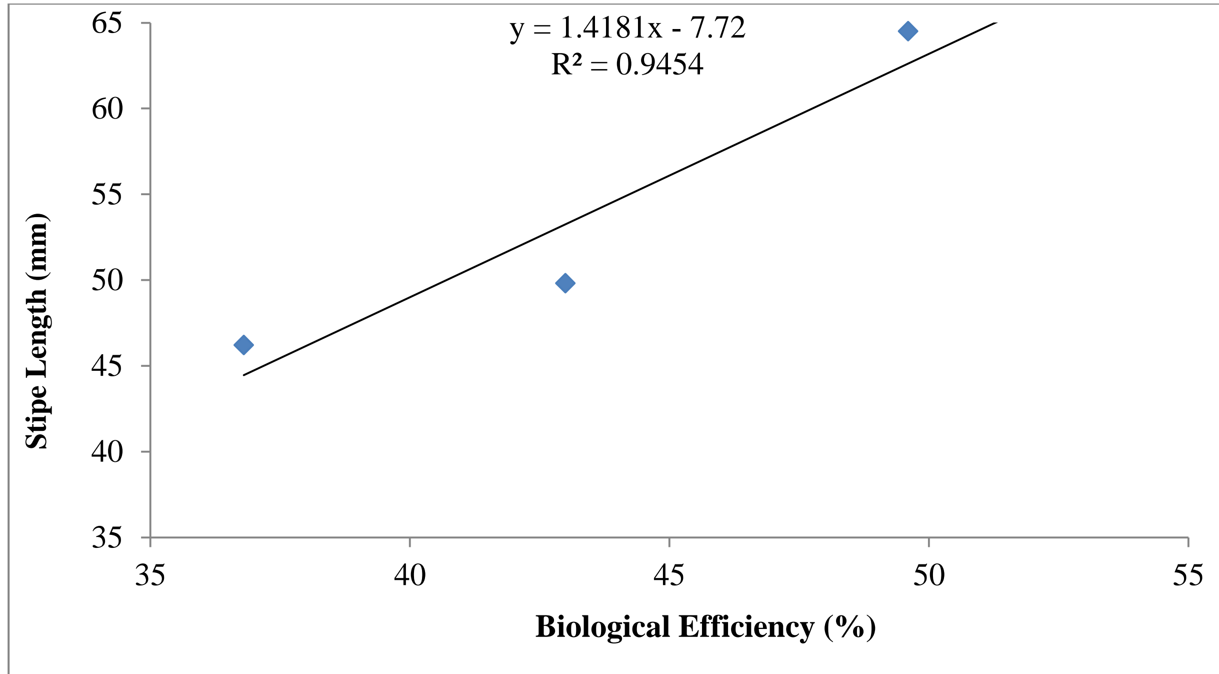


Fig 21: Correlation between Biological Efficiency and Stipe Length (mm) of *P. ostreatus* grown on rice straw only

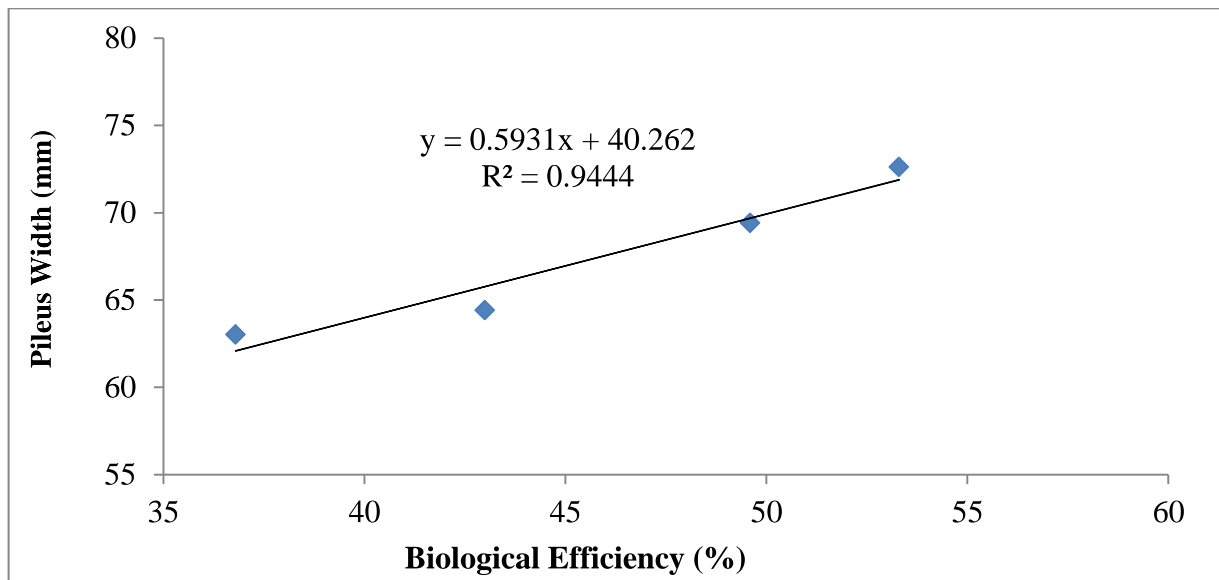


Fig 22: Correlation between Biological Efficiency and Pileus width (mm) of *P. ostreatus* grown on rice straw only

A

B

C

D

Plate 8A: Mycelia ramification of *P. eous* on unfermented rice husk after 3 weeks of inoculation

Plate 8B-D: Mycelia ramification of *P. eous* on 4, 8 and 12 days on fermented rice husk respectively after 3 weeks of inoculation (Mag x 0.05)

A

B

Plates A&B 9: Mycelia ramification of *Pleurotus ostreatus* on rice straw only substrate (top) Unfermented, (bottom) Composted for 4 days at 28±2°C and incubated for 3 weeks. (Mag. x0.05)

A

B

Plates 10A&B: Photograph of the fruit bodies of *Pleurotus ostreatus* grown on uncomposted rice straw substrate only (Top) and composted for 4 days (Bottom) prior to spawning with mycelium and incubated at 28±2˚C for 3 weeks. (Note the better formation of fruit bodies) (Mag. x0.05)

A.

B.

Plates 3a&b: Photograph of fruit bodies of *P. eous* P-31 in unfermented rice straw only (Top) and rice straw only fermented for 4 days (Bottom) prior to spawning with the mycelium of the mushroom and incubated at 28±2°C for 6 weeks (Mag x0.05)

Plate 11c: Photograph showing mature fruit bodies of *P. eous* P-31 growing in raw rice straw compost only fermented for 8 days incubated at 28±2°C for 6 weeks (Note the luxuriant growth of the pileus and the characteristic white colour of stipe and pileus) (Mag. x0.05)

EXPERIMENT 2

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods

The growth of *P. ostreatus* mycelium into the substrates was less prolific in the 8 and 12 days composted substrate in contrasts with *P. eous* where the growth of mycelium was uniformly white (Tables 30 and 31). Interestingly, the spawn run period for *P. ostreatus* on the same substrate with comparatively similar pH after sterilization was three times (9 weeks) as compared to 3 weeks for *P. eous* (Tables 30 and 31). *P. eous* grew almost twice as fast per week as *P. ostreatus*. The average mycelium growth rate / week for *P. eous* ranged between 9.0±0.5 cm to 10.0 ± 0.4cm whereas that of *P. ostreatus* were between 4.8 ± 0.3 to 6.2 ± 0.4 cm (Tables 30 and 31).

(ii & iii) Total number of fruit bodies and pinheads formed

The total number of fruit bodies after 3 flushes in the substrate formulation is shown in Table 32 (*P. ostreatus)* and Table 33 (*P. eous*). The total number of fruit formed by *P. eous* on this substrate (45-63) at all composted periods was nearly double what was formed by *P. ostreatus* (Tables 32 and 33).

Although large numbers of pinheads were formed in the compost seeded with *P. eous* (71-89 depending on the period of composting) fewer numbers (45-63 depending on the period of composting) yielded mature fruit bodies. The same trend was recorded for *P. ostreatus* cultures (Tables 34 and 35). Composting for 4-8 days seems to be adequate for the needed

good growth and fruits-bodies production. In all these instances, *P. eous* performed better than *P. ostreatus* as shown in Tables 34 and 35.

iv. Total yield and Biological Efficiency

Tables 36 and 37 present results obtained. Total yield and Biological Efficiency increased in composting periods at least up to 8 days and thereafter declined. The highest yield was obtained on this substrate after 8 days for *P. ostreatus* gave a yield of 160.6g and BE of 55.4% (Table 36). On the other hand *P. eous* produced the highest total yield of 209- 217.9g and BE of 72.3- 75.19% after 4 - 8 days respectively which was significantly ($P<0.05$) different from that which existed after 4days (131.8g, BE; 45.5%). The total yield of *P. ostreatus* and the BE on this substrate after 4 days was not significantly ($P>0.05$) different from what was obtained on the uncomposted substrate (Tables 36 and 37).

The best Biological Efficiency of 72.3-75.1% in 4 - 8 days obtained for *P. eous* on this substrate, approximates what was recorded on the raw rice straw only (BE 75.6-76.4%) after 4-8 days. The highest Biological Efficiency (55.4%) of *P. ostreatus* after 8days composting of this substrate is marginally higher than the BE 53.5% obtained on rice straw only seeded with the same mushroom on composted substrate.

The correlation coefficient derived from composting stipe length and pileus width with BE were positive and were good between R^2=0.882 to 0.9745. The BE values were high for *P. eous* (74-75%) as compared to 55.5% for *P. ostreatus* (Figs 23-26).

Table 30: Mycelial growth rate of *P. ostreatus* strain EM-1 on rice straw amended with 1% $CaCO_3$ and 10% rice bran at composting and bagging before sterilization and spawning for growth of mycelium at 28±2°C for 63 days

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/week)	Spawn run period (week)	pH of compost	
				Initial	After sterilization
0	+++	4.8 ± 0.3	9	5.1	7.2
4	+++	5.0 ± 0.4	9	5.4	7.4
8	++	4.9 ± 0.3	9	5.0	7.3
12	++	6.2 ± 0.4	9	5.5	7.2

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white in colour
++ Mycelium totally grows through the bag but not uniformly white in colour

Table 31: Mycelial growth rate of *P. eous* strain P-31 on rice straw amended with 1% $CaCO_3$ and 10% rice bran at bagging before sterilization and spawning for growth of mycelium at 28±2°C for 21 days

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/week)	Spawn run period (week)	pH of compost	
				Initial	After sterilization
0	+++	9.2 ± 0.1	3	6.9	7.7
4	+++	9.2 ± 0.1	3	6.8	7.9
8	+++	10.0 ± 0.4	3	6.8	7.9
12	+++	9.0 ± 0.5	3	6.8	7.8

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white in colour

Table 32: Total number of fruit bodies per plush of *P. ostreatus* EM-1growing on rice straw supplemented with 1% $CaCO_3$ and 10% rice bran composted for varying indicated periods before spawning and incubated at 28-30°C for 34 days

Period of composting (day(s)	Total no. of fruit bodies			Total No. of fruit bodies
	1st Flush	2nd Flush	3rd Flush	
0	13	8	6	27
4	12	8	6	26
8	14	11	7	32
12	13	10	9	32

Table 33: Total number of fruit body per flush of *P. eous* strain P-31growing on rice straw amended with 1% $CaCO_3$ and 10% rice bran composted for varying periods and before spawning and incubated at 28-30°C for 21 days

Period of composting / day(s)	Total no. of fruit bodies			Total No. of fruit bodies
	1st Flush	2nd Flush	3rd Flush	
0	32	7	6	45
4	39	17	7	63
8	23	14	9	46
12	30	14	11	55

Table 34: Total number of pinheads per flush of *P. eous* strain P-31 produced on rice straw supplemented with 1% $CaCO_3$ and 10% rice bran composted for varying indicated periods before bagging and sterilization for spawning and incubated at 28-30°C for 34 days

Period of composting	No. of pinheads			Total No. of pinheads
	1st Flush	2nd Flush	3rd Flush	
0	45	14	12	71
4	48	27	14	89
8	37	22	15	74
12	44	21	15	80

Table 35: Total number of pinheads per flush of *P. ostreatus* strain EM-1 produced on rice straw supplemented with 1% $CaCO_3$ and 10% rice bran at bagging and composted for varying indicated periods and sterilization before spawning and incubated at 28-30°C for 34 days

Period of composting	No. of pinheads			Total No. of pinheads
	1st Flush	2nd Flush	3rd Flush	
0	18	10	8	37
4	17	12	10	39
8	22	17	13	52
12	19	22	14	54

Table 36: Total yield and Biological efficiency of *P. ostreatus* strain EM-1 grown on rice straw substrate supplemented with 1% $CaCO_3$ and 10% rice bran at bagging and composted at varying indicated periods before sterilization and spawning for incubation at 28-30°C for 34 days

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	75.6 ± 4.7	31.2 ± 3.4	23.2 ± 3.1	130.0[a]	44.8
4	75.0 ± 6.9	30.4 ± 7.3	26.4 ± 6.2	131.8[a]	45.5
8	70.6 ± 6.4	51.4 ± 3.4	38.6 ± 2.9	160.6[b]	55.4
12	74.4 ± 7.5	54.5 ± 8.2	27.2 ± 8.2	156.1[b]	53.8

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test. Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.

Table 37: Total yield and Biological efficiency of *P. eous* strain P-31 grown on rice straw substrate supplemented with 1% $CaCO_3$ and 10% rice bran at bagging and composted at varying indicated periods before sterilization and spawning for incubation at 28-30°C for 34 days

Period of composting / day(s)	**Yield / Flush (g)**			**Total Yield (g)**	**Biological Efficiency (%)**
	1st Flush	**2nd Flush**	**3rd Flush**		
0	134.1 ± 1.8	37.3 ± 3.5	18.0 ± 1.2	189.4[a]	67.3
4	124.8 ± 2.4	56.3 ± 1.8	28.7 ± 3.8	209.8[b]	72.3
8	125.5 ± 3.8	55.2 ± 2.9	37.2 ± 4.7	217.9[b]	75.1
12	108.8 ± 7.5	56.0 ± 8.7	29.7 ± 6.9	194.5[a]	67.1

The letters indicate significant differences to 95% in accordance with one way ANOVA Test.Values in the same column followed by a different letter do differ significantly from each other. All values are means of five replicates

Table 38: Record of weight of mushroom, stipe and pileus length of *Pleurotus eous* strain P-31 and *P. ostreatus* strain EM-1 grown on different rice straw amended with 1% $CaCO_3$ and 10% rice bran after different periods of composting at 28±2°C up to 12 days

Period of composting (day(s))	Average weight (g) of mushroom ± SE		Average width (mm) of pileus / cap ± SE		Average length (mm) of stipe / stalk ± SE	
	P-31	***EM-1***	***P-31***	***EM-1***	***P-31***	***EM-1***
0	10.9±1.1 ^{a}	10.8±1.9 ^{a}	45.3±2.8^{a}	61.1±13.4^{a}	38.0±4.1 ^{a}	43.6±3.6^{a}
4	12.7±1.8 ^{a}	14.1±1.6 ^{b}	54.2±4.8^{b}	62.3±8.7^{a}	48.7±5.2 ^{b}	51.4±4.7^{b}
8	15.7±2.5 ^{b}	16.7±1.7 ^{c}	55.9±7.4^{b}	86.6±8.1^{b}	53.9±6.6^{b}	63.4±7.9^{c}
12	12.6±0.9 ^{a}	14.3±1.8 ^{b}	48.8±1.7^{a}	77.3±5.8^{c}	40.5±6.7^{a}	58.5±6.6^{c}

Values in the same column followed by a common letter do not differ significantly (P=0.05).

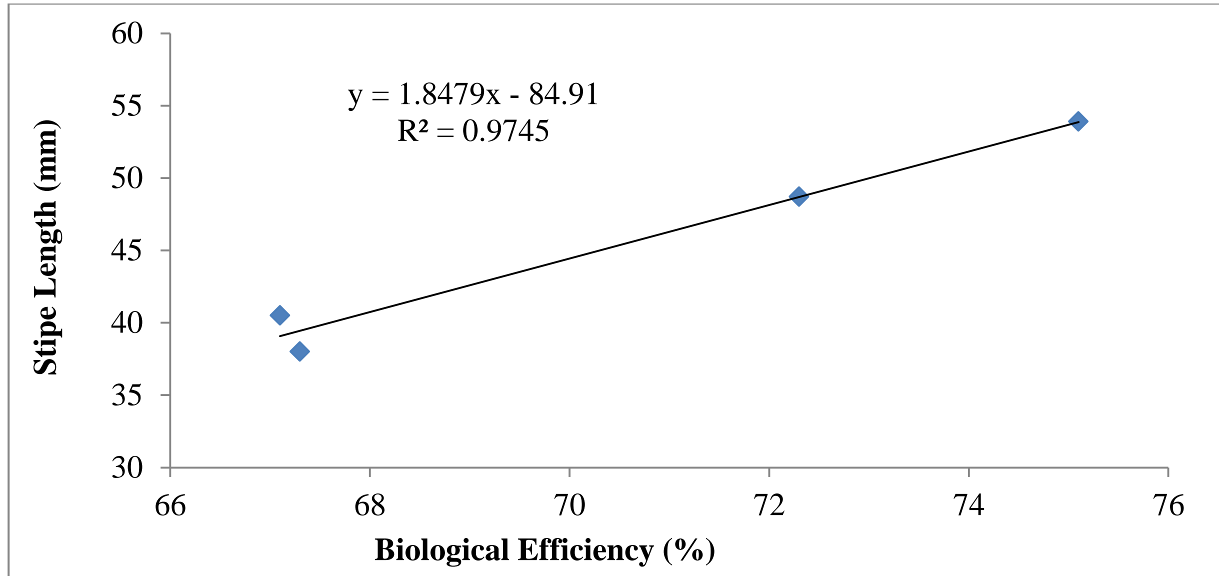


Fig 23: Correlation between Biological Efficiency and Stipe Length (mm) of *P. eous* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran

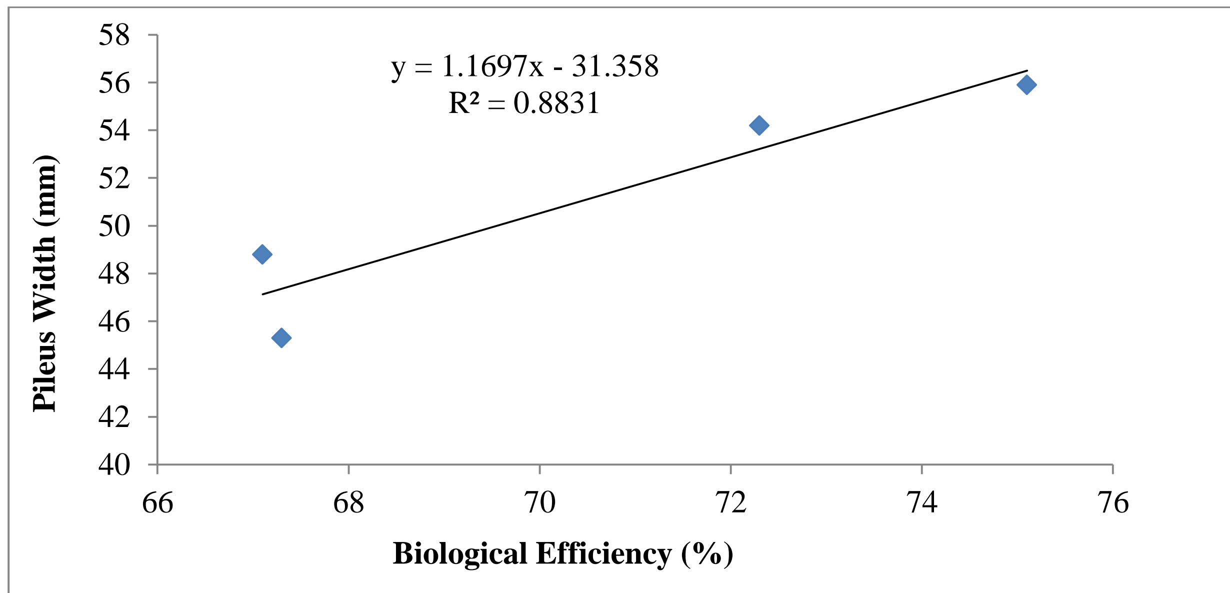


Fig 24: Correlation between Biological Efficiency and Pileus width (mm) of *P. eous* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran

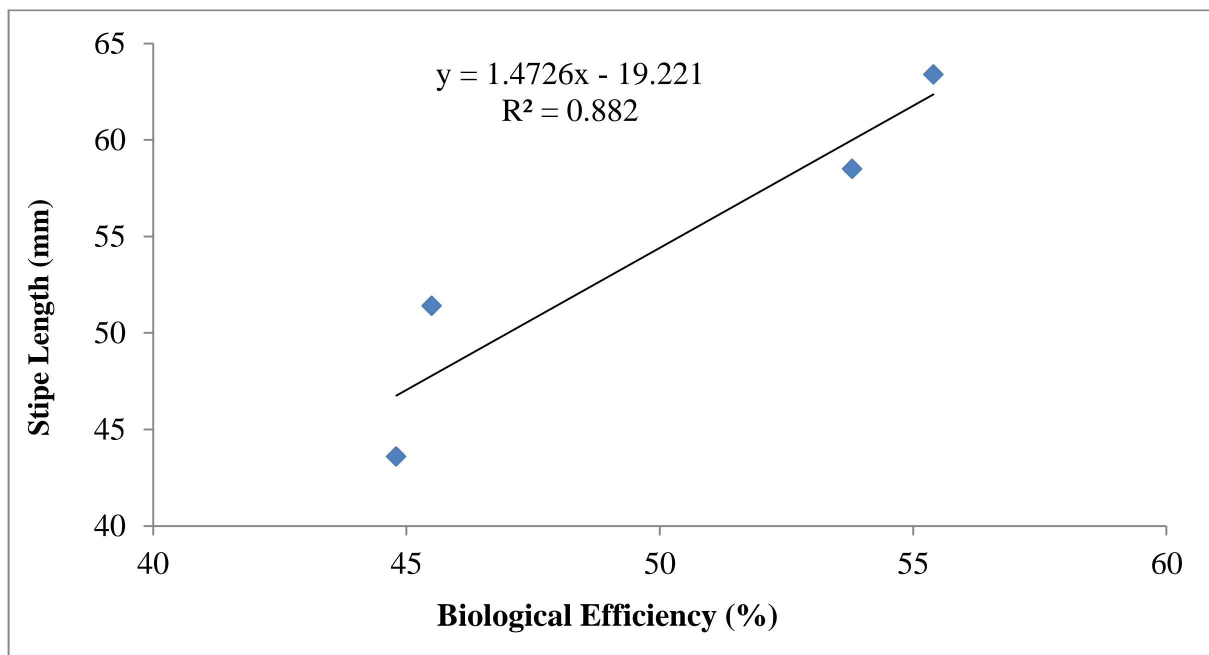


Fig 25: Correlation between Biological Efficiency and Stipe Length (mm) of *P. ostreatus* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran

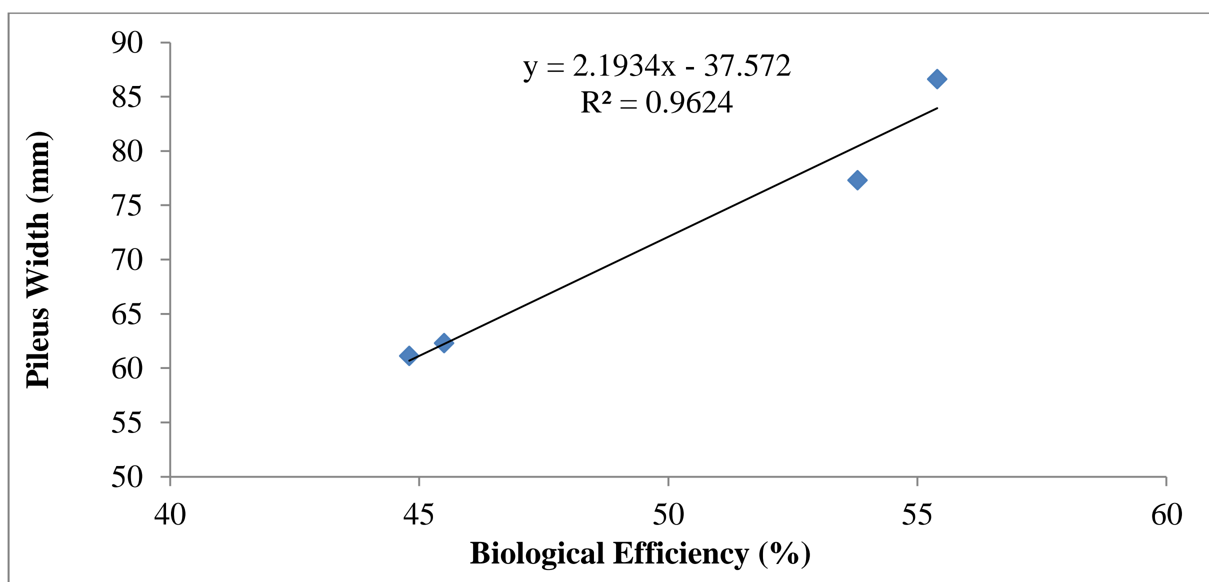


Fig 26: Correlation between Biological Efficiency and Pileus width (mm) of *P. ostreatus* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran

EXPERIMENT 3

Growth yield and Biological Efficiency of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 on rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods (0-12 days) prior to supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization.

i) Mycelial growth rate, mycelial density and spawn run period and pH of substrates

Mycelial growth rate of *P. ostreatus* EM-1 varied from 4.4±0.6cm week^{-1} (B_{15}) to 6.2±0.6cm week^{-1} (A_5) (Table 39). Treatment D_5 also gave nearly the same growth rate of 6.1±0.26cm week^{-1}. The spawn run time was the same (5 weeks) for all treatments but mycelium grew totally through the bags and was uniformly white in all combination of treatments except in C_{15} and $D_{10,}$ where the mycelium in the bags were not uniformly white. The pH of substrate before and after sterilization ranged from pH of 5.5 to 7.1 and after sterilization changed to pH 5.4 to 6.5.

In the case of *P. eous* P-31 the rate of growth of the mycelium varied from 7.9±0.4 cm/week (D_{10}) to 6.3±0.2 cm week^{-1} (D_{15}). Average mycelium growth in the variously treated compost was highest in the sample amended with 5% rice bran and composted 0-12 days (Table 40). The spawn run period was 4 weeks a week faster than what obtained with *P. ostreatus* on the same substrates.

ii) Total yield, Biological Efficiency, no. of fruiting bodies and pinheads formed

There was no statistical difference in Total yield obtained in treatments B_{10} & B_{15}, C_{10} and D_{10}, D_{15} and A_{15} which correspond to yield of 123.5-133.1g. The best yield 164.4g was obtained on C_5 followed by D_{10} (163.4g) (Table 41) for *P. ostreatus*. The best Biological Efficiency of 56.7% was obtained in C_5 followed by D_{10} (56.3%) (Table 41). The least B E was recorded in the uncomposted substrates A_5, $A_{10}$and A_{15} (i.e. BE: 29.0-29.7%) for *P. ostreatus* which does not seem to do well on uncomposted substrate (Table 41). The total number of pinheads formed from three flushes of *P. ostreatus* on the variously treated compost varied from 28 (A_5) to 49 (C_{15}). The no. of pinheads formed was influenced by the period of composting and the percentage of supplementation with rice bran. The same is true for the successful fruit bodies formed. The total no. of fruit bodies formed varied from 18 (A_5) to 34 (C_{15}). Generally the no. of fruit bodies declined. About 8-15 pinheads did not develop to fruiting body depending on the treatment of the compost (Tables 43 and 44). Thus the lowest no. of pinheads and fruit bodies were formed on the same substrate A_5 and the highest on C_{15}.

Flushing and pinhead formation was significantly higher ($p \leq 0.05$) for *P. eous* on the same substrates as *P. ostreatus*. The total no. of pinheads formed on the substrates varied from 60 (D_{15}) to 85 (A_5). This number declined when fruiting bodies were formed (Tables 43 & 44). The highest number of successful fruit bodies formed by *P. eous* was dependent on the treatment of the substrate. For example, the highest no. of fruiting bodies 54 were formed on uncomposted substrate treatments (A_5) 54 followed by 53 (B_{15}) 4 days; 53 (C_{15}) 8 days and

52 (A_{15}); the least formed were 42 (D_5), 41 (B_{15}) 8 days, 40 (D_{15}) 12 days. The number of unsuccessful fruiting body formation from pinhead was also variable ranging from 31 (C_{15}, D_5) to 19 (B_5). Percentage successful fruiting of *P. ostreatus* ranged from 63.4 – 74.4%. The value for successful fruiting of *P. eous* was 59.7 – 76.7% (Table 44).

iii) Correlation of stipe and pileus length and average weight

Table 45 shows the results. No clear cut trend was found in the average of mushroom, average diameter and average length of stipe for each mushroom except that there were significant differences ($p \leq 0.05$) between the values obtained for *P. ostreatus* and *P. eous*. *P. eous* (P-31) was doing better in terms of average weight, average diameter and average length of the stipe in most instances (depending on the amendment of substrate and composting period). However, in all instances, the ratio of cap diameter / stipe length was > 1 or ≤ 1.0.

Correlation of stipe length of *P. eous* with Biological Efficiency, $R^2 = 0.83$; correlation of pileus with BE of *P. eous* gave a high value of 0.9177 (Figs 26 & 27). The correlation coefficient of stipe length and pileus with BE of *P. ostreatus* were $R^2 = 0.6468$ and $R^2 = 0.9245$ respectively.

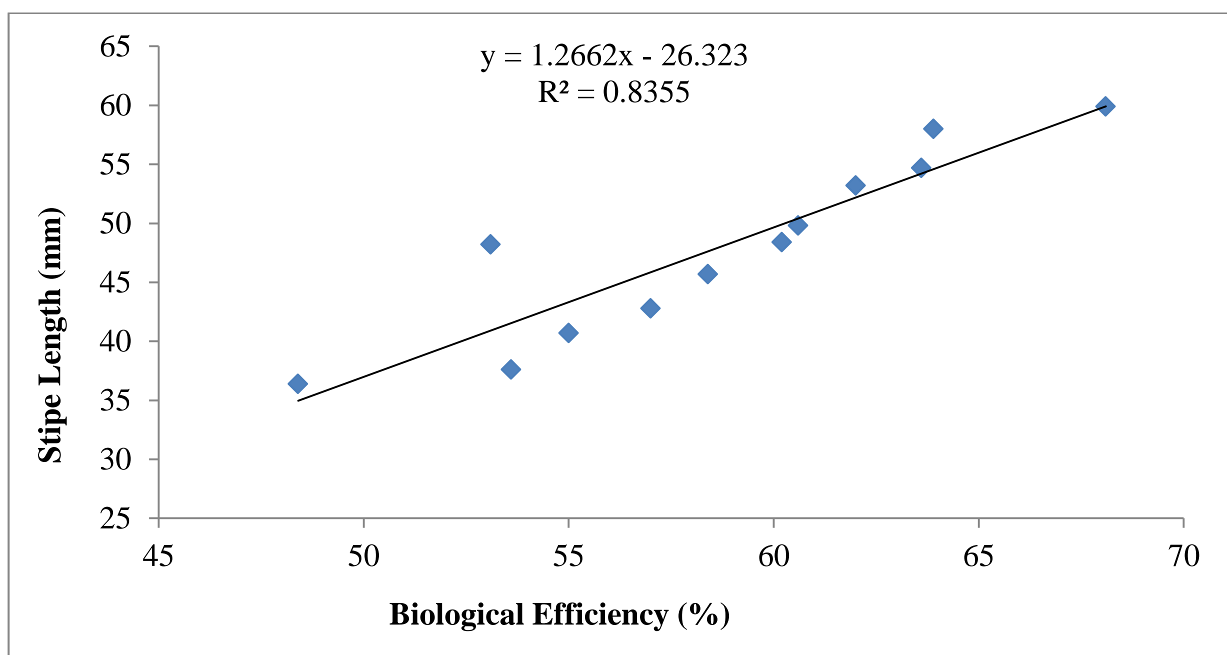


Fig 27: Correlation between Biological Efficiency and Stipe Length (mm) of *P. eous* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

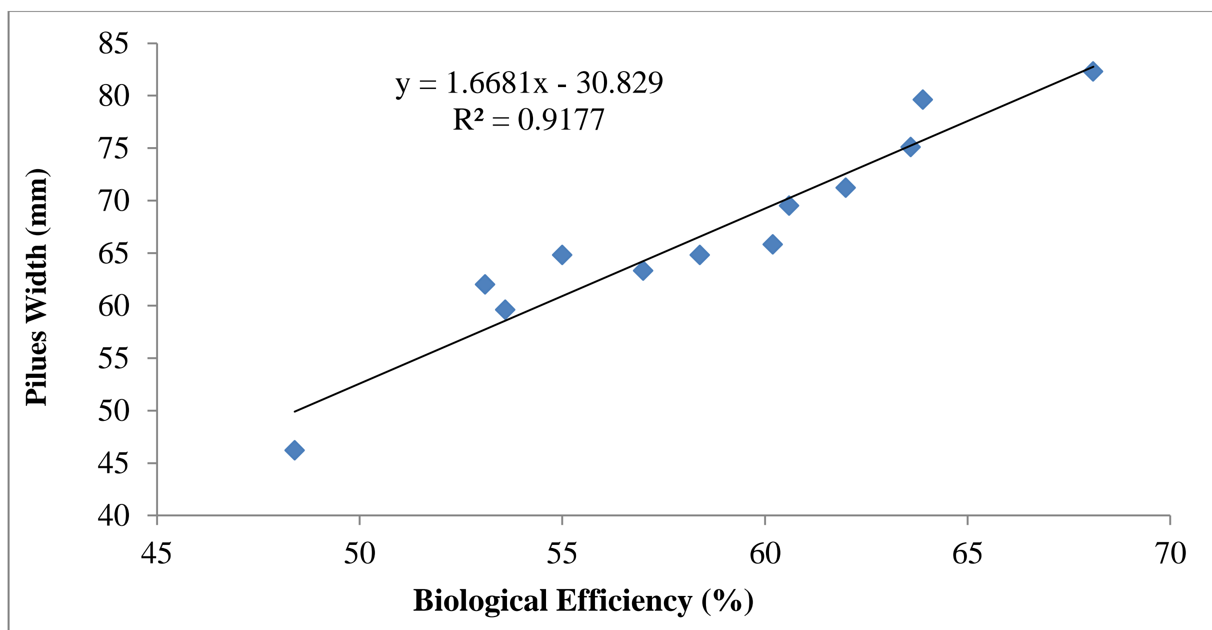


Fig 28: Correlation between Biological Efficiency and Pileus width (mm) of *P. eous* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

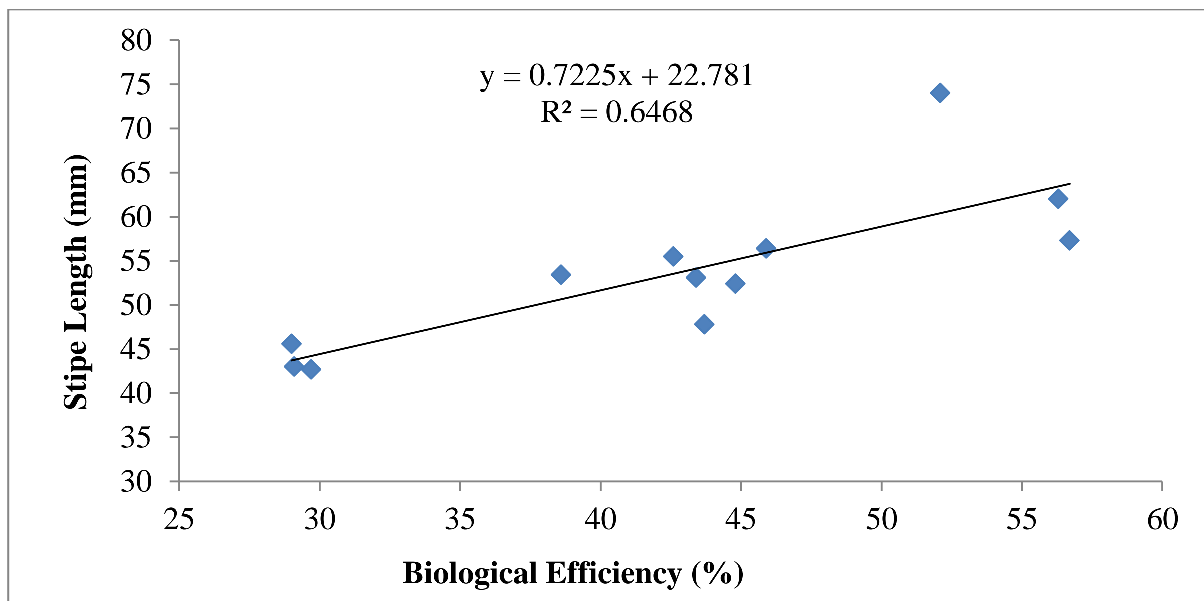


Fig 29: Correlation between Biological Efficiency and Stipe Length (mm) of *P. ostreatus* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

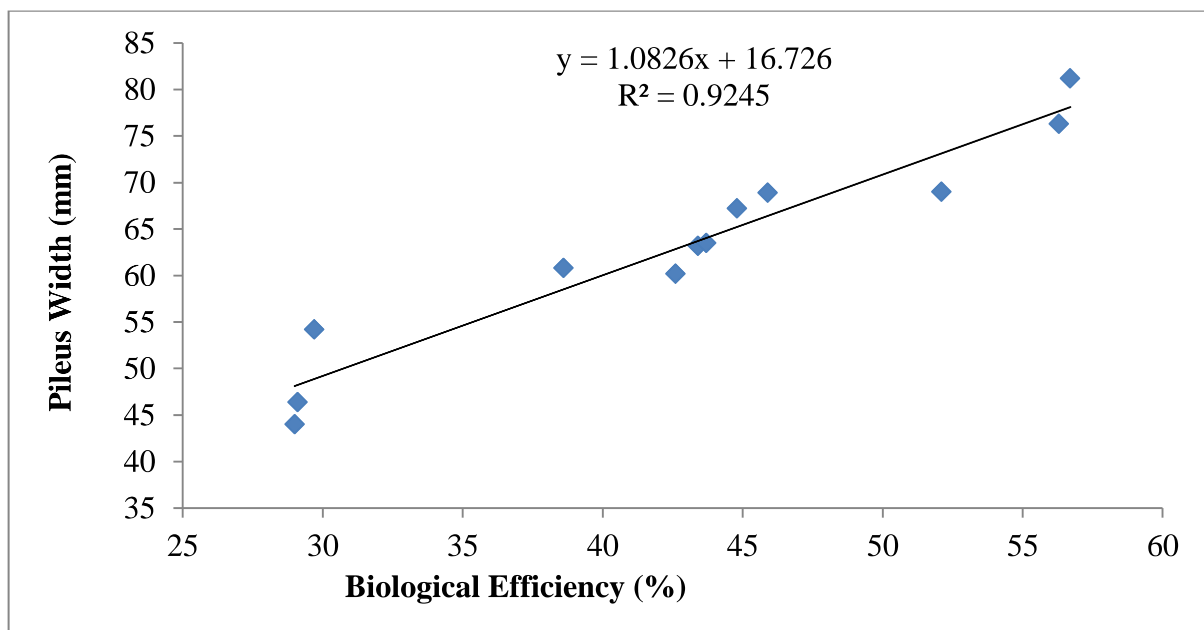


Fig 30: Correlation between Biological Efficiency and Pileus width (mm) of *P. ostreatus* grown on rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

Table 39: Mycelial growth rate of *P. ostreatus* strain EM-1 on rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior to supplementation with different amount of nitrogen (5, 10 and 15% rice bran) at bagging for sterilization.

Period of composting (day(s)	Treatments	Surface mycelial density	Average mycelia growth (cm/week) ± SE	Spawn run period (week)	pH before sterilization	pH after sterilization
0	A_{15}	+++	4.9 ± 0.8	5	6.8	6.5
	A_{10}	+++	5.9 ± 0.9	5	6.6	6.4
	A_5	+++	6.2 ± 0.6	5	6.7	6.5
4	B_{15}	+++	4.4 ± 0.6	5	7.0	6.9
	B_{10}	++	5.5 ± 0.7	5	6.9	6.3
	B_5	+++	4.7 ± 0.6	5	7.0	6.6
8	C_{15}	++	5.9 ±0.4	5	7.1	6.8
	C_{10}	+++	4.6 ± 0.2	5	7.3	6.9
	C_5	+++	5.4 ± 0.3	5	6.8	6.3
12	D_{15}	+++	4.9 ± 0.4	5	7.0	6.8
	D_{10}	++	5.6 ± 0.1	5	7.3	6.9
	D_5	+++	6.1 ± 0.2	5	7.0	6.7

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white
++ Mycelium totally grows through the bag but not uniformly white

Keys:

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 40: Mycelial growth rate of *P. eous* strain P-31 on rice straw amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior to supplementation with different amount of nitrogen (5, 10 and 15% rice bran) at bagging for sterilization

Period of composting (day(s)	Treatments	Surface mycelial density	Average mycelia growth (cm/week) ± SE	Spawn run period (week)	pH before sterilization	pH after sterilization
0	A_{15}	+++	6.4 ± 0.9	4	6.8	6.5
	A_{10}	+++	6.6 ± 0.2	4	6.6	6.4
	A_5	+++	7.4 ± 0.1	4	6.7	6.5
4	B_{15}	+++	5.1 ± 0.1	4	7.0	6.9
	B_{10}	+++	6.5 ± 0.2	4	6.9	6.3
	B_5	+++	7.3 ± 0.2	4	7.0	6.6
8	C_{15}	+++	6.5 ± 0.3	4	7.1	6.8
	C_{10}	+++	6.4 ± 0.2	4	7.3	6.9
	C_5	+++	6.6 ± 0.3	4	6.8	6.3
12	D_{15}	+++	6.3 ± 0.2	4	7.0	6.8
	D_{10}	+++	7.9 ± 0.4	4	7.3	6.9
	D_5	+++	7.7 ± 0.1	4	7.0	6.7

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white
++ Mycelium totally grows through the bag but not uniformly white
Keys:
A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 41: Total yield and Biological efficiency of *P. ostreatus* strain EM-1 on rice straw substrate amended with 1% $CaCO_3$and 10% rice bran during composting and supplemented with different proportions (5, 10 and 15% of rice bran) before sterilization

Period of composting / day(s)	Yield / Flush (g)				Total Yield (g)	Biological Efficiency (%)
	Treatments	1st Flush	2nd Flush	3rd Flush		
0	A_{15}	33.4 ± 6.8	28.7 ± 7.2	23.9 ± 4.0	86.0^{a}	29.7
	A_{10}	39.1 ± 4.8	28.5 ± 2.7	16.8 ± 3.3	$84.4.^{a}$	29.1
	A_5	41.6 ± 8.2	25.1 ± 4.0	23.3 ± 4.5	90.0^{a}	29.0
4	B_{15}	54.1 ± 10.0	42.8 ± 5.2	30.7 ± 4.2	127.6^{b}	43.7
	B_{10}	56.6 ± 5.4	41.3 ± 4.5	27.9 ± 9.2	125.8^{b}	43.4
	B_5	38.6 ± 8.0	45.4 ± 3.6	27.9 ± 5.1	111.9^{c}	38.6
8	C_{15}	45.9 ± 7.7	55.3 ± 6.5	49.8 ± 6.1	151.0^{d}	52.1
	C_{10}	54.8 ± 7.3	39.6 ± 10	38.7 ± 8.3	133.1^{b}	45.9
	C_5	71.5 ± 5.9	52.2 ± 6.5	40.7 ± 7.2	164.4^{e}	56.7
12	D_{15}	52.2 ± 8.1	44.1 ± 4.7	33.6 ± 9.7	129.9^{b}	44.8
	D_{10}	71.8 ± 4.1	49.9 ± 4.7	41.7 ± 3.1	163.4^{e}	56.3
	D_5	51.7 ± 7.1	40.5 ± 7.4	31.3 ± 4.0	123.5^{b}	42.6

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test.

Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.

Keys:

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 42: Total yield and Biological Efficiency (BE) of *P. eous* P-31 grown on rice straw substrate amended with 1% $CaCO_3$ and 10% rice bran supplemented with different (5, 10 and 15%) proportions of rice bran at bagging

Period of composting (day(s)	Treatments	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
		1st Flush	2nd Flush	3rd Flush		
0	A_{15}	91.4 ± 12.3	44.8± 7.0	23.4 ± 1.6	159.6[a]	55.0
	A_{10}	95.1 ± 10.8	39.1± 7.0	31.0 ± 6.0	165.2[b]	57.0
	A_5	125.0 ± 6.7	41.7± 4.4	30.9 ± 3.5	197.6[c]	68.1
4	B_{15}	78.7 ± 4.6	60.9± 3.6	35.1 ± 3.3	174.7[d]	60.2
	B_{10}	92.7± 11.8	46.1± 6.4	41.0 ± 6.4	179.8[d]	62.0
	B_5	101.1±20.0	48.7± 10.3	34.7 ±4.6	184.5[e]	63.6
8	C_{15}	70.3 ± 8.3	49.3± 4.4	34.3 ± 5.4	153.9[a]	53.1
	C_{10}	80.2 ± 5.4	36.5± 2.8	52.8 ± 4.3	169.5[b]	58.4
	C_5	63.7 ± 2.4	49.3± 4.4	42.5 ±7.5	155.5[a]	53.6
12	D_{15}	67.5 ± 12.4	40.4± 6.5	32.4 ± 4.5	140.3[f]	48.4
	D_{10}	94.0 ±7.5	46.6± 6.2	35.0 ± 7.4	175.6[d]	60.6
	D_5	85.7 ± 12.4	59.6± 8.1	40.1 ± 4.6	185.4[e]	63.9

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test.

Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.

Keys:

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 43: Total number of pinheads and fruiting bodies from three flushes by *P. ostreatus* EM-1 raised on rice straw amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportion (5, 10 and 15%) rice bran at bagging prior to sterilization.

Period of composting / day(s)	Treatments	No. of pinheads / fruit bodies per flush			Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
		1st Flush / fruiting bodies	2nd Flush / fruiting bodies	3rd Flush / fruiting bodies		
0	A_{15}	13 (9)	9 (6)	8 (6)	30 (21)	65.6
	A_{10}	15 (10)	9 (7)	8 (6)	32 (23)	71.9
	A_5	16 (10)	6 (4)	6 (4)	28 (18)	75.0
4	B_{15}	18 (12)	16 (11)	8 (6)	42 (28)	66.6
	B_{10}	18 (12)	12 (9)	7 (6)	37 (27)	73.0
	B_5	19 (12)	10 (8)	7 (6)	36 (26)	72.2
8	C_{15}	20 (13)	17 (12)	12 (9)	49 (34)	69.3
	C_{10}	20 (12)	11 (10)	11(7)	42 (29)	69.0
	C_5	20 (11)	13 (10)	8 (5)	41(26)	63.4
12	D_{15}	10 (8)	10 (6)	9 (6)	29 (20)	68.9
	D_{10}	25 (17)	11(8)	9 (7)	45 (32)	71.1
	D_5	22 (15)	11(9)	10 (8)	43 (32)	74.4

Keys: Value of fruit bodies are in parenthesis ( )

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 day

Table 44: Total number of pinheads and fruiting bodies from three flushes by *P. eous* P-31 raised on rice straw amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportion (5, 10 and 15%) rice bran at bagging prior to sterilization

Period of composting / day(s)	Treatments	No. of pinheads / fruit bodies per flush			Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
		1st Flush / fruiting bodies	2nd Flush / fruiting bodies	3rd Flush / fruiting bodies		
0	A_{15}	42 (33)	21 (13)	11 (6)	74 (52)	70.3
	A_{10}	38 (25)	20 (12)	14 (6)	72 (43)	59.7
	A_5	51 (35)	15 (10)	19 (9)	85 (54)	63.5
4	B_{15}	49 (30)	20 (14)	15 (9)	84 (53)	63.1
	B_{10}	42 (23)	13 (10)	15 (8)	70 (53)	75.7
	B_5	41(29)	18 (13)	14 (8)	73 (41)	56.2
8	C_{15}	42 (34)	15 (10)	15 (9)	72 (53)	73.6
	C_{10}	35 (27)	17 (8)	17 (9)	69 (44)	63.8
	C_5	34 (21)	18 (13)	20 (12)	72 (46)	63.9
12	D_{15}	31 (22)	17 (11)	12 (7)	60 (40)	66.7
	D_{10}	39 (27)	15 (8)	13 (6)	67 (41)	61.2
	D_5	34 (22)	17 (11)	17 (9)	68 (42)	61.8

Keys: Value of fruit bodies are in parenthesis ( )

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 day

Table 45: Record of weight of mushroom, stipe and pileus length of *Pleurotus eous* strain P-31 and *P. ostreatus* strain EM-1 grown on different rice straw amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportion (5, 10 and 15%) of rice bran prior to sterilization after different periods of composting at 28±2°C up to 12 days.

Compost treatment	Average weight (g) of mushroom ± SE		Average width (mm) of cap ± SE		Average length (mm) of stipe ± SE	
	P-31	***EM-1***	***P-31***	***EM-1***	***P-31***	***EM-1***
D_5	$24.0±6.2^a$	$9.7±1.9^a$	$82.3±10.2^a$	$44.0±6.8^a$	$59.9±14.0^a$	$42.7±6.7^a$
D_{10}	$12.9±1.4^b$	$10.3±2.8^a$	$63.3±7.7^b$	$46.4±5.7^a$	$42.8±5.9^b$	$43.0±3.7^a$
D_{15}	$12.8±3.3^b$	$11.1±2.7^a$	$64.8±8.4^b$	$54.2±10.2^b$	$40.7±2.6^b$	$45.6±4.7^a$
C_5	$17.8±3.1^c$	$12.5±3.2^a$	$75.1±20.7^c$	$60.8±7.8^c$	$54.7±4.7^c$	$47.8±5.2^a$
C_{10}	$17.4±4.8^c$	$13.4±3.5^a$	$71.2±7.2^c$	$63.2±9.9^c$	$53.2±6.2^c$	$53.1±7.8^b$
C_{15}	$14.2±2.5^d$	$13.9±2.1^a$	$65.8±4.0^b$	$63.5±11.9^c$	$48.4±6.7^d$	$53.4±1.8^b$
B_5	$11.4±2.7^e$	$20.2±6.7^b$	$59.6±3.6^c$	$81.2±13.4^d$	$37.6±5.7^e$	$74.0±8.2^c$
B_{10}	$13.9±2.1^d$	$15.5±3.3^c$	$64.8±10.4^b$	$68.9±4.7^e$	$45.7±9.0^d$	$56.4±9.5^b$
B_{15}	$10.4±2.2^e$	$16.1±3.4^c$	$62.0±5.6^b$	$69.0±10.3^e$	$48.2±3.7^d$	$57.3±7.1^b$
A_5	$17.8±4.7^c$	$12.7±2.6^a$	$79.6±17.3^a$	$60.2±2.9^c$	$58.0±6.7^a$	$52.4±7.1^b$
A_{10}	$14.4±2.6^d$	$18.2±6.4^d$	$69.5±10.2^c$	$76.3±4.9^d$	$49.8±11.8^d$	$62.0±10.5^c$
A_{15}	$10.1±1.5^e$	$14.5±2.6^c$	$46.2±5.5^d$	$67.2±13.2^e$	$36.4±5.0^e$	$55.5±5.4^b$

Values in the same vertical column followed by a common letter do not differ significantly (P=0.05).

EXPRERIMENT 4

Growth yield and Biological Efficiency of *P. ostreatus* EM-1 and *P. eous* P-31 on rice straw and rice husk (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization.

This experiment was same as Experiment 3. However in this instance rice husk was mixed with the rice straw in the ratio of 1:1 w/w and amended with 1% $CaCO_3$ and 10% rice bran and composted for 12 days. Results obtained are presented in Tables 46 to 52

i) **Mycelial growth rate, mycelial density and spawn run period and pH of substrate**

Mycelial growth rates of *P. ostreatus* EM-1 varied from 4.0± 0.2cm per week (D_{10}, D_{15}) to 6.0 ±0.3cm week^{1} (B_5) (Table 46). Treatment B_{15} also gave almost the same growth rate of mycelium 6.0 ±0.3cm week^{-1}. The spawn run period was the same (6weeks) for all but two of the treatments (B_5, B_{10}) where they lagged behind 1-2 weeks. The surface mycelial density was nearly the same for all treatments except C_{15} and D_{10} where the mycelium grew through the bags and was uniformly white. The pH of the substrate after sterilization ranged between 5.4 and 6.5.which falls within the optimum pH for growth of the two *Pleurotus* species.

The average mycelial growth rate of *P. eous* P-31 ranged from 4.8±0.2 cm week^{-1} (D_{10}) to 10.3± 0.2 cm week^{-1} (A_5) (Table 47). Treatment A_{10} allowed nearly the same growth rate of 10.1±0.2 cm week^{-1}. The unfermented compost (A_5) generally allowed better growth than

fermented compost (B-D). The spawn run period, 3 weeks was half of what obtained with *P. ostreatus* on the same substrate (Tables 46 and 47) except those grown on substrates fermented for 8-12 days, the surface mycelial density for *P. eous* was the same for all the treatments (A-D) where mycelium totally grew through the bag and was uniformly white. The pH of sterilized substrate ranged from pH 5.5 to 6.5.conducive for the growth of the mycelium.

ii) Total Yield Biological Efficiency, Number of Fruiting bodies and pinheads formed

Total yield of *P. ostreatus* in the variously formulated substrates and different composting periods using a mixture of rice straw and husk (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran varied with the period of composting. For example, the total yield of *P. ostreatus* EM-1 was between 179.5g- 183.5g obtained on substrate composted for 12 days (i.e. D_5, D_{10} and D_{15}). The lowest yields were recorded in uncomposted substrates (99.5-120.3g) and substrates composted for 4 days (112-134g) i.e. A_5– A_{15} and B_5-B_{15}. Values obtained on the substrates composted for 8 days were intermediate (Table 48). The Biological Efficiency recorded followed the same trend i.e. highest in substrates composted for 12 days (BE.57.8 - 63.39%) i.e. (D_5-D_{15}) and the lowest were recorded in uncomposted substrates BE 34.3 – 41.5 % and substrates composted for 4 days (BE 38.8-46.3%). Data for 8 days composted substrates were intermediate (BE 45.3 – 60.6%). The differences observed were statistically ($P<0.05$) significant. Thus *P. ostreatus* seen to do better on substrates composted for longer period than in the uncomposted medium (Table 48).

The converse was true, what was generally observed in *P. ostreatus* was the reverse recorded for *P. eous* P-31. Thus *P. eous* P-31 yielded better flushes and fruiting bodies on the uncomposted substrates i.e. A_5-A_{15} (154.7 – 211.0g) than in 12 days composted

substrates D_5-D_{15} (i.e. 108.4 – 113.9g) (Table 49). Thus, *P. eous* P-31 grows and yields better in uncomposted substrate formulation than in same medium composted for 4-12 days. Biological Efficiency values were highest (53.3-72.8%) in the uncomposted formulation than in the composted substrates (37.4-45.9%).

ii) Number of Pinheads and fruit bodies formed

Pinheads were formed by all the differently composted and formulated substrates and greater proportions were successfully converted into fruity bodies (Tables 50 and 51). The successful conversion from pinheads to mature fruiting bodies ranged from 31-71.1% (in *P. ostreatus* EM-1) and 64.1 – 74.1% (in *P. eous*. No generalization can be made. However the percentage conversion to pinhead to mature fruiting bodies was higher in 8-12 days composted substrate i.e. C_5-C_{15}; D_5-D_{15} (60.5 – 71.1 %) than in the uncomposted, A_5-A_{15} (60.5 – 68.9%). The lowest conversion of pinheads to mature fruiting bodies (31%) was recorded in 4days composted C_{15} samples in the case of *P. ostreatus*. Total number of pinheads and fruiting bodies formed was generally higher for *P. eous* than what existed for *P. ostreatus* (Table 51).

iii) Correlation of stipe and pileus length and average weight of mushroom

Generally there were marginal or no significant difference (P>0.05) within the same treatments block irrespective of period of composting for both species in relation to the average weight, average diameter of cap and average length of stipe (Table 52).

There was a strong positive correlation coefficient between stipe length, pileus width with BE of *P. eous* strain P-31 giving $R^2 = 0.935$ (stipe-BE) and $R^2 = 0.9107$ (pileus-BE) respectively (Figs 30 and 31). Lower correlation coefficient between stipe length values were obtained for *P. ostreatus* strain EM-1 on the same substrates for stipe length ($R^2 = 0.6346$) and pileus width ($R^2 = 0.570$) (Figs 32 and 33).

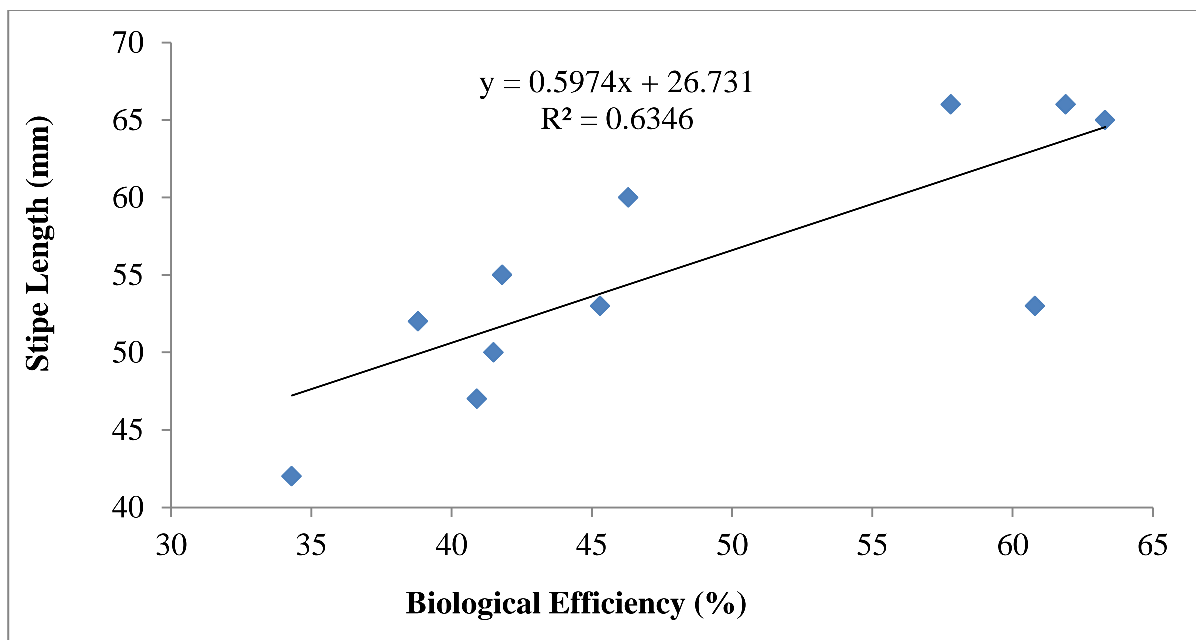


Fig 31: Correlation between Biological Efficiency and Stipe Length (mm) of *P. ostreatus* grown on rice straw and rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

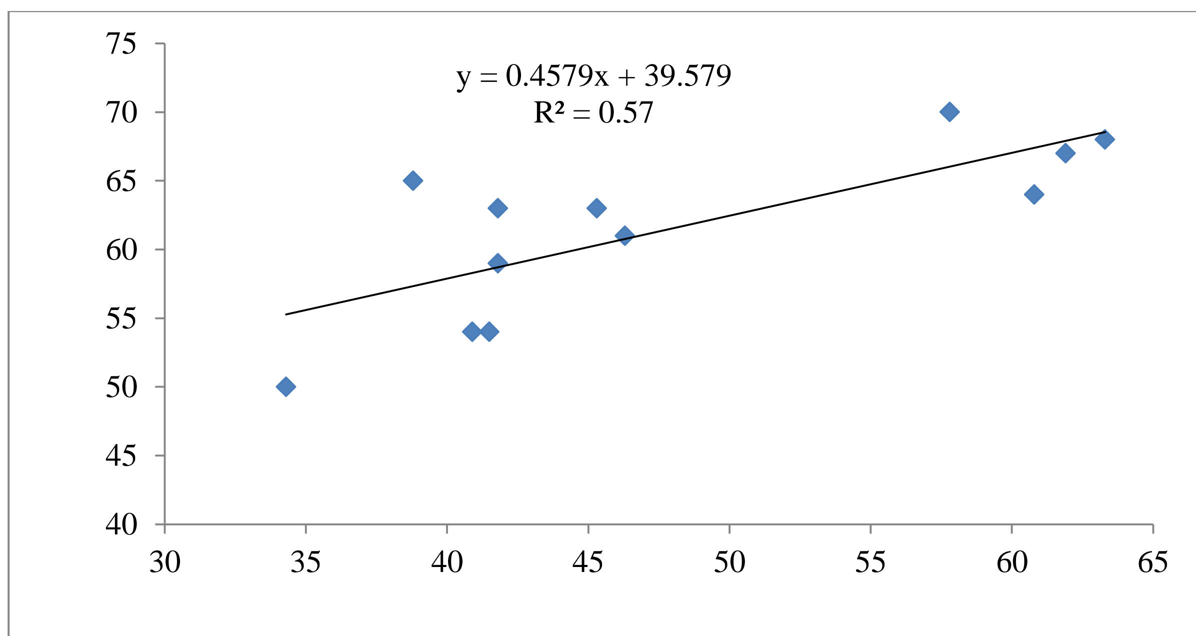


Fig 32: Correlation between Biological Efficiency and Pileus width (mm) of *P. ostreatus* grown on rice straw and rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

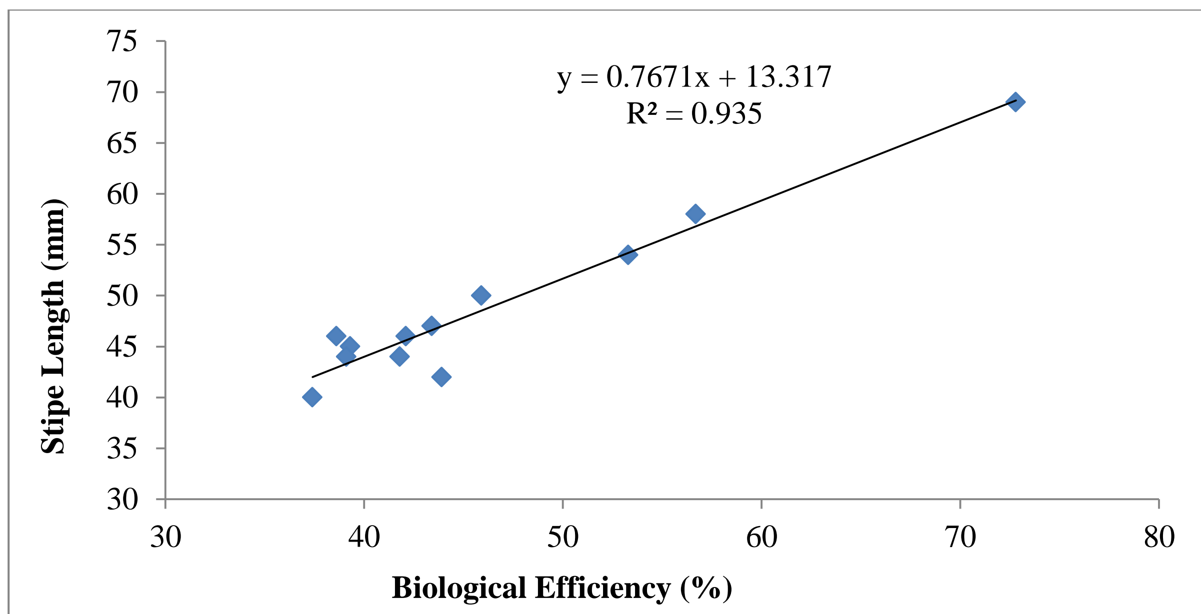


Fig 33: Correlation between Biological Efficiency and Stipe Length (mm) of *P. eous* grown on rice straw and rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

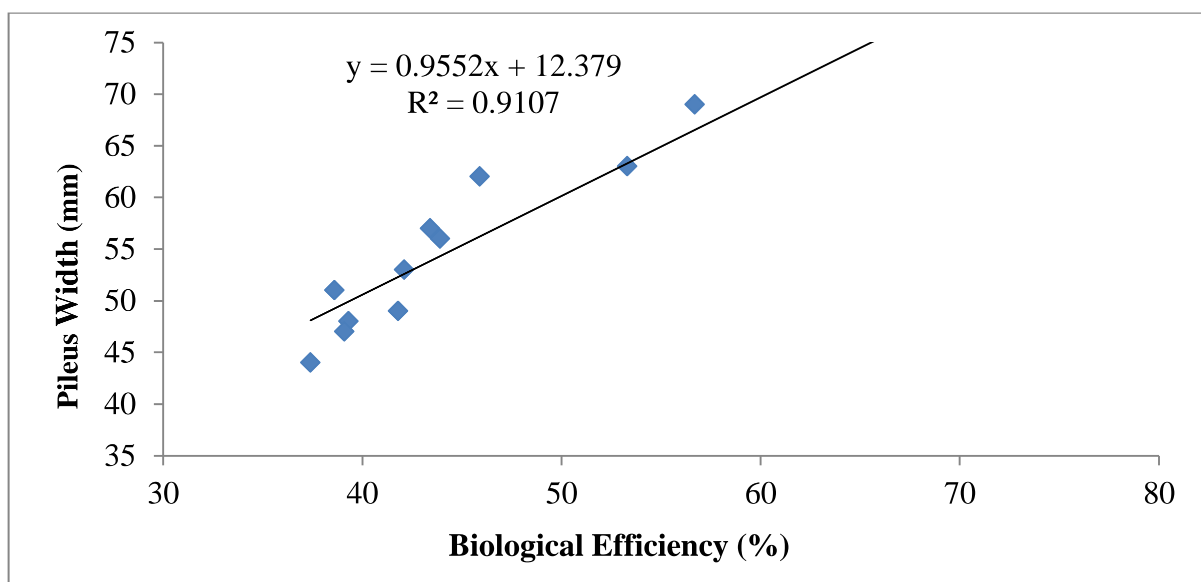


Fig 34: Correlation between Biological Efficiency and Pileus width (mm) of *P. eous* grown on rice straw and rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10, 15%) of rice bran was added

Table 46: Mycelial growth rate of *P. ostreatus* EM-1 on a mixture of rice straw and rice husk (1:1w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for the indicated period (0-12 days) prior to supplementation with different amount of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s))	Treatments	Surface mycelial density	Average mycelia growth (cm/weeks) ± SE	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	A_{15}	+++	5.8 ± 0.2	6	6.8	6.5
	A_{10}	+++	5.7 ± 0.2	6	6.6	6.4
	A_5	+++	7.2 ± 0.7	6	6.7	6.0
4	B_{15}	+++	6.0 ± 0.3	6	7.0	6.3
	B_{10}	+++	5.2 ± 0.4	5	6.7	6.3
	B_5	+++	6.2 ± 0.3	4	6.6	6.1
8	C_{15}	++	4.2 ± 0.1	6	6.9	6.4
	C_{10}	+++	5.3 ± 0.3	6	6.7	6.5
	C_5	+++	4.4 ± 0.3	6	6.8	6.3
12	D_{15}	+++	4.0 ± 0.2	6	7.0	6.5
	D_{10}	++	4.0 ± 0.3	6	7.1	6.1
	D_5	+++	4.8 ± 0.6	6	7.0	6.3

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white
++ Mycelium totally grows through the bag but not uniformly white

Keys:

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 47: Mycelial growth rate of *P. eous* P-31 on a mixture of rice straw and rice husk (1:1w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for the indicated period (0-12 days) prior to supplementation with different amount of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s))	Treatments	Surface mycelial density	Average mycelia growth (cm/weeks) ± SE	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	A_{15}	+++	9.7±0.4	3	6.8	6.5
	A_{10}	+++	10.1±0.2	3	6.6	6.4
	A_5	+++	10.3±0.2	3	6.7	6.0
4	B_{15}	+++	6.1±0.3	3	7.0	6.3
	B_{10}	+++	7.8±0.5	3	6.7	6.3
	B_5	+++	8.1±0.2	4	6.6	6.1
8	C_{15}	+++	7.4±0.6	4	6.9	6.4
	C_{10}	+++	5.9±0.3	4	6.7	6.5
	C_5	+++	5.7±0.3	4	6.8	6.3
12	D_{15}	+++	5.1±0.2	5	7.0	6.5
	D_{10}	+++	4.8±0.2	5	7.1	6.1
	D_5	+++	5.6±0.1	5	7.0	6.3

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white

Keys:
A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 days

Table 48: Total yield and Biological Efficiency of *P. ostreatus* EM-1 cultivated on rice straw amended with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior to supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s)	**Treatments**	**Yield / Flush (g)**			**Total Yield (g)**	**Biological Efficiency (%)**
		1st Flush	**2nd Flush**	**3rd Flush**		
0	A_{15}	43.8	31.2	24.5	99.5^{a}	34.3
	A_{10}	53.2	41.6	23.8	118.6^{b}	40.9
	A_{5}	75.6	30.5	14.2	120.3^{b}	41.5
4	B_{15}	51.7	51.0	31.6	134.3^{c}	46.3
	B_{10}	50.2	36.6	34.3	121.1^{b}	41.8
	B_{5}	52.2	34.9	25.5	112.6^{b}	38.8
8	C_{15}	61.9	57.4	56.5	175.8^{ab}	60.8
	C_{10}	65.3	40.7	15.2	121.2^{b}	41.8
	C_{5}	53.7	49.8	27.8	131.3^{c}	45.3
12	D_{15}	78.1	53.6	47.8	179.5^{ab}	61.9
	D_{10}	78.9	55.0	33.8	167.7^{ab}	57.8
	D_{5}	84.9	67.1	31.5	183.5^{ab}	63.3

Values in the same column followed by a common letter do not differ significantly.

All values are means of five replicates.

Table 49: Total yield and Biological Efficiency of *P. eous* P-31 cultivated on rice straw amended with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior to supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s))	Treatments	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
		1st Flush	2nd Flush	3rd Flush		
0	A_5	110.8 ± 3.0	66.3 ± 9.8	33.9 ± 5.0	211.0^a	72.8
	A_{10}	80.7 ± 1.9	51.2 ± 6.0	32.5 ± 4.6	164.4^b	56.7
	A_{15}	78.6 ± 5.2	49.8 ± 3.7	26.3 ± 2.5	154.7^c	53.3
4	B_5	65.1 ± 2.1	38.5 ± 4.5	29.9 ± 4.5	133.5^d	45.9
	B_{10}	64.0 ± 9.6	41.9 ± 3.0	20.5 ± 1.9	126.4^e	43.4
	B_{15}	60.8 ± 2.2	42.3 ± 3.5	24.1 ± 0.9	127.2^e	43.9
8	C_5	63.5 ± 4.6	38.4 ± 4.2	20.2 ± 3.5	122.1^e	42.1
	C_{10}	56.5 ± 6.2	34.9± 3.3	20.4 ± 3.6	111.8^f	38.6
	C_{15}	58.8 ± 4.2	42.2 ± 2.8	20.2 ± 3.5	121.2^e	41.8
12	D_5	53.8 ± 3.7	38.8 ± 5.1	21.3 ± 4.3	113.9^f	39.3
	D_{10}	53.6 ± 3.1	37.4 ± 4.4	22.8 ± 3.7	113.4^f	39.1
	D_{15}	52.6 ± 4.3	36.3 ± 5.8	19.3 ± 2.7	108.4^g	37.4

Values in the same column followed by a common letter do not differ significantly.

All values are means of five replicates.

Table 50: Total number of pinheads and fruiting bodies from three flushes by *P. ostreatus* EM-1 raised on rice straw and rice husk mixture (1:1w/w) amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportions (5, 10 and 15% of rice bran at bagging prior to sterilization.

Period of composting / day(s)	**Treatments**	**No. of pinheads / fruit bodies per flush**			**Total number of pinheads / fruiting bodies**	**% Conversion to fruiting bodies**
		1st Flush / fruiting bodies	**2nd Flush / fruiting bodies**	**3rd Flush / fruiting bodies**		
0	A_{15}	20 (15)	14 (9)	11(7)	45 (31)	68.9
	A_{10}	18 (10)	14 (10)	11 (6)	43 (26)	60.5
	A_5	24 (16)	14 (9)	11 (5)	49 (30)	61.2
4	B_{15}	18 (10)	15 (9)	12 (7)	45 (26)	57.8
	B_{10}	14 (8)	13 (7)	11 (7)	38 (22)	57.9
	B_5	18 (13)	08 (5)	11 (6)	37 (24)	64.9
8	C_{15}	19 (13)	15 (9)	15 (8)	49 (30)	61.2
	C_{10}	19 (12)	11 (7)	08 (4)	38 (23)	60.5
	C_5	11 (9)	12 (7)	10 (6)	33 (22)	66.7
12	D_{15}	23 (17)	12 (8)	13 (8)	48 (33)	68.8
	D_{10}	19 (15)	14 (10)	12 (7)	45 (32)	71.1
	D_5	23 (16)	30 (20)	10 (7)	63 (43)	68.3

Keys: Values of fruiting bodies are in parenthesis ( )

A_{15} rice straw supplemented with 15% rice bran at bagging; No composting (Day 0)
A_{10} rice straw supplemented with 10% rice bran at bagging; No composting (Day 0)
A_5 rice straw supplemented with 5% rice bran at bagging; No composting (Day 0)
B_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 4 days
B_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 4 days
B_5 rice straw supplemented with 5% rice bran at bagging; Composted for 4 days
C_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 8 days
C_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 8 days
C_5 rice straw supplemented with 5% rice bran at bagging; Composted for 8 days
D_{15} rice straw supplemented with 15% rice bran at bagging; Composted for 12 days
D_{10} rice straw supplemented with 10% rice bran at bagging; Composted for 12 days
D_5 rice straw supplemented with 5% rice bran at bagging; Composted for 12 day

Table 51: Total number of pinheads and fruiting bodies from three flushes by *P. eous* P-31 raised on rice straw and rice husk mixture (1:1w/w) amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportions (5, 10 and 15% of rice bran at bagging prior to sterilization.

Period of composting / day(s)	**Treatments**	**No. of pinheads / fruit bodies per flush**			**Total number of pinheads / fruiting bodies**	**% Conversion to fruiting bodies**
		1st Flush / fruiting bodies	**2nd Flush / fruiting bodies**	**3rd Flush / fruiting bodies**		
0	A_{15}	50 (38)	27 (20)	12 (8)	89 (66)	74.1
	A_{10}	34 (23)	21 (14)	16 (9)	71 (46)	64.8
	A_5	37 (24)	25 (15)	16 (12)	78 (51)	65.4
4	B_{15}	24 (14)	14 (10)	12 (8)	50 (34)	68.0
	B_{10}	25 (18)	16 (13)	12 (7)	53 (38)	71.7
	B_5	26 (15)	14 (10)	10 (8)	50 (33)	66.0
8	C_{15}	26 (17)	16 (11)	9 (6)	51 (34)	66.7
	C_{10}	21 (14)	10 (10)	9 (5)	40 (29)	72.5
	C_5	22 (16)	14 (10)	11 (6)	47 (32)	68.1
12	D_{15}	30 (20)	13 (8)	10 (8)	53 (30)	58.5
	D_{10}	27 (18)	14 (10)	10 (7)	51 (35)	68.2
	D_5	27 (18)	15 (10)	11 (6)	53 (34)	64.1

Keys: Values of fruiting bodies are in parenthesis **( )**

Table 52: Record of weight of mushrooms, stipe length and pileus width of *P. eous* strain P-31 and *P. ostreatus* strain EM-1grown on rice straw amended with rice husk (1:1 w/w) and supplemented with 1% $CaCO_3$ and 10% rice bran at composting and amended with varying proportion (5, 10 and 15%) of rice bran prior to bagging prior to sterilization

Compost treatment	**Average weight (g) of mushroom ± SE**		**Average width (mm) of cap ± SE**		**Average length (mm) of stipe ± SE**	
	P-31	***EM-1***	***P-31***	***EM-1***	***P-31***	***EM-1***
$\mathbf{A_5}$	$15.9±3.9^a$	$12.0±2.6^a$	$79.0±9.0^a$	$54.0 ±5.0^a$	69.0±9.0a	$50.0±8.0^a$
$\mathbf{A_{10}}$	$14.5±2.3^a$	$12.4±2.6^a$	$69.0±10.0^a$	$54.0±6.0^a$	$58.0±2.0^b$	$47.0±4.0^a$
$\mathbf{A_{15}}$	$14.1±3.0^a$	$13.4±3.5^a$	$63.0±10.0^b$	$50.0±3.0^a$	$54.0±4.0^b$	$42.0±8.0^b$
$\mathbf{B_5}$	$11.5±2.1^b$	$13.5±3.7^a$	$62.0±10.0^b$	$65.0±5.0^b$	$50.0±7.0^b$	$52.0±10.0^a$
$\mathbf{B_{10}}$	$8.3±1.7^c$	$14.8±2.2^a$	$57.0±8.0^c$	$63.0±8.0^b$	$47.0±6.0^b$	$55.0±5.0^a$
$\mathbf{B_{15}}$	$12.3±1.4^b$	$9.9±1.8^b$	$56.0±11.0^c$	$61.0±12.0^b$	$42.0±6.0^c$	$60.0±6.0^c$
$\mathbf{C_5}$	$11.0±1.7^b$	$14.9±2.6^a$	$53.0±8.0^c$	$63.0±8.0^b$	$46.0±.6.0^b$	$53.0±8.0^a$
$\mathbf{C_{10}}$	$11.8±1.2^b$	$13.3±2.2^a$	$51.0±5.0^c$	$59.0±6.0^c$	$46.0±5.0^b$	$52.0±11.0^a$
$\mathbf{C_{15}}$	$11.5±2.9^b$	$10.3±2.5^c$	$49.0±9.0^c$	$64.0±9.0^b$	$44.0±5.0^c$	$53.0±8.0^a$
$\mathbf{D_5}$	$8.0±2.0^c$	$13.7±2.2^a$	$48.0±6.0^c$	$68.0±14.0^b$	$45.0±7.0^b$	$65.0±12.0^c$
$\mathbf{D_{10}}$	$10.5±1.5^b$	$14.6±3.7^a$	$47.0±5.0^c$	$70.0±6.0^a$	$44.0±7.0^c$	$66.0±9.0^c$
$\mathbf{D_{15}}$	$9.4±1.6^c$	$13.8±2.8^a$	$44.0±8.0^d$	$67.0±9.0^b$	$40.0±6.0^c$	$66.0±8.0^c$

Values in the same vertical column followed by a common letter do not differ significantly (P=0.05).

EXPERIMENT 5

Growth yield and Biological Efficiency of *P. ostreatus* EM-1 and *P. eous* P-31 on unamended Engelberg Mixture (rice husk and rice bran 2:1w/w) composted for varying periods prior to bagging.

i. Total yield and Biological Efficiency

The best yield and Biological Efficiency of *P. ostreatus* on unsupplemented Engelberg mixture was obtained on the uncomposted substrate (88.4g and BE=35.2%) and the least was obtained on both the composted for 8days and 12days respectively (55.6g and BE=22.2%). The differences observed were statistically significant ($P<0.05$) (Table 53).

On the other hand, the best yield of *P. eous* on the same unsupplemented Engelberg mixture was obtained on uncomposted substrate (131.5g BE=52.6%). Total yield from three flushes on substrate composted for 4 - 8days were slightly inferior but close to the value on the composted substrate (121.8-109.7g BE=48.7-43.9%). Clearly, *P. eous* performed better on Engelberg mixture only and the difference obtained between the performance of the two *Pleurotus* species on same substrate were statistically ($P\leq0.05$) significant (Table 54).

ii. Spawn run and mycelial growth rate and pH of substrate

It took 4 weeks for the spawn of *P. ostreatus* and *P. eous* to cover the substrate. However, the mycelium totally grew through the uncomposted substrate and was uniformly white (Tables 55&56).

The average mycelia growth by *P. ostreatus* was 7.3±0.9cm/week on the uncomposted substrate with the least recorded on the substrate composted for 12days (5.1±0.3 cm $week^{-1}$).

The same trend was obtained with *P. eous* on the same substrate. However, average growth of mycelium on the uncomposted substrate was significantly higher ($8.9 \pm 0.4 cmwk^{-1}$) ($P<0.05$). The pH of the substrate ranges from 5.4 - 6.6 which provides optimum condition for growth of the mycelium.

iii) Number of Pinheads and fruit bodies formed

Pinheads were formed by all the differently formulated composted and uncomposted substrates and more than 50% of pinheads formed were successfully converted into fruiting bodies (Tables 57 and 58) in both *Pleurotus* species. The successful conversion from pinheads to mature fruiting bodies in *P. ostreatus* ranged from 52.1-67.1% and 60.9 –73.5% for *P. eous*. There was higher percentage conversion of pinheads into mature fruiting bodies in the uncomposted substrate for *P. ostreatus* (67.1%) and *P. eous* (73.5%) respectively. However, *P. eous* had the highest conversion of pinheads to mature fruit bodies than *P. ostreatus* on the same substrates (Tables 57 and 58).

Table 53: Total yield and Biological Efficiency of *P. ostreatus* strain EM-1 grown on unsupplemented Engelberg mixture (rice husk: rice bran 2:1w/w) composted for varying periods

Period of composting / day(s)	**Yield / Flush (g) after**			**Total Yield (g)**	**Biological Efficiency (%)**
	1st Flush	**2nd Flush**	**3rd Flush**		
0	51.1 ± 4.7	26.8 ± 4.8	10.5 ± 2.6	88.4[a]	35.2
4	46.4 ± 5.4	20.7 ± 3.9	11.4 ± 2.0	78.5[a]	31.4
8	25.7 ± 4.8	20.3 ± 3.6	9.6 ± 2.0	55.6[b]	22.2
12	28.9 ± 3.6	14.9 ± 2.8	11.8 ± 3.0	55.6[b]	22.2

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test. Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.

Table 54: Total yield and Biological Efficiency of *P. eous* P-31 grown on unsupplemented Engelberg mixture (rice husk: rice bran 2:1w/w) composted for varying periods

Period of composting / day(s)	Yield / Flush (g) after			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	63.9±11.0	45.5±4.3	22.1±7.5	131.5[a]	52.6
4	57.5±7.2	42.6±9.0	21.7±5.8	121.8[b]	48.7
8	59.0±3.2	30.4±7.3	29.1±4.4	118.5[b]	47.4
12	65.2±7.5	27.1±3.1	17.4±5.1	109.7[c]	43.9

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test. Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.

Table 55: Mycelial growth rate of *P. ostreatus* strain EM-1 on Engelberg mixture substrate only composted for varying period of days

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/week)±SE	Spawn run period (weeks)	pH of substrate	
				before sterilization	after sterilization
0	+++	7.3±0.90	4	5.5	5.4
4	++	6.0±0.80	4	6.6	6.4
8	+	5.5±0.25	4	6.5	6.3
12	+	5.1±0.38	4	6.4	6.0

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white

+ Poor patchy growth

Table 56: Mycelial growth rate of *P. eous* strain P-31 on Engelberg mixture substrate only composted for varying period of days

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/weeks)±SE	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	+++	8.9±0.44	4	5.5	5.4
4	++	8.4±0.38	4	6.6	6.4
8	+	6.2±0.24	4	6.5	6.3
12	+	5.1±0.13	4	6.4	6.0

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white++ Mycelium totally grows through the bag but not uniformly white

+ Poor patchy growth

Table 57: Total number of pinheads and fruiting bodies from three flushes by *P. ostreatus* EM-1 cultivated on unsupplemented Engelberg mixture (rice husk: rice bran 2:1w/w) composted for varying periods

Period of composting (days)	No. of pinheads/ fruiting bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion to fruiting bodies
	1st Flush	2nd Flush	3rd Flush		
0	40 (26)	24 (15)	12 (10)	76 (51)	67.1
4	38 (24)	21 (10)	14 (8)	73 (42)	57.5
8	37 (21)	19 (10)	15 (7)	71 (38)	53.5
12	38 (19)	21 (10)	14 (9)	73 (38)	52.1

Table 58: Total number of pinheads and fruiting bodies from three flushes by *P. eous* P-31 cultivated on unsupplemented Engelberg mixture (rice husk: rice bran 2:1w/w) composted for varying periods

Period of composting (days)	No. of pinheads / fruiting bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion to fruiting bodies
	1st Flush	2nd Flush	3rd Flush		
0	18 (10)	15 (9)	11 (6)	34 (25)	73.5
4	28 (19)	25 (17)	9 (4)	62 (40)	64.5
8	38 (27)	16 (11)	14 (10)	68 (48)	70.6
12	17 (11)	14 (8)	15 (9)	46 (28)	60.9

EXPERIMENT 6

Growth yield and Biological Efficiency of *P. ostreatus* EM-1 and *P. eous* P-31 on Engelberg Mixture (rice husk and rice bran 2:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran at composting before bagging for sterilization and inoculation

i) Total yield and Biological Efficiency

The best yield and Biological Efficiency of *P. ostreatus* on supplemented Engelberg Mixture was obtained on unfermented compost (yield=113.8g; BE=45.5%) and the least was recorded on both 8 and 12 days respectively (yield=107.0 and 107.1; BE= 42.8%). The differences observed were statistically significant ($p \leq 0.05$) (Table 63).

P. eous performed best on uncomposted substrate (yield=122.2g; BE= 48.9%) followed by substrate composted for 4 days (yield 92.9g; BE=36.8%) (Table 64). There was no statistical difference $p \geq 0.05$ between growth yield and Biological efficiency obtained on the substrate composted for 8 and 12 days respectively.

ii) Mycelial growth rate, mycelial density, spawn run period and pH of substrate

Mycelial growth was marginally better in the case of *P. eous* (7.0±0.75 cm $week^{-1}$) growing on the uncomposted amended Engelberg mixture as compared to *P. ostreatus* on the same substrate (6.1±0.56 cm $week^{-1}$) (Tables 59 and 60). The surface mycelial density in this instance was the best for both species growing on the amended Engelberg mixture composted for 4 days (Tables 59 & 60). The average mycelial growth was poor and patchy on the amended Engelberg mixture composted for 8-12 days in both *Pleurotus* species (i.e. 4.6±0.27 cm $week^{-1}$ 6.0±0.4 cm $week^{-1}$ although it took 4 weeks to cover the bags in all

instances (Tables 59 and 60). The pH of the substrate before and after sterilization was similar.

iii) Total number of pinheads and fruit bodies formed

The highest number of pinheads and fruit bodies was formed in the uncomposted substrate by both species *P. ostreatus* (64.7%) and *P. eous* 64.2% conversion of pinhead to fruit bodies. The conversion of pinheads to mature fruit bodies in both species decreased with increased days of composting. However, there was a constant conversion of pinheads to fruiting bodies in the case of *P. eous* whereas there was a marginal decrease for *P. ostreatus*. These were not statistically significant ($p \leq 0.05$) (Tables 61-62).

Table 59: Mycelial growth rate of *P. ostreatus* strain EM-1 on Engelberg mixture substrate amended with 1% $CaCO_3$ and 10% rice bran and composted for the indicated periods prior to bagging for sterilization

Period of composting / day(s) Treatment	Surface mycelial density	Average mycelia growth (cm/week)±SE	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	+++	6.1±0.6	4	5.9	5.5
4	++	6.0±0.5	4	6.9	6.5
8	+	5.0±0.4	4	6.6	6.3
12	+	4.6±0.3	4	6.7	6.5

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white

+ Poor patchy growth

Table 60: Mycelial growth rate of *P. eous* strain P-31 on Engelberg mixture substrate amended with 1% $CaCO_3$ and 10% rice bran prior to bagging

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/week) ± SE	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	+++	7.0±0.75	4	5.9	5.5
4	++	6.7±0.45	4	6.9	6.5
8	+	6.0±0.41	4	6.6	6.3
12	+	4.6±0.15	4	6.7	6.5

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white

+ Poor patchy growth

Table 61: Total number of pinheads and fruiting bodies formed from three flushes of *P. ostreatus* EM-1 cultivated on Engelberg mixture amended with 1% $CaCO_3$ and 10% rice bran at composting before bagging for sterilization.

Period of composting	No. of pinheads / fruiting bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion / Successful fruiting
	1st Flush	2nd Flush	3rd Flush		
0	28 (18)	15 (10)	8 (5)	51 (33)	64.7
4	21 (13)	12 (7)	6 (4)	39 (24)	61.5
8	16 (10)	14 (8)	6 (2)	36 (20)	55.6
12	15 (10)	11 (5)	4 (2)	30 (17)	56.7

Keys: Value of fruit bodies are in parenthesis **( )**

Table 62: Total number of pinheads and fruiting bodies formed from three flushes of *P. eous* P-31 cultivated on Engelberg mixture amended with 1% $CaCO_3$ and 10% rice bran at composting before bagging for sterilization.

Period of composting	No. of pinheads / fruiting bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion / Successful fruiting
	1st Flush	2nd Flush	3rd Flush		
0	34 (24)	12 (7)	7 (3)	53 (34)	64.2
4	18 (11)	12 (5)	10 (4)	40 (20)	50.0
8	15 (7)	12 (7)	9 (4)	36 (18)	50.0
12	15 (10)	9 (3)	10 (4)	34 (17)	50.0

Keys: Value of fruit bodies are in parenthesis **( )**

Table 63: Total yield and Biological Efficiency of *P. ostreatus* strain EM-1 grown on Engelberg mixture substrate amended with 1% $CaCO_3$ and 10% rice bran before bagging for sterilization and inoculation

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	58.9±7.5	32.1±4.9	22.8±1.9	113.8^{a}	45.5
4	57.4±2.2	29.4±4.8	25.1±3.1	111.9^{a}	44.8
8	51.9±3.2	35.4±3.8	19.7±1.4	107.0^{b}	42.8
12	55.1±4.8	34.1±4.2	17.9±2.4	107.1^{b}	42.8

The letters indicate significant differences to 95% in accordance with one way ANOVA Test. Values in the same column followed by a different letter do differ significantly from each other. All values are means of five replicates

Table 64: Total yield and Biological Efficiency of *P. eous* P-31grown on Engelberg mixture substrate amended with 1% $CaCO_3$ and 10% rice bran before bagging for sterilization and inoculation

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	55.5 ± 3.3	30.5 ± 4.1	36.2 ± 4.6	122.2[a]	48.9[a]
4	39.3 ± 8.2	35.5 ± 8.2	17.2 ± 2.6	92.0[b]	36.8[b]
8	33.6 ± 2.9	24.5 ± 4.8	18.1 ± 2.5	76.2[c]	30.5[c]
12	37.5 ± 6.2	24.8 ± 4.3	18.5 ± 1.5	80.8[c]	32.3[c]

The letters indicate significant differences to 95% in accordance with one way ANOVA Test. Values in the same column followed by a different letter do differ significantly from each other. All values are means of five replicates

EXPERIMENT 7

Growth yield and Biological Efficiency of *P. ostreatus* EM-1 and *P. eous* P-31 on 'wawa' sawdust (*Triplochiton scleroxylon*) amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods (0-12days) prior to bagging for sterilization.

i. Mycelial growth rate, mycelia density, spawn run period and pH of substrate

The mycelia growth rate for both *Pleurotus species* was higher on composted substrates (4-12days) than on the uncomposted (4.5±0.2 to 6.1±0.2cm week^{-1} and 5.1±0.9c to 6.2±0.3cm wk^{-1} respectively). It took 5-6 weeks for spawn to cover the compost. The pH of the compost before and after sterilization was same for the growth of both species. (Tables 65 & 66)

ii. Total yield, Biological Efficiency

The highest yield of *P. ostreatus* was on the 8days composted substrate (222.8g) and the least was in the uncomposted substrate (175.6g). The differences observed were statistically significant ($p<0.05$). The same trend was obtained in the Biological Efficiency data. It was highest BE=63.7% in the 8 days composted substrate and least in the uncomposted substrate (BE=50.2%) (Table 67)

The highest yield of *P. eous* (226.9g) was obtained in 8 days composted substrate on the same compost and the least yield of 194.6g was obtained on the uncomposted substrate. The BE value also followed the same trend i.e. BE=64.8% on the 8 days composted

substrate and BE=55.6% on the uncomposted substrate (Table 68). The differences obtained were statistically ($P \leq 0.05$) significant.

iii. Correlation of stipe and pileus length and average weight

The average diameter of pileus and average length of stipe did not vary significantly ($P>0.05$) for *P. eous* in the 0-8days composted substrate and the same was true for *P. ostreatus* (Tables 69&70). The highest average length, diameter of pileus, and average length of stipe was obtained on the 12 days composted substrate (Table 69 & 70). Figs 34 -37 shows the correlation between stipe length, pileus width and Biological Efficiency of *P. ostreatus* and *P. eous* on the various substrates. The correlation coefficient (R^2) of the relationship between stipe length and Biological Efficiency for *P. eous* was $R^2 = 0.7083$ and $R^2 = 0.8801$ for *P. ostreatus* on the same substrates (Figs 34 and 36).

The correlation coefficient (R^2) of the relationship between pileus width and BE for *P. eous* $R^2 = 0.778$ and $R^2 = 0.6031$ for *P. ostreatus* on the same substrates (Figs 35 and 37). The Summary Tables 72a&b show the Total Yield and Biological Efficiencies obtained in Experiments 1-7 for the two *Pleurotus* species. Uncomposted rice straw only and especially those composted for short period on 4- 8 days gave the best BE (75.6-76.4%) and yield (161.5- 221.5g) and the shortest spawn run period (3 weeks) for *P. eous*. Scheffe Average obtained from Analysis of Variance showed that the difference observed were statistically significant ($p<0.05$). On the other hand long composting periods favoured high B E by *P. ostreatus* on the tested composts in Experiments 1-7. BE varied from 22.2- 63.7% depending on the compost. Spawn run period varied from 4- 9 weeks.

Total no. of pinhead and fruit bodies formed on substrate

The no. of pinheads and fruiting bodies formed varies with species of *Pleurotus* and period of composting. Successful fruiting from pinheads formed on the substrate varied from 57.9%-67.6% on the composted substrate used for cultivation of *P. eous* and 62.8-75% in the case of *P. ostreatus* (Tables 71 and 72). The most successful conversion of pinheads to fruit bodies was obtained on compost fermented for 8days (Table 71 and 72.). There was no statistical difference (P>0.05) between pinhead and fruit bodies formed by *P. eous* on the composted (0-12 day(s) substrates.

Table 65: Mycelial growth rate of *P. ostreatus* strain EM-1 on sawdust amended with 1% $CaCO_3$ and 10% rice bran composted for the indicated periods before bagging for sterilization

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/weeks)	Spawn run period (weeks)	pH before sterilization	pH after sterilization
0	+++	4.5 ± 0.2	6	6.6	6.4
4	+++	6.1 ± 0.5	5	6.9	6.5
8	+++	5.5 ± 0.1	5	7.0	6.7
12	+++	6.1 ± 0.2	5	7.0	6.7

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white

Table 66: Mycelial growth rate of *P. eous* P-31 on sawdust amended with 1% $CaCO_3$ and 10% rice bran composted for the indicated periods before bagging for sterilization

Period of composting / day(s)	Surface mycelial density	Average mycelia growth (cm/weeks)	Spawn run period (weeks)	pH of substrate	
				pH before sterilization	pH after sterilization
Treatment					
0	+++	5.1 ± 0.93	6	6.6	6.4
4	+++	5.9 ± 0.79	5	6.9	6.5
8	+++	6.1 ± 0.35	5	7.0	6.7
12	+++	6.2 ± 0.33	5	7.0	6.7

Degree of mycelial density when mycelia fully colonize the substrate
+++ Mycelium totally grows through the bag and is uniformly white

Table 67: Total yield per three flushes and Biological Efficiency of *P. ostreatus* strain EM-1 grown on sawdust amended with 1% $CaCO_3$ and 10% rice bran at composting

Period of composting (day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	71.7±6.2	56.3 ± 5.8	47.6±9.7	175.6[a]	50.2
4	75.6±6.8	67.3± 12.5	44.3±5.3	187.2[b]	53.5
8	98.6±6.1	66.3 ± 6.4	57.9±6.3	222.8[c]	63.7
12	87.7±11.9	61.9 ± 6.9	37.2±8.2	186.6[b]	53.3

The letters in vertical column indicate significant ($p \leq 0.05$) all values are means of five replicates.

Table 68: Total yield per flush on *P. eous* strain P-31on sawdust compost amended with 1% $CaCO_3$ and 10% rice bran at composting

Period of composting (day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	113.4±6.9	52.1±4.0	28.8±6.0	194.3[a]	55.6
4	104.7±6.1	56.0±2.6	36.2±8.0	196.7[a]	56.2
8	122.3±5.4	62.1±9.0	42.5±4.3	226.9[b]	64.8
12	119.8±2.2	57.2±4.7	41.4±7.6	218.4[b]	62.4

The letters in vertical column indicate significant ($p \leq 0.05$) all values are means of five replicates.

Table 69: Correlation of stipe / stalk length and pileus (cap) width of *Pleurotus eous* (strain P-31) and *P. ostreatus* (strain EM-1) grown on *Triplochiton scleroxylon* ('wawa') sawdust amended with 1% $CaCO_3$ and 10% rice bran

Period of composting (day(s)	**Average weight (g) of mushroom ± SE**		**Average diameter (mm) of pileus / cap ± SE**		**Average length (mm) of stipe / stalk ± SE**	
	P-31	***EM-1***	***P-31***	***EM-1***	***P-31***	***EM-1***
0	7.3 ± 0.8[a]	7.4 ± 1.0[c]	63.6 ± 2.1[b]	61.6 ± 2.6[a]	49.6 ± 2.3 [c]	60.6 ± 2.6[e]
4	10.0 ± 0.9[a]	7.6 ± 0.9[c]	62.9 ± 3.5[b]	70.3 ± 3.7[e]	57.4 ± 4.9 [c]	62.9 ± 3.5[a]
8	15.3 ± 1.5[b]	13.1 ± 1.2[d]	67.7 ± 2.0[b]	74.7 ± 5.7[e]	62.8 ± 2.9 [d]	67.7 ± 2.0[b]
12	19.3 ± 1.6[b]	9.9 ± 1.2[c]	64.5 ± 2.7[b]	72.0 ± 6.5[e]	59.0 ± 4.3 [c]	64.5 ± 2.7[a]

Values in the same column followed by a common letter do not differ significantly (P=0.05).

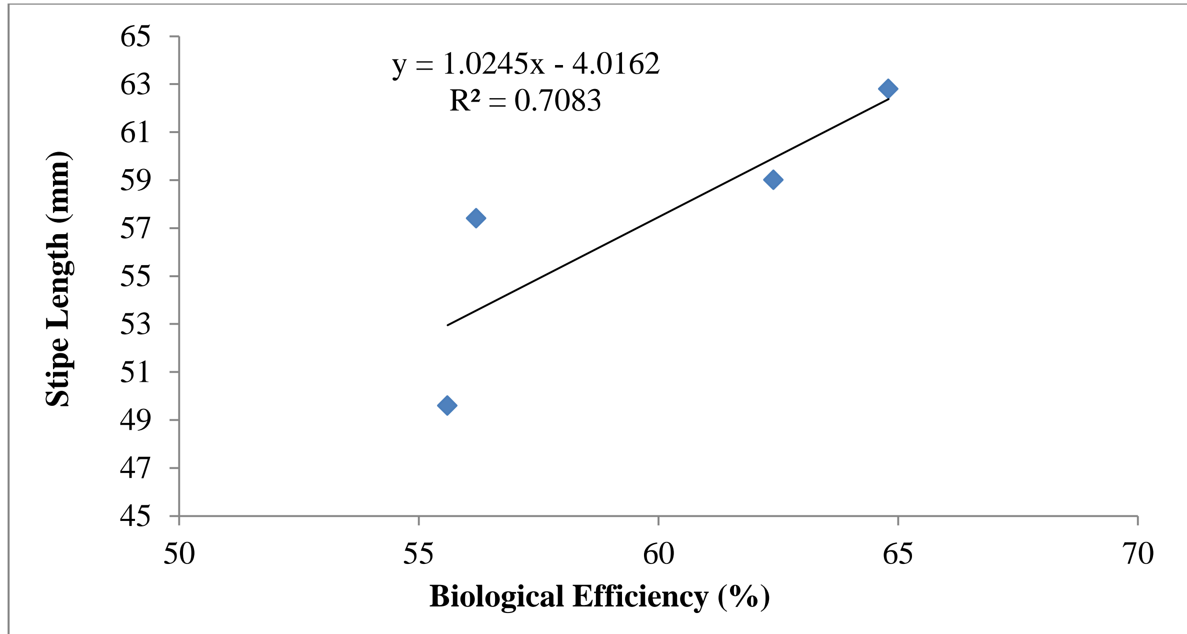


Fig 35: Correlation between Biological Efficiency and Stipe Length (mm) of *P. eous* grown on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran

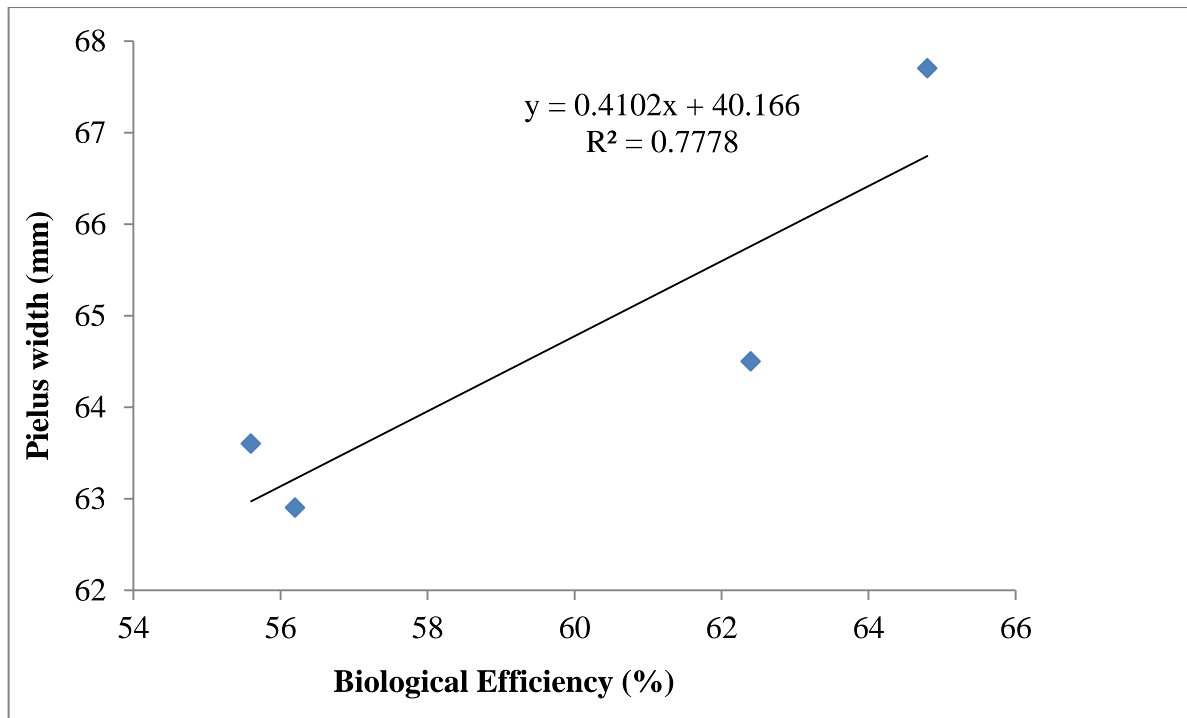


Fig 36: Correlation between Biological Efficiency and Pileus width (mm) of *P. eous* grown on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran

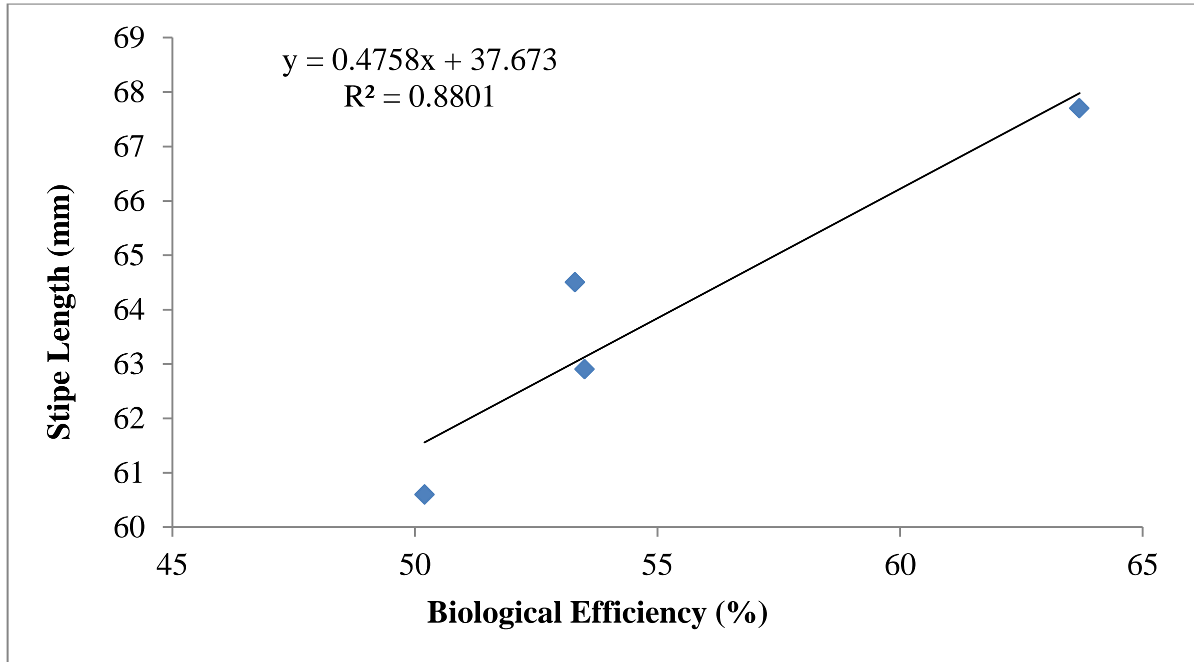


Fig 37: Correlation between Biological Efficiency and Stipe Length (mm) of *P. ostreatus* grown on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran

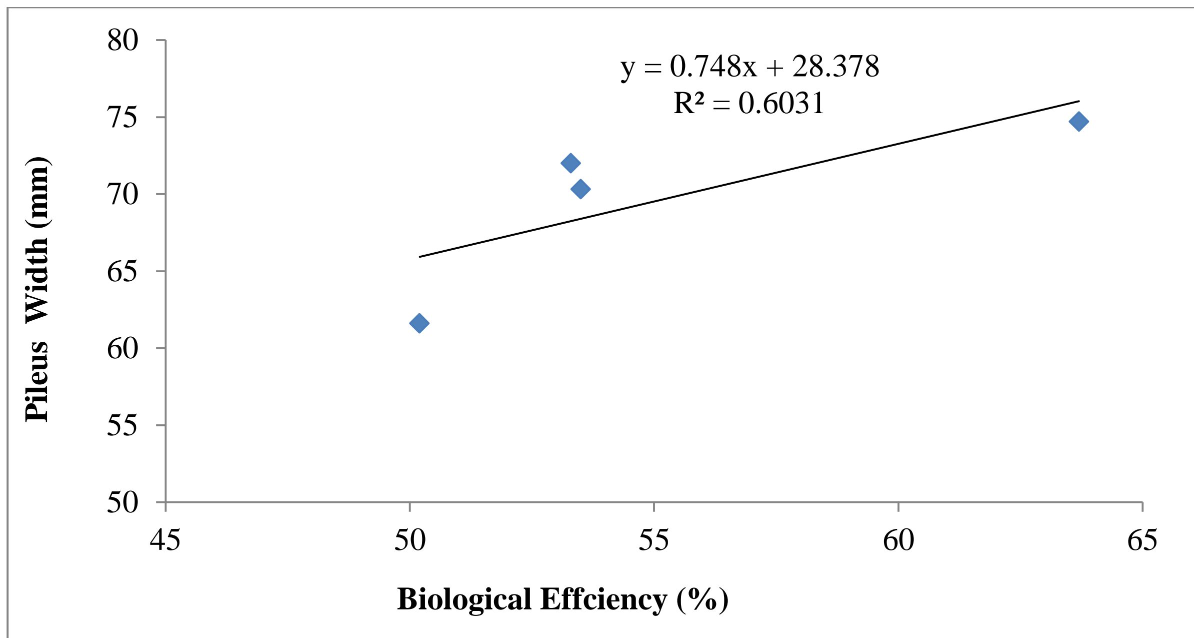


Fig 38: Correlation between Biological Efficiency and Pileus width (mm) of *P. ostreatus* grown on 'wawa' sawdust amended with 1% $CaCO_3$ and 10% rice bran

Table 70: Total number of pinheads and fruit bodies per three flushes of *P. ostreatus* EM-1 on sawdust amended with 1% $CaCO_3$ and 10% rice bran

Period of composting	No. of pinheads / fruit bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion to fruit bodies
	1st Flush	2nd Flush	3rd Flush		
0	29 (16)	30 (19)	19 (14)	78 (49)	62.8
4	24 (15)	17 (12)	21 (12)	62 (39)	62.9
8	26 (21)	17 (13)	22 (15)	65 (49	75.4
12	24 (16)	18 (12)	15 (9)	57 (37)	64.9

Fruiting bodies are in parenthesis **( )**

Table 71: Total number of pinheads and fruit bodies per three flushes of *P. eous* P-31 on sawdust amended with 1% $CaCO_3$ and 10% rice bran

Period of composting	No. of pinheads / fruit bodies per flush			Total No. of pinheads / fruiting bodies	% Conversion to fruit bodies
	1st Flush	2nd Flush	3rd Flush		
0	40 (23)	24 (15)	12 (6)	76 (44)	57.9
4	38 (25)	21 (13)	14 (8)	73 (46)	63.0
8	37 (23)	19 (14)	15 (11)	71 (41)	67.6
12	38 (27)	21 (10)	14 (10)	73 (47)	64.4

No. of fruiting bodies are in parenthesis **( )**

Table 72a: Summary of Table of spawn run period, mycelial growth, total yield and biological efficiency of *Pleurotus eous* P-31 cultivated on the indicated formulated lignocellulose wastes

Type of substrate	Treatment / Period of composting (days)	Spawn run period (weeks)	Mycelial growth rate (cm / week)	Total yield (g)	Biological Efficiency (%)
Rice straw only	0	3	8.8 ± 1.8	161.5	55.7
	4	3	9.5 ± 0.9	219.3	75.6
	8	3	9.6 ± 0.1	221.5	76.4
	12	3	7.5 ± 0.3	185.5	64.0
Rice straw + 1% $CaCO_3$ + 10% Rice bran	0	3	9.2 ± 0.1	189.4	67.3
	4	3	9.2 ± 0.1	209.8	72.3
	8	3	10.0 ± 0.4	217.9	75.1
	12	3	9.0 ± 0.5	194.5	67.1
Rice straw + 1% $CaCO_3$ + 10% Rice bran + 5, 10, 15% RB	A5	4	7.4 ± 0.1	197.6	68.1
	A10	4	6.6 ± 0.2	165.2	57.0
	A15	4	6.4 ± 0.9	159.6	55.0
	B5	4	7.3 ± 0.2	184.5	63.5
	B10	4	6.5 ± 0.2	179.8	62.0
	B15	4	5.1 ± 0.1	174.7	60.2
	C5	4	6.6 ± 0.3	155.5	53.6
	C10	4	6.4 ± 0.2	169.5	58.4
	C15	4	6.5 ± 0.3	153.9	53.1
	D5	4	7.7 ± 0.1	185.4	63.9
	D10	4	7.9 ± 0.4	175.6	60.6
	D15	4	6.3 ± 0.2	140.3	48.4
Rice straw + Rice husk 1% $CaCO_3$ + 10% Rice bran + 5, 10, 15% RB	A5	3	10.3 ± 0.2	211.0	72.8
	A10	3	10.1 ± 0.2	164.4	56.7
	A15	3	9.7 ± 0.4	154.7	53.3
	B5	3	8.1 ± 0.2	133.5	45.9
	B10	3	7.8 ± 0.5	126.4	43.4
	B15	4	6.1 ± 0.3	127.2	43.9
	C5	4	5.7 ± 0.3	122.1	42.1
	C10	4	5.9 ± 0.3	111.8	38.6
	C15	4	7.4 ± 0.6	121.2	41.8
	D5	5	5.6 ± 0.1	113.9	39.3
	D10	5	4.8 ± 0.2	113.4	39.1
	D15	5	5.1± 0.2	108.4	37.4

Table 72a (cont):

Unamended Engelberg mixture (Rice husk + bran)	0	4	8.9±0.44	131.5	52.6
	4	4	8.4±0.38	121.8	48.7
	8	4	6.2±0.24	118.5	47.4
	12	4	5.1±0.13	109.7	43.9
Engelberg mixture (Rice husk + bran) + 1% $CaCO_3$ + 10% RB	0	4	7.0±0.75	122.2	48.9
	4	4	6.7±0.45	92.0	36.8
	8	4	6.0±0.41	76.2	30.5
	12	4	4.6±0.15	80.8	32.3
Sawdust + 1% $CaCO_3$ + 10% RB	0	6	5.1 ± 0.93	194.3	55.6
	4	5	5.9 ± 0.79	196.7	56.2
	8	5	6.1 ± 0.35	226.9	64.8
	12	5	6.2 ± 0.33	218.4	62.4

Key

Exp. 1: rice straw only compost

Exp. 2: rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Exp. 3: rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 4: rice straw with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 5: Unamended Engelberg mixture (rice husk and bran 2:1w/w)

Exp. 6: Engelberg mixture (rice husk and bran 2:1w/w) amended with 1% $CaCO_3$ and 10% rice bran

Exp. 7: Sawdust amended with 1% $CaCO_3$ and 10% rice bran

Table 72b: Summary of Table of spawn run period, mycelial growth, total yield and biological efficiency of *Pleurotus ostreatus* EM-1 cultivated on the indicated formulated lignocellulose wastes

Type of substrate	Treatment / Period of composting (days)	Spawn run period (weeks)	Mycelial growth rate (cm / week)	Total yield (g)	Biological Efficiency (%)
Rice straw only	0	8	5.4 ± 0.3	106.6	36.8
	4	8	6.3± 0.5	154.7	53.3
	8	8	7.8 ± 0.7	143.7	49.6
	12	8	4.7 ± 1.1	124.7	43.0
Rice straw + 1% $CaCO_3$ + 10% Rice bran	0	9	4.8 ± 0.3	130.0	44.8
	4	9	5.0 ± 0.4	131.8	45.5
	8	9	4.9 ± 0.3	160.6	55.4
	12	9	6.2 ± 0.4	156.1	53.8
Rice straw + 1% $CaCO_3$ + 10% Rice bran + 5, 10, 15% RB	A5	5	6.2±0.6	90.0	29.0
	A10	5	5.9±0.9	84.4	29.1
	A15	5	4.9±0.8	86.0	29.7
	B5	5	4.7±0.8	111.9	43.7
	B10	5	5.5±0.7	125.8	43.4
	B15	5	4.4±0.6	127.6	38.4
	C5	5	5.4±0.3	164.4	52.1
	C10	5	4.6±02	133.1	45.9
	C15	5	5.9±0.4	151.0	56.7
	D5	5	6.1±0.2	123.5	44.8
	D10	5	5.6±0.1	163.4	56.3
	D15	5	4.9±0.4	129.9	42.6
Rice straw + Rice husk 1% $CaCO_3$ + 10% Rice bran + 5, 10, 15% RB	A5	6	5.8±0.2	120.3	41.5
	A10	6	5.7±0.2	118.6	40.9
	A15	6	5.8±0.2	99.5	34.3
	B5	4	6.2±0.3	112.6	38.8
	B10	5	5.2±0.4	121.1	41.8
	B15	6	6.0±0.3	134.3	46.3
	C5	6	4.4±0.3	131.3	45.3
	C10	6	5.3±0.3	121.2	41.8
	C15	6	4.2±0.1	175.8	60.8
	D5	6	4.8±0.6	183.5	63.3
	D10	6	4.0±0.3	167.7	57.8
	D15	6	4.0±0.2	179.5	61.9

Table 72b (cont'd):

Unamended Engelberg mixture (Rice husk + bran)	0	4	7.3±0.90	88.4	35.2
	4	4	6.0±0.80	78.5	31.4
	8	4	5.5±0.25	55.6	22.2
	12	4	5.1±0.38	55.6	22.2
Engelberg mixture (Rice husk + bran) + 1% $CaCO_3$ + 10% RB	0	4	6.1±0.6	113.8	45.5
	4	4	6.0±0.5	111.9	44.8
	8	4	5.0±0.4	107.0	42.8
	12	4	4.6±0.3	107.1	42.8
Sawdust + 1% $CaCO_3$ + 10% RB	0	6	4.5 ± 0.2	175.6	50.2
	4	5	6.1 ± 0.5	187.2	53.5
	8	5	5.5 ± 0.1	222.8	63.7
	12	5	6.1 ± 0.2	186.6	53.3

Key

Exp. 1: rice straw only compost

Exp. 2: rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Exp. 3: rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 4: rice straw with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 5: Unamended Engelberg mixture (rice husk and bran 2:1w/w)

Exp. 6: Engelberg mixture (rice husk and bran 2:1w/w) amended with 1% $CaCO_3$ and 10% rice bran

Exp. 7: Sawdust amended with 1% $CaCO_3$ and 10% rice bran

EXPERIMENT F:

Chemical, nutritional and mineral composition of the different lignocellulose agro-wastes and the fruiting bodies of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 cultivated on the different formulation of the lignocellulose materials

a) Lignin, cellulose, hemicellulose, silica, dry matter and moisture

i) Rice straw only and rice straw amended with 1% $CaCO_3$ and 10% rice bran

Only the percentage (%) gross moisture content of both raw rice straw and its amendment with 1% $CaCO_3$ and 10% rice bran declined during the 12 days composting period. The rest of the parameter gross dry weight, fine dry weight, hemicellulose, cellulose, lignin, crude protein and silica remained nearly the same (Figs 38 and 39). The dry unamended rice straw had the following: Hemicellulose (15.63%), Cellulose (34.55%), Lignin (6.36%), Moisture (7.34%), Acid Neutral Detergent Fibre (49.61%) and Neutral Detergent Fibre (65.24%), (Table 73).

ii) Engelberg Mixture only and Engelberg Mixture amended with 1% $CaCO_3$ and 10% rice bran

Lignin, Hemicellulose, Cellulose increased with higher period of composting of the Engelberg Mixture only while silica remained also marginally the same (Table 74). There was an attendant increase in crude protein from 4.48% in the uncomposted to 4.61% after 12days of composting (Table 74).

In the case of the Engelberg Mixture amended with 1% $CaCO_3$ and 10% rice bran Dry matter increased from 88.82 to 90.79% within 12days period. Crude protein decreased from 5.06 to 4.19% in 12days. Hemicellulose increased from 9.60% to13.03% in the same period

of 12days. Lignin content and cellulose increased marginally from 15.89-18.14% (Lignin) and 36.27-38.71% (cellulose) (Table 74). Silica content on the other hand decreased from 21.06-18.35% (Table 74).

iii) Raw 'wawa' (*Triplochiton scleroxylon*) sawdust amended with 1% $CaCO_3$ and 10% rice bran

Moisture content decrease from 71.62% to 66.58% in 12 days; so did the dry matter decrease from 91.67-86.63% (Table 76). The following parameters also decreased in 12days of composting: Crude protein 4.25-3.55%; Hemicellulose (9.44-6.39%); Cellulose (44.69-38.84%); Lignin increased from 20.76-22.10% while Silica increased marginally from 9.06-9.45%.

b) Mineral Element Estimation: Heavy metals [Copper (Cu); Zinc (Zn); Lead (Pb); Manganese, (Mn) and Calcium (Ca); Iron (Fe); Magnesium (Mg) and Sodium (Na); Phosphorus (P) and Potassium (K)].

i) Mineral Content

The mineral component (Ca, Fe, K, Mg, Na, P) of the fruiting bodies of *P. eous* P-31 and *P. ostreatus* EM-1 cultivated on seven different lignocellulose substrates are presented in Tables 77 and 78.

The fruiting bodies of *P. ostreatus* contained minerals such as Calcium, Iron, Potassium, Magnesium, Sodium and Phosphorus in the range 0.0327-1.076, 0.0312-0.350, 12.90-17.00, 1.067-1.491, 0.306-8.00, 5.22-14.50mg/kg dry weight of fruit bodies respectively. The highest Ca content was obtained in Experiment 1 (1.0760mg/kg) and the least was recorded in Experiment 7 (0.0327mg/kg) (Table 78).

The mineral component of the fruit bodies of *P. eous* contained Ca, Fe, K, Mg, Na and P in the range 0.102-0.395; 0.2310-0.320; 11.70-32.00; 0.1163-0.86; 6.00-14.90; 6.32-10.08mg/kg respectively. The highest concentration of Ca 0.395mg/kg was recorded in Exp. 1 (rice straw only) (0.3950mg/kg) and the least concentration 0.1017mg/kg was obtained in Exp. 7 (sawdust supplemented with 1% $CaCO_3$ and 10% rice bran). The highest concentration of Fe 0.35omg/kg in *P. eous* cultivated in Exp. 7 and the lowest, 0.231 - 0.234mg/kg was obtained on Exp. 1 (rice straw only) and Exp. 2 (rice straw + 1% $CaCO_3$ + 10% rice bran). Potassium level was highest; 32mg/kg in Exp. 5 (Engelberg Mixture only) and least, 11.70mg/kg in Exp. 1 (rice straw only). Sodium concentration was highest (14.90mg/kg) in Exp. 7 (sawdust + 1% $CaCO_3$ + 10% rice bran) and lowest (6.00mg/kg) in Exp. 1, 2 and 3. Fruiting bodies of *P. eous* cultivated in Exp. 3 (rice straw + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%) contained the highest level (10.08mg/kg) of phosphorus while same cultivated some cultivated in Exp. 7 (sawdust + 1% $CaCO_3$ + 10% rice bran) contained the least level of phosphorus. Finally, Magnesium was highest (0.86mg/kg) in fruit body of *P. eous* cultivated in Exp. 3 (rice straw + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%) and the least (0.11-0.13mg/kg) was detected in fruit bodies harvested in Exp. 4, 5 and 7 (rice straw and rice husk mixture + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%; unamended Engelberg Mixture and sawdust + 1% $CaCO_3$ + 10% rice bran) Table 77.

ii) Heavy metals (Copper, Zinc, Lead and Manganese)

P. ostreatus

Some heavy metals were detected in the fruit bodies cultivated on the variously formulated rice lignocellulose in Experiments 1-7 albeit low. For example, Cu could not be detected in the fruit bodies of *P. ostreatus* cultivated in Experiments 3 and 4 but was detected in low quantities (0.005-0.08mg/kg) in the rest of the substrates (Table 78). Zinc concentration ranging from 0.0144-2.250mg/kg was detected in the fruit body of *P. ostreatus* in all the tested substrates. The lowest 0.0144mg/kg was detected in Exp. 7 (sawdust + 1% $CaCO_3$ + 10% rice bran) and the highest (0.250mg/kg) was in the fruit bodies cultivated in Exp. 1(rice straw only) (Table 78). Lead was detected in fruit bodies harvested from all the variously formulated substrates used in Exp. 1-7. The concentration range from 0.0014mg/kg (Exp. 5 unamended Engelberg Mixture) to 0.160-0.165mg/kg (Exp. 1 and 2; rice straw only and rice straw amended with 1% $CaCO_3$ + 10% rice bran) (Table 78). Manganese was also present in the harvested mushroom and the concentrations varied from 0.031mg/kg (Exp. 5 unamended Engelberg Mixture) to 1.272mg/kg (Exp. 7 sawdust + 1% $CaCO_3$ + 10% rice bran).

***P. eous* fruit body**

Copper, Zinc, Lead and Manganese were found in fruit bodies of *P. eous* cultivated in the variously formulated compost used in Experiments 1-7. The value of copper ranged from none in Experiments 3 and 4 (rice straw + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%; rice straw and rice husk mixture + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%) respectively to 0.0237mg/kg (Experiments 5 & 7 unamended Engelberg Mixture; sawdust + 1% $CaCO_3$ + 10% rice bran). The concentration of zinc varied from 0.0144mg/kg (Exp. 7 sawdust + 1% $CaCO_3$ + 10% rice bran) to 0.250mg/kg (Exp. 1rice straw only) (Table 77). Lead was also detected in the fruit bodies of *P. eous* cultivated on Experiments 1-7. The values varied from 0.014mg/kg (Exp.7 sawdust + 1% $CaCO_3$ + 10% rice bran) to 0.250mg/kg (Exp. 1 rice straw only). Manganese detected in the fruit bodies of *P. eous* cultivated on variously composted substrates ranged from 0.013mg/kg (Exp. 3 (rice straw + 1% $CaCO_3$ + 10% rice bran + different proportions of rice bran 5, 10 and 15%) to 1.272mg/kg (Exp. sawdust + 1% $CaCO_3$ + 10% rice bran) (Table 77).

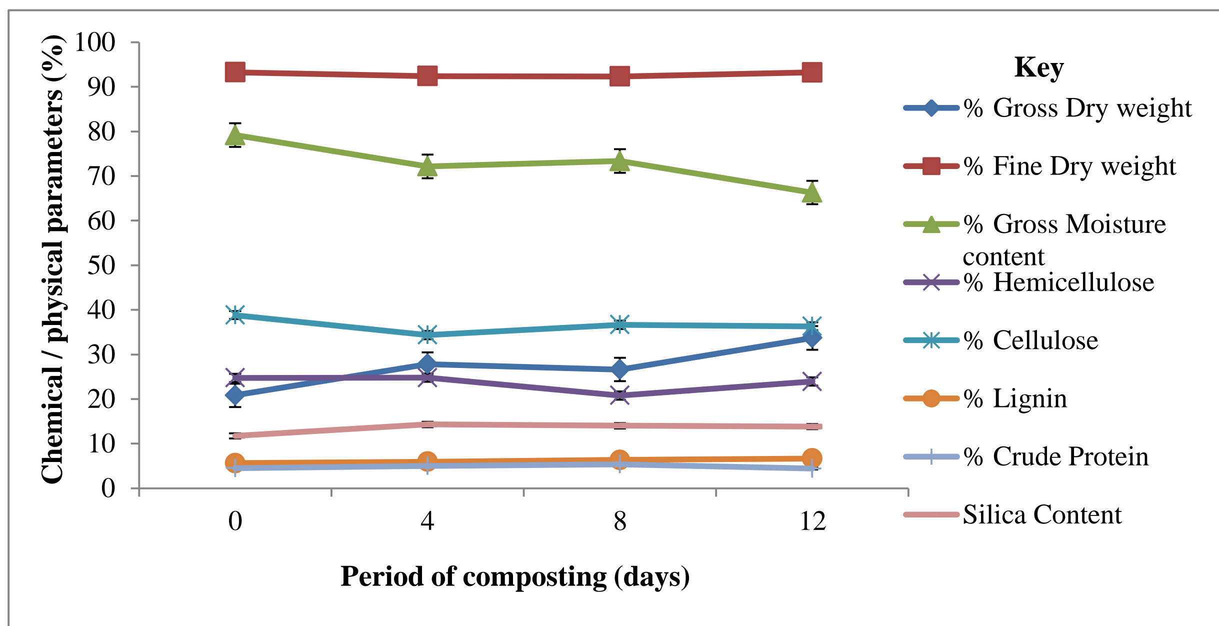


Fig 39: Chemical analysis of fermented and unfermented rice straw only with the indicated period of composting days

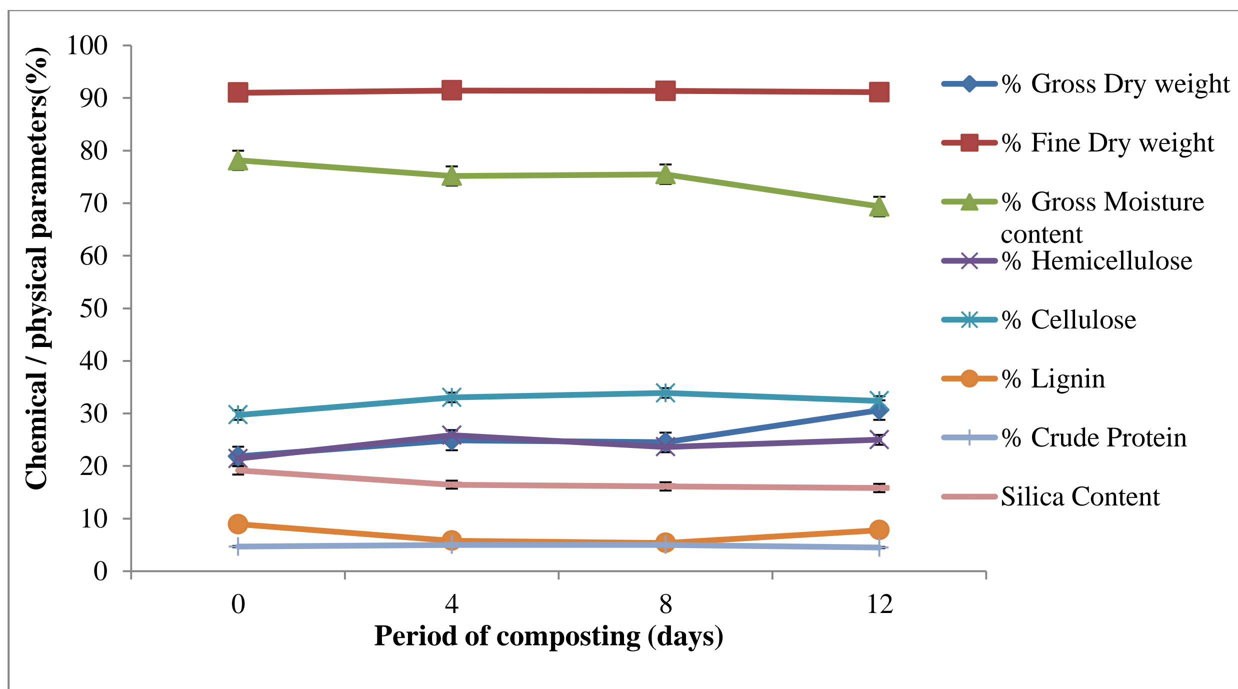


Fig 40: Chemical analysis of fermented and unfermented rice straw amended with 1% $CaCO_3$ and 10% rice bran with the indicated period of composting days

Table 73: Chemical analysis of dry rice straw used for the cultivation of the two *Pleurotus* species

Chemical / physical parameters	% Dry Matter	NDF	ADF	Crude Protein	Hemicellulose	Cellulose	Lignin	MC (%)
Rep 1	92.72	60.59	46.29	6.55	14.30	32.06	5.82	7.34
Rep 2	92.59	60.31	45.65	6.63	14.66	31.95	5.95	7.34
ADB	92.66	60.45	45.97	6.59	14.48	32.01	5.89	7.34
DMB	92.66	65.24	49.61	7.11	15.63	34.55	6.36	7.34
Mean± SE	92.66 ±0.65	65.24 ±0.14	49.61 ±0.32	7.11 ±0.40	15.63 ±0.18	34.55 ±0.06	6.36 ±0.06	7.34 ±0.04

Table 74: Chemical composition of unamended Engelberg mixture used for the cultivation of the two *Pleurotus* species

Period of composting (days)	Chemical composition				
	%Crude Protein (DMB)	%Hemi-cellulose (DMB	% Cellulose (DMB)	% Lignin (DMB)	% Silica (DMB
0	4.48	11.60	32.76	16.20	19.24
4	4.92	13.79	38.25	17.93	18.77
8	4.52	14.21	38.56	17.44	18.99
12	4.61	13.03	39.11	17.32	19.85

Table 75: Chemical analysis of unfermented and fermented Engelberg Mixture substrate amended with 1% $CaCO_3$ and 10% rice bran before inoculation with mycelia of two Pleurotus species

Period composting / day(s)	% Dry Matter	%Crude Protein	%Hemi-cellulose	% Cellulose	% Lignin	% Silica
0	88.82	5.06	9.60	36.27	15.89	21.06
4	90.46	4.92	14.19	38.25	16.73	18.09
8	91.23	4.75	14.32	37.88	17.44	17.39
12	90.79	4.19	13.03	38.71	18.14	18.35

Table 76: Chemical analysis of unfermented and fermented *Triplochiton scleroxylon* 'wawa' substrate amended with 1% $CaCO_3$ and 10% rice bran before inoculation with mycelia of two *Pleurotus* species

Period composting / day(s)	% Moisture content	% Dry Matter	%Crude Protein	%Hemi-cellulose	% Cellulose	% Lignin	% Silica
		Chemical parameters (% Dry Matter Basis DMB)					
0	71.62	91.67	4.25	9.44	44.69	20.76	9.06
4	70.58	90.63	3.78	7.83	44.19	21.21	9.08
8	69.44	89.49	3.69	6.49	41.12	21.68	9.58
12	66.58	86.63	3.55	6.39	38.84	22.10	9.45

Table 77: Total mineral content and heavy metals detection of *Pleurotus eous* strain P-31 grown on different formulation of lignocellulose materials.

Treatment per substrate	Mineral content (mg/kg)									
	Ca	Cu	Fe	K	Mg	Mn	Na	P	Pb	Zn
Exp. 1	0.3950	0.0020	0.2340	11.7000	0.7650	0.0180	6.0000	7.5900	0.0500	0.1900
Exp. 2	0.3690	0.0130	0.2310	12.1500	0.7580	0.0160	6.0010	7.5950	0.1015	0.1870
Exp. 3	0.3780	0.0150	0.2680	12.2500	0.8600	0.0060	6.0000	10.0750	0.2020	0.1760
Exp. 4	0.1301	0.0000	0.3200	24.0000	0.1163	0.0011	13.000	8.2025	0.0134	0.0184
Exp. 5	0.1017	0.0018	0.3080	32.0000	0.1262	0.0000	14.900	7.9850	0.0108	0.0150
Exp. 6	0.3170	0.0030	0.2744	16.1200	0.2445	0.0096	14.940	6.0800	0.0003	0.0175
Exp. 7	0.1250	0.0000	0.3500	16.1020	0.1289	0.0598	14.0000	6.3150	0.0000	0.0130

Key

Exp. 1: rice straw only compost

Exp. 2: rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Exp. 3: rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 4: rice straw with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 5: Unamended Engelberg mixture (rice husk and bran 2:1w/w)

Exp. 6: Engelberg mixture (rice husk and bran 2:1w/w) amended with 1% $CaCO_3$ and 10% rice bran

Exp. 7: Sawdust amended with 1% $CaCO_3$ and 10% rice bran

Table 78: Total mineral content of *Pleurotus ostreatus* strain EM-1 grown on different lignocellulose materials.

Treatment per substrate	Mineral content (mg/kg)									
	Ca	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**Pb**	**Zn**
Exp. 1	1.0760	0.0800	0.3080	14.9000	1.2440	0.0180	7.5000	5.2200	0.1650	0.2500
Exp. 2	0.4830	0.0050	0.3500	17.0000	1.3800	0.0260	8.0000	14.2500	0.1600	0.1820
Exp. 3	0.8910	0.0000	0.3220	15.5000	1.4910	0.0130	7.0000	10.6750	0.1410	0.1970
Exp. 4	0.4150	0.0000	0.3060	16.7500	1.1510	0.6240	7.5000	12.7950	0.1570	0.1500
Exp. 5	0.0415	0.0237	0.2010	12.9000	1.3170	0.0310	6.0050	14.5000	0.0014	0.0327
Exp. 6	0.5100	0.0265	0.0120	15.4600	1.3700	0.0330	7.2010	9.2400	0.0040	0.0110
Exp. 7	0.0327	0.0237	0.0312	16.3000	1.0670	1.2720	0.3060	11.0000	0.0160	0.0144

Key

Exp. 1: rice straw only compost

Exp. 2: rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Exp. 3: rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 4: rice straw with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 5: Unamended Engelberg mixture (rice husk and bran 2:1w/w)

Exp. 6: Engelberg mixture (rice husk and bran 2:1w/w) amended with 1% $CaCO_3$ and 10% rice bran

Exp. 7: Sawdust amended with 1% $CaCO_3$ and 10% rice bran

c) The proximate analyses on Dry matter basis of the fruit bodies of *P. ostreatus* EM-1 and *P. eous* P-31 growing on the best substrate composition and composting periods

Tables 79 and 80 show the results obtained.

P. ostreatus

Fat content ranged from 11.1- 27.8%; Crude fibre, 5.56- 15.26%; Crude protein, 1.46-33.15%; total Ash, 6.53-10.54%; Carbohydrate, 17.37- 26.69%; Neutral detergent fibre, 34.39- 50.31%. Energy value varied from 387.80kcal/100g to 305.81kcal/100g.

P. eous

Fat content varied from 5.97-19.9%; Crude fibre, 12.19-24.54%; Crude protein, 21.63-35.99%; total Ash, 3.39- 5.73%; Carbohydrate, 9.35- 28.6%; Neutral detergent fibre, 36.99-54.00%. Energy value varied from 195.52kcal/100g to 366.22kcal/100g. The ranges observed may be due to types of substrate and period of composting before use.

Table 79: Proximate Analysis of *Pleurotus ostreatus* strain EM-1 mushroom on dry matter basis (DMB) grown on the best formulation of lignocellulosics substrates and composted for different periods

Experiment No.	% Dry Matter	% Fat	% Crude Fibre	% Crude Protein	% Total Ash	% Carbohydrate	% NDF	Energy (kcal/100g)
Exp. 1	83.44	12.97	14.92	24.07	9.30	23.20	50.31	305.81
Exp. 2	88.98	11.74	12.38	32.93	10.54	17.37	39.80	306.86
Exp. 3	84.40	11.18	15.26	33.15	9.83	19.87	34.39	312.70
Exp. 4	84.36	11.36	13.32	31.91	10.38	20.65	44.24	312.44
Exp. 5	85.50	20.8	5.56	28.55	7.46	21.60	43.87	387.80
Exp. 6	85.38	13.24	6.18	26.75	7.76	25.64	35.25	328.72
Exp. 7	76.57	16.62	11.47	30.16	6.53	26.69	50.59	376.98

ADF: Acid Detergent Fibre

NDF: Neutral Detergent Fibre

Table 80: Proximate Analysis of *Pleurotus eous* strain P-31 mushroom on dry matter grown on the best different formulation of lignocellulose compost and composted for varying periods before use

Experiment No.	% Dry Matter	% Fat	% Crude Fibre	% Crude Protein	% Total Ash	% Carbohydrate	% NDF	Energy (kcal/100g)
Exp. 1	85.55	3.99	24.54	21.63	5.26	28.25	47.91	235.43
Exp. 2	86.73	5.91	21.78	23.28	4.97	18.11	54.00	218.75
Exp. 3	88.44	14.87	16.37	28.83	5.73	11.54	45.41	295.31
Exp. 4	85.14	19.05	12.19	35.99	5.63	9.35	39.55	352.81
Exp. 5	86.52	19.90	13.50	37.22	3.39	9.56	36.92	366.22
Exp. 6	88.35	3.96	12.35	31.32	8.66	11.87	39.24	208.40
Exp. 7	85.50	5.56	17.25	27.82	4.89	8.55	44.60	195.52

Key

Exp. 1: rice straw only compost

Exp. 2: rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Exp. 3: rice straw amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 4: rice straw with rice husk mixture (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and prior to bagging different proportions (5, 10 and 15%) of rice bran was added

Exp. 5: Unamended Engelberg mixture (rice husk and bran 2:1w/w)

Exp. 6: Engelberg mixture (rice husk and bran 2:1w/w) amended with 1% $CaCO_3$ and 10% rice bran

Exp. 7: Sawdust amended with 1% $CaCO_3$ and 10% rice bran

ADF: Acid Detergent Fibre

NDF: Neutral Detergent Fibre

EXPERIMENT G

Preliminary molecular characteristics of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 using PCR Amplification and Restrictive Digestion Technique

The morphological differences in the two species is hard to separate except for size and length of stipe, colour of pileus (cap) which is white in *P. eous* P-31and greyish brown to black in *P. ostreatus* EM-1 not excepting the habit of the fruiting body on the compost (substrate). The photograph of the gill regions of *P. ostreatus* EM-1 and *P. eous* P-31are shown in Plates 12 and 13 and they look rather similar. But the cultural characteristic on agar is also different (Plate 14). The appearance of the two *Pleurotus* in the cropping house is also different (Plates 15 and 16.).

PCR performed with ITS 1 and ITS 4 primers to characterise the ITS region of the mushroom samples grown in different substrates showed that the two species of *Pleurotus* produced a characteristic band size of 650bp when run on ITS 1 and ITS 4 primer pair. Both *P. ostreatus* EM-1 and *P. eous* P-31 could not be segregated at this stage (Fig 41a.).

ITS characterisation of the two *Pleurotus* species

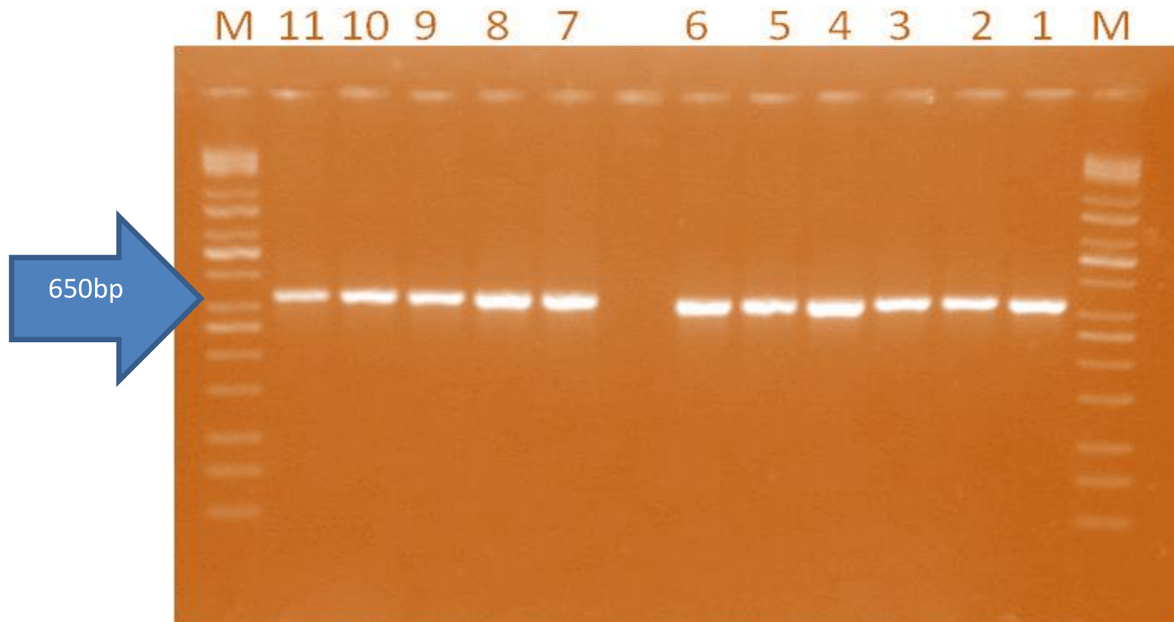


Fig 41a: Gel profile showing ITS 1 and ITS 4 amplification of Mushroom isolates (1 - 11) run alongside Kapa universal DNA ladder (M) i.e. left band strain EM-1 and right band P-31 respectively

All mushroom samples produced a characteristic band size of 650bp when run with ITS 1 and ITS 4 primer pair. Both mushroom species could not be segregated at this stage.

2. Restriction digestion

Hha I, Hinf I and *Rsa I* restriction enzymes were unable to distinguish the two mushroom species. *Hae III* restriction endonuclease was the most effective in segregating the two mushroom species (Fig 41b).

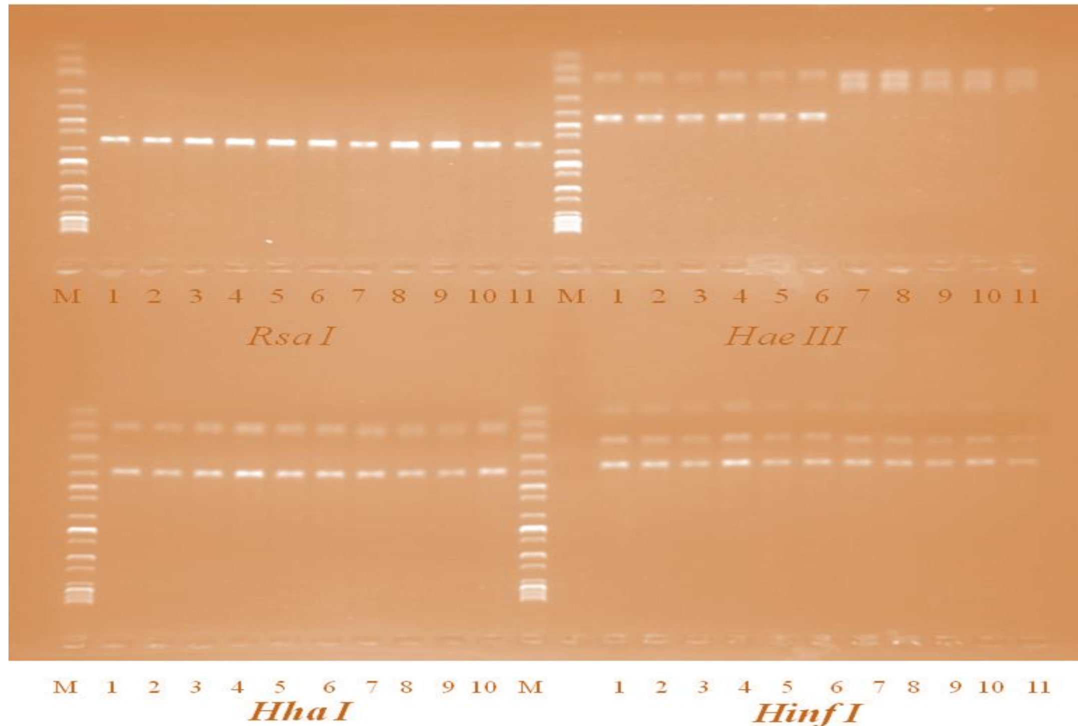


Fig 41b: Gel profile showing restriction patterns generated by four restriction enzymes; *Hha I, Hinf I, Rsa I* and *Hae III* on samples; 1 (EM-1 Exp. 1), 2 (EM-1 Exp. 2), 3 (EM-1 Exp. 3), 4 (EM-1 sawdust), 5 (EM-1 Exp. 4), 6 (EM-1 Mixture substrate),7 (P-31 Exp. 1 Mixture. substrate), 8 (P-31 Exp. 2), 9 (P-31 Exp. 3), 10 (P-31 Exp. 1) and 11 (P-31 sawdust). Alongside kappa universal DNA ladder (M). Mushrooms are however segregated into 2 main groups based on *Hae III* restriction pattern of the ITS regions. The 2 groups are however confirmed to include the *Pleurotus ostreatus* strain EM-1 isolates on 1 side and the *P. eous* strain P-31 isolates on the other side.

Plate 12: Photograph showing the gill regions of *P. ostreatus* EM-1 (Mg. x1/2)

Plate 13: Photograph showing the gill regions of *P. eous* P-31 (Mg. x1/2)

Plate 14: Photograph showing the growth of the two *Pleurotus* species used in the investigation on Potato Dextrose Agar **Top**; *Pleurotus eous* P-31; **Bottom**: *P. ostreatus* EM-1 (Mg. x1/2)

Plate 15: Photograph showing the appearance of the two *Pleurotus eous* P-31 in the cropping house (Mg. x0.05)

Plate 16: Photograph showing the appearance of the two *Pleurotus ostreatus* EM-1 in the cropping house (Mg. x0.05)

EXPERIMENT H

Studies on mycoflora and some physicochemical elemental and nutrient content of the soil and Spent Mushroom Compost before utilization as mixtures in different proportions for the cultivation of tomato, pepper and cowpea

The soil sample did not contain copper (Cu) and Iron (Fe), but Zn, Mn, Pb, Ca, Mg, Na, P, K and N were detected in very low quantities (Table 81). The spent mushroom compost did not contain Fe but Zn, Cu, Mn, Pb, Ca, Mg, Na, P, K, N were detected (Table 81) albeit in low quantities. The presence of heavy metals such as Pb, Zn and Cu was noted. The pH of the soil was 6.8 and spent mushroom compost, pH 6.6 was within the favourable pH for the growth of both *P. ostreatus* and *P. eous* and the plants as well. There were marginal differences between the population of soil mycoflora isolated on Cooke's medium and DRBC (about 0.1 log cycles). In the spent mushroom compost the difference was larger (0.4 log cycles) albeit small (Fig 42).

The use of two media enables a wider spectrum of fungi to be detected. Eighteen (18) different fungi belonging to 10 genera (*Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Rhodotorula, Trichoderma, Yeasts, Scopulariopsis*) and *Mycelia sterilia* were encountered in both samples. *A. flavus, A. niger, P. citrinum* and *T. harzianum* predominated in the soil samples while *Rhodotorula, T. harzianum, P. citrinum* and *A. candidus* and *A. flavus* formed 5-48% of the population (Fig 43); *T. harzianum* contributed 20-30%, *Rhodotorula* 33-47%; *P. citrinum* (8-9%) (Fig 43).

Table 81: Some physicochemical properties and elemental content of soil and spent compost used as growing medium for tomato, pepper and cowpea seedlings

Growing medium	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Na (mg/kg)	P (mg/kg)	K (mg/kg)	N (mg/kg)	pH	Moisture content (%)
Soil	0.016	0.000	0.137	0.014	0.471	0.444	0.000	0.217	0.890	0.118	0.819	6.80	0.98
Spent Compost only	0.017	0.003	0.013	0.013	0.597	0.773	0.000	0.196	0.795	0.281	0.978	6.60	11.51

Table 82: Proximate analyses of the combined Spent Mushroom Compost from *P. eous* strain P-31 used as fertilizer medium for the cultivation of pepper, tomato and cowpea in the greenhouse

% Composition of Spent Mushroom Compost						
% Dry matter	**% Crude protein**	**% Hemicellulose**	**% Cellulose**	**% Lignin**	**% NDF**	**% ADF**
90.89±1.79	7.76±0.48	3.79±1.26	32.34±1.97	7.26±1.94	53.3±6.07	50.42±4.64

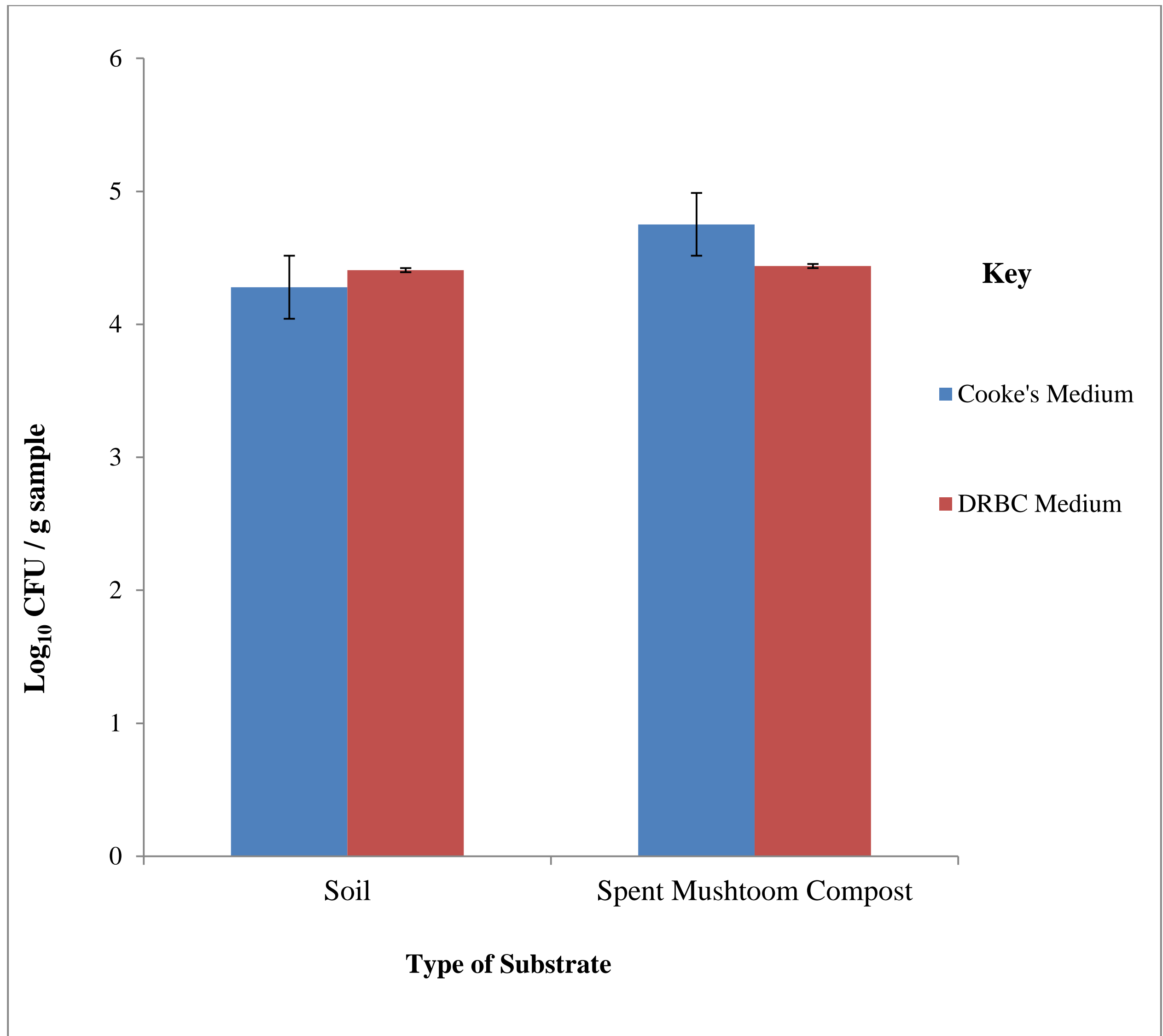
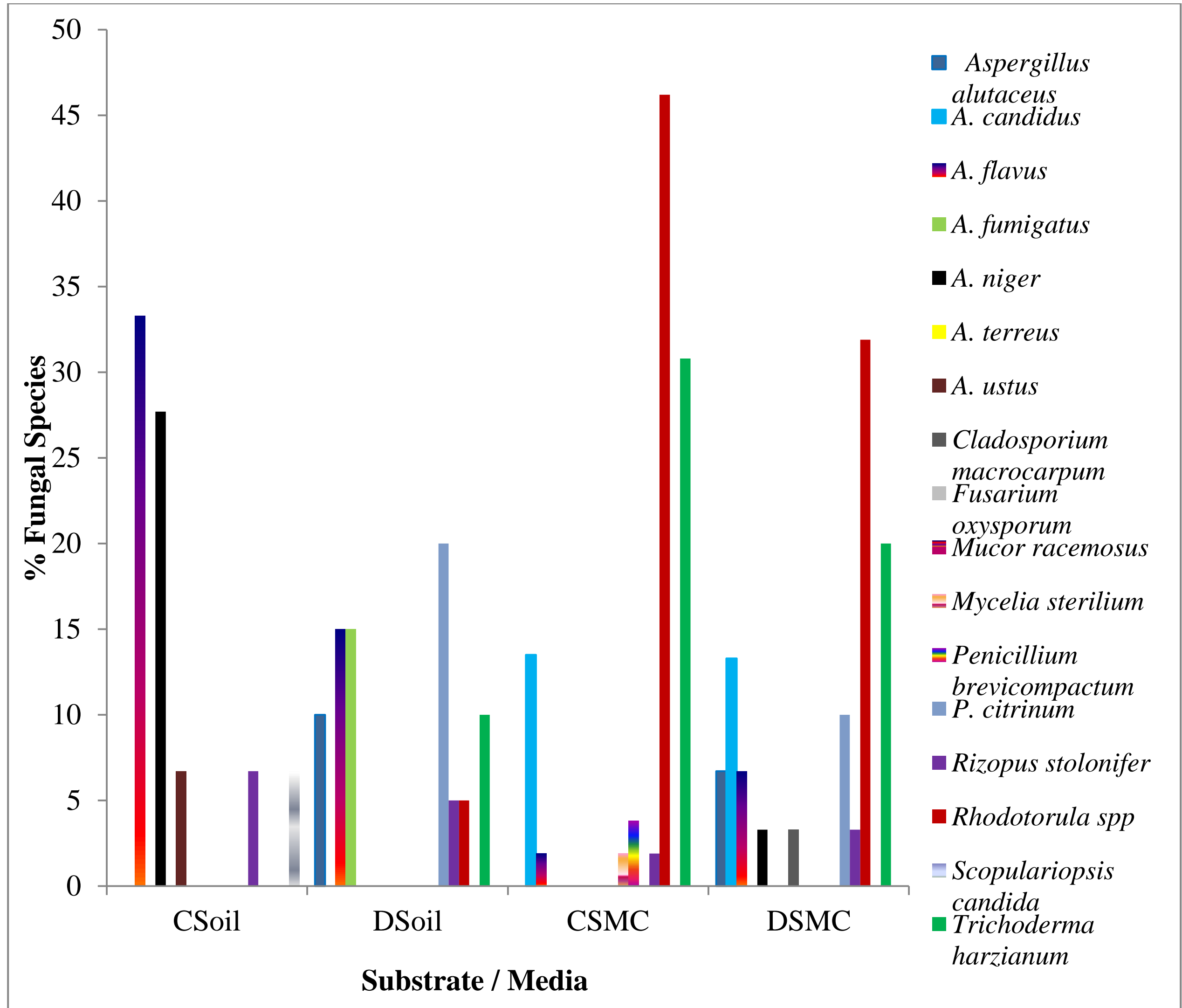


Fig 42: Initial mycoflora population resident in raw soil and raw spent mushroom compost used for the cultivation of cowpea, pepper and tomato



Key

C Soil: Cooke's Medium (Soil) C SMC: Cooke's Medium (Spent Compost)

D Soil: DRBC Medium (Soil) D SMC: DRBC (Spent Compost)

Fig 43: Mycoflora profile of soil and spent mushroom compost estimated on two mycological media at 28±2◦C for 7 days

EXPERIMENT I

Influence of the Spent Mushroom Compost SMC of *P. eous* P-31 (used as bio-fertilizer) on the growth and development of tomato (*Lycopersicon esculentum* Mill.) seedlings under greenhouse conditions

Results obtained are presented in Figs 44A-D and Plates 17 and 18 and Appendix 1(a-d).

i) Plant height

The height of tomato seedlings growing in soil only lagged behind and was inferior to the rest of the other plants growing in soil : SMC mixtures (5-30% SMC) (Fig 44A and Plate 17. The height of plants growing in the 5-15%, 20, 25% SMC were not significantly different ($p>0.05$) (similar 56-58cm after 8 weeks) (Fig 44A). The best height attained in 10% SMC was significantly ($p\leq0.05$) different from the rest.

ii) Leaf area

The leaf area measurements of the developing seedling of tomato followed the same trend as the plant height. Plants growing in the control (soil only) attained an average area of about 250mm^2 in 8 weeks as compared to seedlings growing in pots with 15 and 20% SMC (Fig 43B) with a similar leaf area of 700-720mm^2. The differences observed after 8 weeks were statistically significant ($p<0.05$). The largest net photosynthetic area of the leaf was obtained in plants growing in 10% SMC similar to what was obtained for 15 and 20% SMC. Plate 18 shows the general view of the luxuriant growth of the treated plants growing in soil containing spent mushroom compost.

iii) Number of leaves

There was no statistical differences ($p>0.05$) between no. of leaves formed by plant growing in soil amended with 5-30% SMC. However leaf formation in soil only lagged behind the rest of the treatments and the differences observed were statistically significant ($p<0.05$) (Fig 44C).

iv) Chlorophyll content

Increasing proportions of SMC in soil affected the development of chlorophyll such that Chlorophyll content in leaves decreased with increasing % SMC in this order 5% >10% >15% >20% >25% >30%. The chlorophyll contents of plants growing in soil with varying proportions of SMC as bio-fertilizer were statistically ($p\leq0.05$) higher than the control (Fig 44D).

Thus one can say that high percentage of SMC in the soil was attended with a lowering of chlorophyll content of plants but were still superior statistically ($p\leq0.05$) to the control. Appendix 2 shows the statistical analysis of the data.

v) Dry weight of plants

The least dry matter accumulation by the plant was obtained in the unamended soil shoot (90.3±5.9g) roots (45.3±2.9g). There was commensurate increase in dry weight of shoot and roots as the concentration (%) of SMC increased from 5-25% shoot and thereafter declined to 94.9± 3.90g (shoot) and root (48.9 ± 2.48g) in pots containing spent mushroom compost only. High concentration of the compost therefore affected the growth of the plant (Table 83) and Plates 17 and 18.

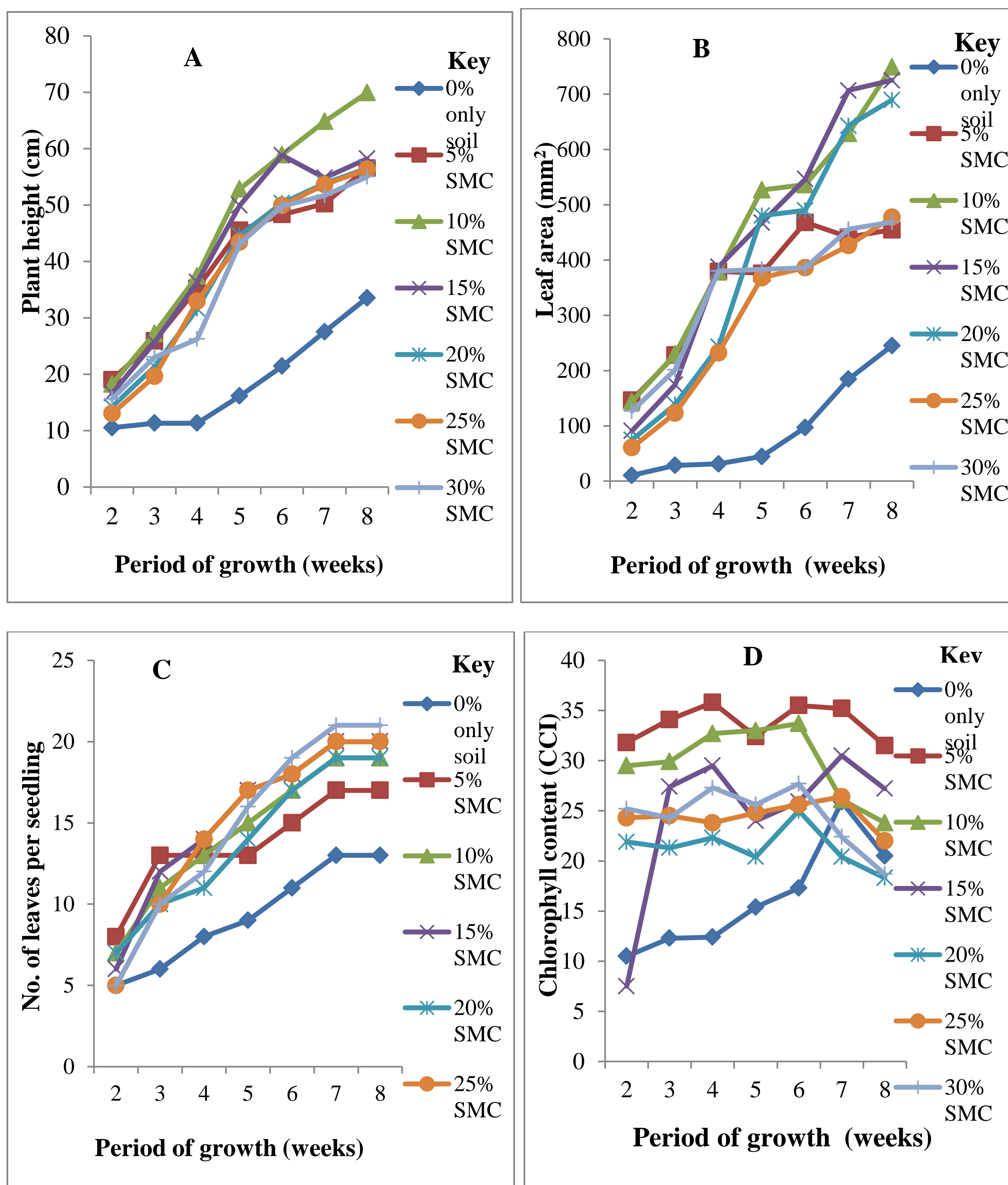


Fig 44(A-D): Influence of varying percentages of Spent Mushroom Compost (SMC) in soil on the growth and development of tomato seedling grown in varying proportions of soil: SMC under greenhouse conditions at 30±2˚C for 8 weeks

Table 83: Dry matter accumulation by tomato seedlings after12 weeks of growth in pots containing indicated percentage of soil: SMC mixtures in the greenhouse at 28-32°C

Substrate Treatment (%SMC)	**Mean dry weight (g) ±SE**	
	Shoot system	**Root system**
0 (Soil)	90.3± 5.86[a]	45.3 ± 2.99[a]
5	160.6 ± 6.3 [b]	83.6 ± 5.13[b]
10	185.5± 3.8[c]	92.3 ± 2.20[c]
15	185.2 ± 4.0[c]	84.1 ± 1.37[b]
20	167.9 ± 2.8[d]	82.1 ± 2.52[b]
25	165.4 ± 5.7[d]	78.8 ± 4.10[d]
30	159.0 ± 7.3[e]	77.0 ± 2.79[d]
100	94.9 ±3.9[a]	48.9 ± 2.48[a]

Plate 17: Vegetative growth of tomato seedlings in the indicated soil: SMC mixtures of different percentages of SMC growing in the greenhouse at 30±2°C for 4 weeks after transplanting. (Note the diminutive height of the plants growing in soil only; control) (Mg. x1/10)

Plate 18: Photograph showing general view of the luxuriant growth and development of tomato seedlings in soil amended with varying percentages of (0-30% SMC) Spent Mushroom Compost of *P. eous* at 30±2°C (Mg. x1/5)

EXPERIMENT J

Influence of Spent Mushroom Compost, SMC of *P. eous* P-31 (used as bio-fertilizer) on the growth and development of pepper (*Capsicum annum* L.) seedlings under greenhouse conditions

The seeds germinated in all the pots with the different proportions of the Spent Mushroom Compost. Dry matter accumulation by the shoot system (leaves and stems) increased with increasing proportion of the SMC up to 25% SMC (Table 84) The root system also increased in dry matter accumulation in a similar manner but declined after 20% SMC. The poorest growth was obtained in soil only (24.7g) Fig 44c-d, Plate 19 and Appendix 2.

i) Plant height

The growth of the pepper seedling in height followed near sigmoid curves in all the treatments (Fig 44A). There was no statistical differences ($p>0.05$) in the height of plants growing in soil amended with 5-25% SMC and were the best recorded after 8 weeks of growth. The extensional growth of the seedlings in soil only (control) was the lowest and statistically ($p<0.05$) different from what was recorded in the soil amended with 5-25% SMC (Fig 44A). Growth of pepper seedling in soil amended with 30% SMC was intermediate and statistically ($p<0.05$) superior to the control (Fig 44A) although lower than the heights attained in the soil amended with 5-25% SMC.

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ii) Leaf area

Data on the leaf area of plants growing in soils amended with 5-30% SMC is presented in Fig 44B. It also followed a nearly sigmoid curve. Mean leaf area of the plants cultivated in soil amended with 5-25% SMC were initially not significantly different ($p>0.05$) after 2 - 4 weeks but could be distinguished after 8weeks growth. The highest leaf area was obtained in soil amended with 5% SMC which was statistically ($p>0.05$) different from the leaf areas of plants in soil amended with 15% SMC. The higher the concentration (proportion) of the SMC: soil the smaller the leaf area (Fig 44B). Interestingly, the smallest leaf area ≥ $20cm^2$ was recorded in plants growing in the unamended native soil.

iii) Chlorophyll content

The development of chlorophyll in the plants sown in soil amended with 5-30% SMC was erratic albeit produced the highest recorded chlorophyll content up to 30% SMC (18-22 CCI) in 8 weeks. However, chlorophyll levels in leaves sown in unamended soil (control) 11-15 CCI never approximated those in the amended soils (18-22 CCI) throughout the 8weeks growth. The difference observed were statistically significant ($p<0.05$) (Fig 44C).

iv) Number of leaves per plant

Data obtained for the development of leaves by the control and plants growing in soil amended with 5-30% SMC also followed a near sigmoid curve. Soil amended level with SMC ranging from 5-25% proportionately increased the no. of leaves up to 70-80 per seedling. However, at 30% SMC there was a dramatic decline in the no. of leaves / plant to about 50 in 8weeks (Fig 44D). The difference observed were statistically ($p\leq0.05$)

significant. The differences in the stimulation of leaf production by 5-20% SMC were initially indistinguishable after 2-6 weeks and remained so until 8 weeks at least for 5% SMC concentrations up to 20% SMC (Fig 44D). Plate 19 shows the comparative appearance of the plants after 6 weeks. Appendix 2 shows the statistical analysis of the data obtained.

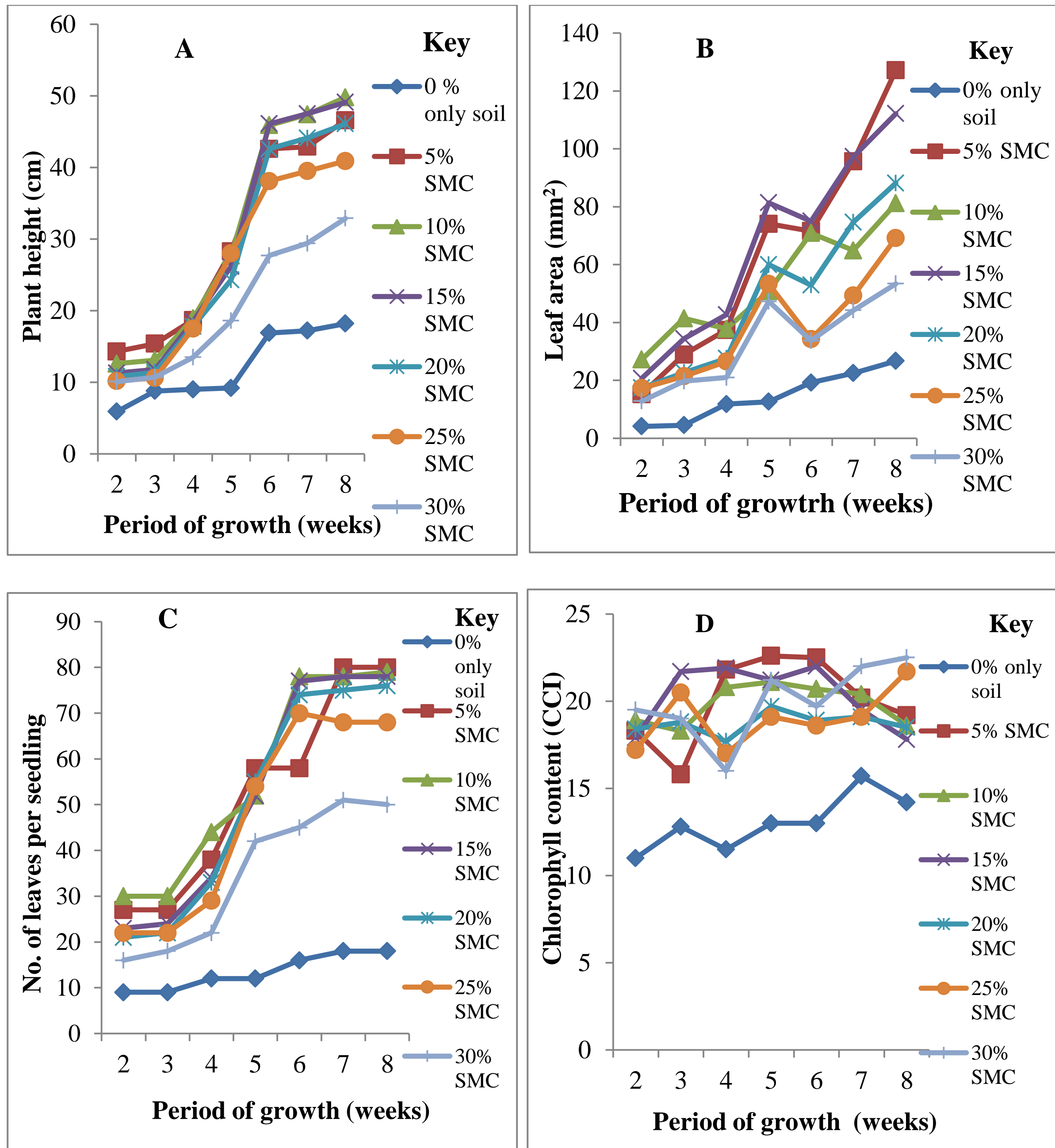

Fig 45(A-D): Influence of spent mushroom compost (SMC) on the growth and development of pepper seedlings grown in varying proportions of soil: SMC under greenhouse conditions

Table 84: Dry matter accumulation by pepper seedlings after12 weeks of growth in pots containing different indicated percentage of soil: SMC mixtures in the greenhouse at 28-32°C

Substrate Treatment (%)	Mean dry weight (g) ±SE	
	Shoot system	Root system
0 (Soil only)	27.1 ± 1.25[a]	24.7 ± 2.66[a]
5	70.0 ± 1.65 [b]	41.5 ± 2.13[b]
10	89.5 ± 5.38[c]	46.9 ± 2.39[b]
15	98.7 ± 3.92[d]	45.2 ± 3.12[b]
20	103.8 ± 2.66[e]	44.4 ± 3.86[b]
25	133.8 ± 16.44[f]	42.4 ± 3.81[b]
30	146.6 ± 13.56[g]	40.7 ± 1.88[b]

Plate 19: Photographs showing comparative height and leaves formation by pepper plants growing in the indicated varying percentage mixture of soil and Spent Mushroom Compost after 6 weeks growth at 30±2°C in the greenhouse (Note the diminutive height of the plants growing in unamended soil; control) (Mg. x1/10)

EXPERIMENT K

Influence of Spent Mushroom Compost, SMC of *P. eous* P-31 (used as bio-fertilizer) on the growth and development of cowpea (*Vigna unguiculata* Walp) seedlings under greenhouse conditions

The seeds germinated in all pots. Dry matter accumulation of shoots (leaves and stem) and the root system in 12 weeks commensurate with the percentage of SMC used up to 15% SMC and thereafter decreased. The highest dry weight of shoot, 43.4±2.0g and root system (0.75±0.36g) was recorded in 15% SMC and there was no statistical difference ($p<0.05$) between this and the 10% SMC (Table 87). The poorest growth was obtained in the pots containing compost only (16.2 ± 1.18g) (100% SMC) followed by pots containing soil only (20.6 ± 1.58g) (Table 86.). Plates 20 and 21 show the growth of the seedlings after 6 and 12 weeks respectively.

The trend in the growth and development parameters i.e. height of plant (cm), leaf area (i.e. height of plant (cm), leaf area (mm^2), number of leaves formed, no. of axillary branches, number of floral buds, no. of flowers, no. of pods setting, weight of the pods, and weight of seeds followed this general trend: 5% < 10% < 15% < 20% < 25% < 30% < 100% in descending order.

The differences observed were statistically significant ($p< 0.05$) (Appendix 3). Fig 45A-D and Appendix 3 show results obtained.

i) Plant height

Although plant heights after 2 weeks was nearly same after 2 weeks there was clear distinction after 3-4weeks resulting in the depression of growth in the soil amended with 5-25% SMC as percentage of SMC increased. There was no statistical difference ($p \geq 0.05$) between the plant height of seedlings cultivated in 30%, 100% (SMC only) and 0% (unamended soil) Fig 45A. Generally plant height (dry matter accumulation) followed a sigmoid curve and the decline in plant height was apparent (Plates 20&21).

ii) Number of leaves

This parameter of measurement of the efficiency of the Spent Mushroom Compost as a bio-fertilizer also followed a near sigmoid curve. Higher concentrations of SMC (15-100% SMC) depressed leaf formation and there was no statistical difference ($p \geq 0.05$) between plants sown in 15-100% SMC and the compost free control soil (Fig 45C).

iii) Leaf area

The data of the leaf area of the plants cultivated in the control soil and soil amended with the varying %SMC over 8weeks followed a sigmoid curve. There were two categories of the effect on the leaf area of the seedlings. Concentration (%) of SMC 5, 10, 15, 20% increased the leaf area (550-680mm^2) Fig 45B while concentration (%) 25% and beyond depressed leaf area (300-400 mm^2). Interestingly the growth parameters recorded for plants that were growing in spent mushroom compost only (100% SMC) were comparable to what was obtained in soil only.

iv) Chlorophyll content

There was an initial decline in chlorophyll content after 3weeks but was followed by an increase reaching its maximum levels after 5weeks and thereafter declined (Fig 45D). High concentrations 10-100% SMC reduced chlorophyll content while 5-10% gave the highest concentration of chlorophyll in the leaves after 4 and 6 weeks respectively and thereafter declined (Fig 45D). Amendment of soil with 30-100% SMC severely depressed chlorophyll formation ($p<0.05$) Plate 20 shows the external morphology of the plant during growth in the greenhouse. Appendix 3 shows the statistical analyses.

v) Nitrogen content of compost and Plant during growth

The nitrogen content of the soil: compost mixtures increased with increasing percentage of the spent composts from 5-100% SMC (Table 87). The increase in Nitrogen content of the plant during growth was commensurate with the increase in percentage of the SMC in soil (Table 87). Thus there was a transfer of nitrogen to the growing plant during cultivation for 12 weeks.

Percentage dry matter of the Spent Mushroom Compost did not change significantly ($p<0.05$) in all the varying mixtures with soil (0-30% SMC) and was attended by corresponding dry matter accumulation of the cowpea seedlings (93.18-93.80%) (Table 87).

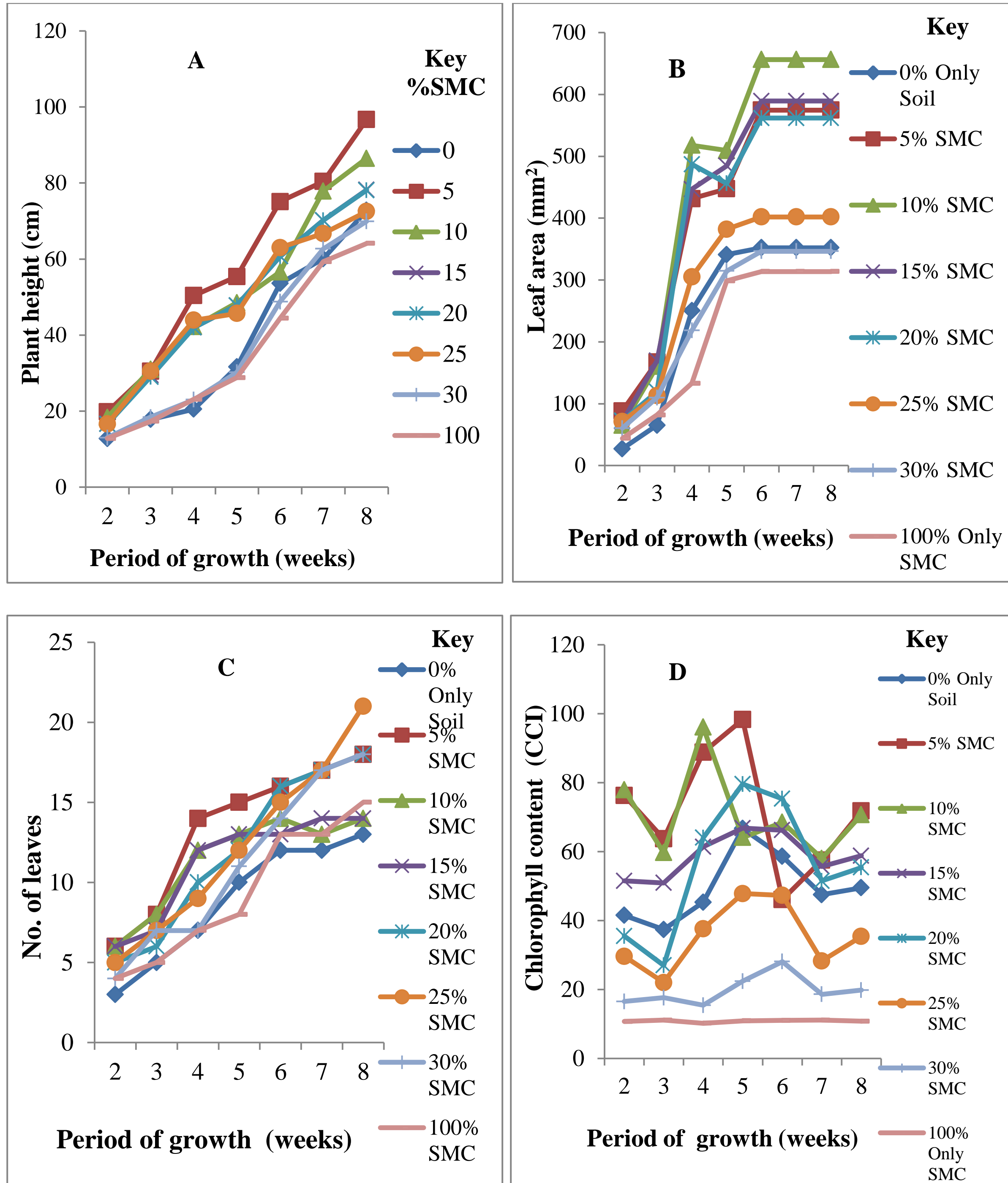

Fig 46(A-D): Influence of spent mushroom compost (SMC) on the growth and development of cowpea seedlings grown in varying proportions of soil: SMC under greenhouse conditions

Table 85: Dry matter accumulation by cowpea seedlings after12 weeks of growth in pots containing indicated percentage of soil: SMC mixtures in the greenhouse at 28-32°C

Substrate Treatment (%SMC)	**Mean dry weight (g ± SE) of**	
	Shoot system	**Root system**
0 (Soil only)	20.6 ± 1.58[a]	0.39 ± 0.14[a]
5	24.2 ± 1.58[a]	0.50 ± 0.16[b]
10	42.7 ± 1.50[b]	0.71 ± 0.32[c]
15	43.4 ± 2.00[b]	0.75 ± 0.36[c]
20	36.6 ± 2.76[c]	0.41 ± 0.20[d]
25	34.4 ± 4.29[c]	0.33 ± 0.11[a]
30	22.9 ± 0.11[a]	0.30 ± 0.05[a]
100	16.2 ± 1.18[d]	0.21 ± 0.02[e]

Table 86: Correlation between nitrogen content of growing medium and assimilation of nitrogen by cowpea seedlings cultivated in the indicated proportions of spent mushroom compost with soil at 28-32°C for 12 weeks

Type of treatment (% SMC) Mixture with soil	Total (%) Nitrogen content				Moisture Content (%)	
	% Dry Matter of growing media	% Dry Matter of Plant Sample	Compost (%)	Plant (%)	Growing media	Plant
0 (Soil only)	98.68	93.76	0.07	1.55	3.15	83.37
5	98.59	93.27	0.16	1.62	5.87	87.13
10	98.41	93.20	0.19	1.92	7.46	87.59
15	98.48	93.80	0.25	2.18	8.83	87.90
20	98.40	93.43	0.30	2.34	11.04	87.45
25	98.03	93.74	0.43	2.25	12.54	87.30
30	97.99	93.18	0.40	2.08	20.63	88.20
100 (SMC only)	94.10	94.18	1.16	1.60	44.71	89.34

Plate 20: Vegetative growth of cowpea in varying proportions (percentages) of soil: SMC in pots in the greenhouse at 28-32°C for 6 weeks (Note the luxuriant growth in 5-25% SMC pots and the diminutive growth in pots with higher concentration 30-100% SMC) (Mag x1/10)

Plate 21: Vegetative growth and pod formation of cowpea growing in pots containing the indicated proportions (percentages) of soil: SMC mixtures at 28-32°C for 12 weeks. (Note the growth and pod formation in the 5-20% SMC: Soil mixture) (Mag x1/5)

EXPERIMENT L.

Assessment of cowpea seed germination and Nodule Index of nodules formed in soil amended with varying proportions (%) of Spent Mushroom Compost (SMC) under greenhouse conditions.

It was anticipated that successful nodulation and pod setting of the cowpea plant will confirm the efficacy of the SMC as a bio-fertilizer.

Table 89 shows the record of percentage *in vitro* germination of cowpea seeds, mean length of radicles / rootlets, mean no. of nodules, mean diameter of nodules, colour and mean weight of nodules. Plate 22 shows the root system of the harvested plants in the various combination percentages of soil: SMC. The calculated Nodule Indices are summarised in Table 88.

Percentage germination of the seeds sown in the soil amended with 5-30% of SMC was 100% whereas in the unamended and SMC only 80% was obtained in the pots. The total weight, diameter (size) and number of nodules produced increased with increasing percentage of SMC in soil up to 10% and thereafter declined (Table 87.). The calculated Nodule Index (a product of Nodule Size x Nodule Colour x No. of Nodules) show that it was highest at 5% followed by 10% SMC and thereafter declined (Table 88). The colour of the nodules were pink to red in 5-30% SMC and white in the soil only while no nodules were formed in the 100% SMC growing medium. Plate 22 shows the morphology of the root system with the attendant nodules formed.

There was a corresponding slight increase in population of rhizobium in the root nodules with increasing % of SMC reaching peak of $\log_{10}$ 7.87 CFU /g at 25% SMC. However, the differences were not statistically significant ($p<0.05$). There were no rhizobia in the 100% SMC medium.

Table 87: Record of seed germination, nodule formation, nodule size and weight, no. of nodules and colour of nodule in the varying soil: spent mushroom compost used as growing medium for cowpea at 28±2°C for 9 weeks

SMC: Soil combination (%)	Percentage germination of seed	Weight(mg) of nodule	Diameter(mm) Size of nodule	No. of nodules per size			Total No. of Nodules	Colour Index
				Small	Medium	Large		
0	80	102.8	1.21	46	12	0	58	1
5	100	509.5	3.70	10	17	62	89	2
10	100	435.1	3.56	5	21	27	53	2
15	100	129.2	2.79	12	16	18	46	2
20	100	111.8	2.25	23	8	2	33	2
25	100	119.3	2.23	15	5	2	22	2
30	100	31.3	1.60	2	7	9	18	2
100 (SMC only)	80	0	0	0	0	0	0	-

Scale

Nodule Size (mm)	**No. of Nodules**	**Color Index**
0 - 1.5……….Small (1)	0 – 30……..Few (1)	1………..White
1.6 - 2.21…....Medium (2)	31 – 60……Several (2)	2……….Pink - Red
2.22 – 3.70….Large (3)	61 – 90…...Many (3)	

Table 88: Estimation of Nodule Index of nodules formed in the varying compost: soil mixtures used as growing medium for cowpea at 28±2°C for 9 weeks (63 days)

Growing Medium (%SMC) (Compost : Soil Mixture)	Nodule Size (mm) Nodule Index	Nodule Weight (mg) Nodule Index
0	2	4
5	18	18
10	12	12
15	8	8
20	8	8
25	4	4
30	4	2
100	0	0

Nodule Index = A x B x C ≤ 18 (Rehab *et al*. 2002)

A = Nodule Size; **B** = Nodule Colour; **C**= No. of Nodule

Nodule size………………..Value of A

Small……………………………1

Medium…………………………2

Large……………………………3

No. of Nodule………………..Value C

Few………………………………..1

Several…………………………..…2

Many…………………………........3

Nodule Colour………………Value B

White……………………………...1

Pink to Red…………………………..2

Plate 22: Root system of the harvested plants in the various combination percentages of soil: spent mushroom compost (SMC). Mag. x1/10

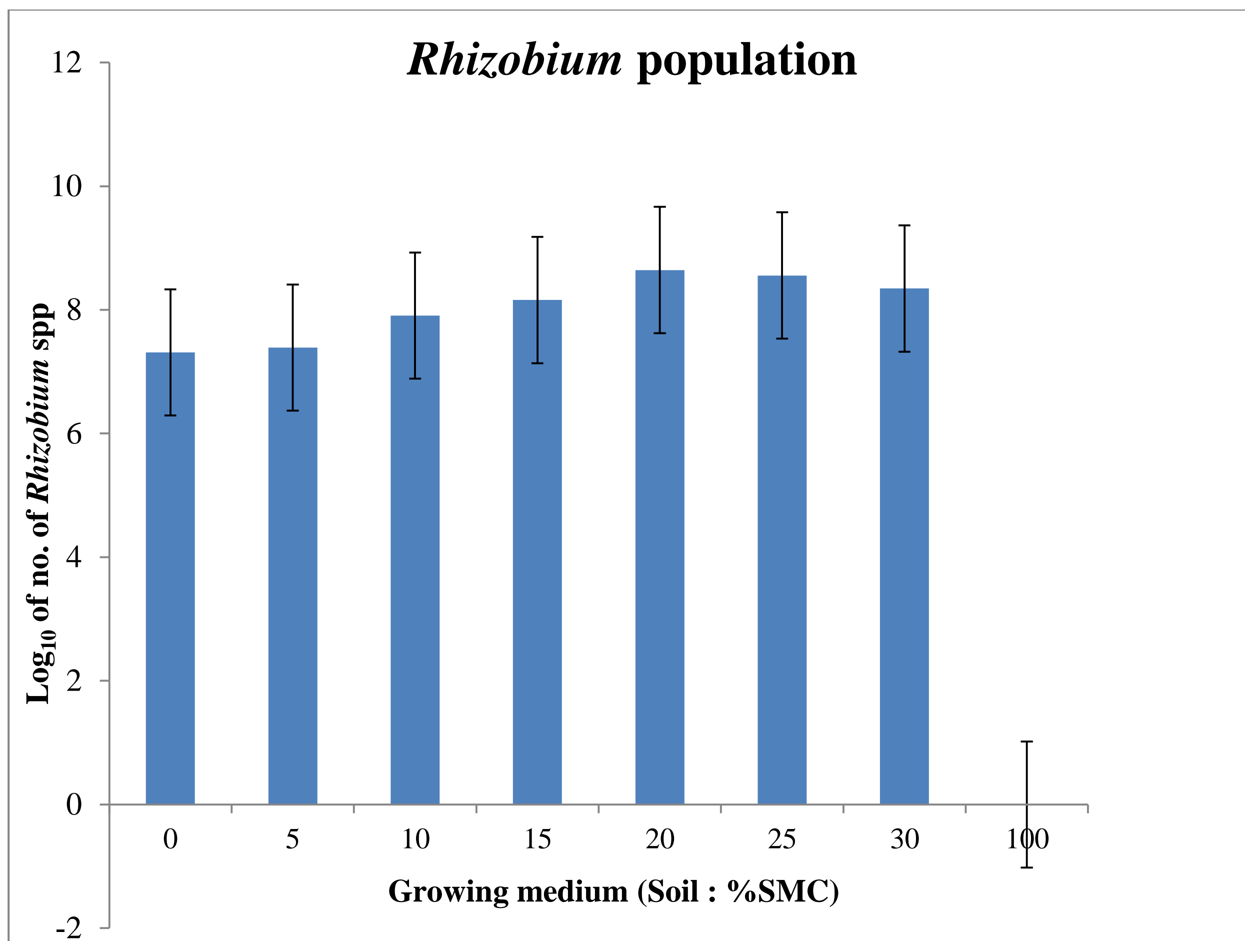

Fig 47: *Rhizobium* population resident in the root nodules of cowpea seedlings growing in different proportion (%) of the Soil: SMC mixtures after 12 weeks at 28-32°C

GENERAL DISCUSSION

Mushrooms have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fibre, and also poor in fat. Edible mushroom also provides a significant content of vitamins (B_1, B_2, B_{12}, C and E (Heleno *et al*. 2010; Mattila *et al*. 2001). Edible mushrooms are a source of many different nutraceutical such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids (Ferreira *et al*. 2009; Vaz *et al*. 2010; Perreira *at al*. 2012).

More than 3000 mushrooms are considered to be edible of which about 100 species are cultivated commercially and only 10 of these are on industrial scale. Their global economic value is now staggering, and the prime reason for the increased consumption is the combination their value as food as well as medicinal and nutraceutical values (Chang and Miles, 2004). The most cultivated mushrooms worldwide is *Agaricus bisporus*, followed by *Lentinula edodes* (Shiitake), *Pleurotus* species, *Flammulina velutipes*, *Volvariella volvacea* (domo) to mention but a few (Aida *et al*. 2009); Chang and Miles, 2004).

Cultivation of oyster mushroom (*Pleurotus* spp) has increased tremendously throughout the world because of their ability to grow over a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). These mushrooms can reduce lignin, cellulose, hemicellulose, tannins and crude fibre content of agricultural waste materials making it ideal for animal feed as well (Ortega *et al*. 1992).

In Africa, there is a large scale pollution of the environment owing to the methods of handling agricultural waste. Africa, not excepting Ghana, is yet to take advantage of the use agro-lignocellulose on a wider industrial scale to produce oyster mushrooms to supplement protein and nutritional deficiencies. Strengthening oyster mushroom production by diversifying the use of agro-residues from field crops could be essential in order to enable the rural economy to keep to its vibrancy and development (Sharma *et al*. 2013). This will increase diversify business and employment opportunities in the rural areas, by providing income opportunities in mushroom cultivation to assist the disadvantage small family farm dwellers. It could also give additional or alternative income for farmers looking for a value-added product (Non-Timber Forest Products NTFP) and a way to supplement farm income while making use of by products (lignocellulose residues) or co-products from other crops (Sharma *et al*. 2013).

This thesis provides novel information on the use of rice lignocellulose (rice straw, rice bran and rice husk) and its supplementation to boost the production of oyster mushroom from *Pleurotus ostreatus* strain EM-1 and *Pleurotus eous* strain P-31. While *P. ostreatus* cultivation has been extensively tried on substrates like rice husk, sorghum stover, sawdust, cotton waste, cocoa bean shell in Ghana and elsewhere, the use of rice lignocellulose and other agro-waste for the cultivation of *P. eous* has not been used extensively in Ghana.

Most plant wastes are composed of lignin, cellulose and hemicellulose (which include pectin starch etc.). Large amounts of these including hemicellulose and some other materials in plant waste are undesirable and so in the process of preparing the substrate by composting the resident microflora contribute to the environment of the substrate to make it digestible by the mushroom. This phenomenon was investigated in Chapter A of the Results Section.

Fungal population and phenology profiles in the different agricultural lignocellulose wastes used in the cultivation of *P. ostreatus* and *P. eous* were studied.

Rice straw only

The initial fungal population increased by 2-3log cycles after 4-8 days composting. The use of the two media enabled a wide range of fungal species to be isolated from the compost (Figs 3&4). Six *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. niger, A. panamensis* and *A. penicilloides*) predominated followed by *Penicillium* (*P. glabrum*, and *P. citrinum*) (Fig 3). Fungi belonging to nine genera (*Aspergillus, Fusarium, Cladosporium, Curvularia, Rhizopus*, *Rhodotorula, Trichoderma*) and *Mycelia sterilia* were isolated from the composting rice straw after 12days (Figs 3 and 4). There was no clear-cut difference in the species diversity and percentage occurrence of the fungal species with top (5cm-10cm), middle (15cm-20cm) and bottom (25cm-30cm) of the pile because the compost was stirred at 4days intervals.

The most frequently encountered species were *A. flavus*, *Rhodotorula* sp., *A. niger, Trichoderma harzianum* and *Penicillium citrinum* (Figs 5&6).

Sandhu and Sidhu (1980) identified certain fungi associated with the composting process. These included *Aspergillus fumigatus, A. terreus, Mucor pusillus, Penicillium* species, *Rhizopus microsporus, Trichoderma* spp and an agaric. *A. fumigatus* and *A. terreus* predominated over the others. Generally, it has been shown that the underlisted genera were encountered in most compost. *Alternaria*, *Cladosporium, Coprinus, Dactylium, Epicoccum*, *Fusarium, Geotrichum, Monilia, Mucor, Mycelia sterilia, Mycogyne, Neurospora, Papulospora, Penicillium, Rhizopus, Scopulariopsis, Sepedium, Trichoderma, Tricothecium, Verticillium* and *Yeasts* (Stamets, 1993). About six of these (*Aspergillus, Cladosporium*,

Fusarium, Penicillium, Trichoderma and *Mycelia sterilia*) genera were isolated in this thesis but Obodai (1992) showed that *Mucor pusillus* predominated in rice straw used for the cultivation of *Pleurotus* spp., followed by *Paecilomyces varotii* and *Penicillium oxalicum*, *A. flavus, A. terreus and Cladosporium herbarium* in Ghana. The differences in resident mycoflora may be due to the source of the raw rice straw.

Rice straw amended (supplemented) with 1% $CaCO_3$

Supplements added to substrates earmarked for mushroom cultivation may or may not benefit the mushroom. If it does benefit the mushroom, it acts by enhancing the growth of the resident microorganisms for satisfactory composting (Obodai *et al*. 2011). Rice bran, chicken manure and other by products are commonly mixed to improve pH and nitrogen level and enhance texture, quality and mycoflora development (Chang and Miles, 1987).

Liming of compost with 1% $CaCO_3$ only marginally increased the initial fungal population in the rice compost (Figs 7&8) and composting for 4-12days increased population by 0.5-1.2 log cycles on both media. Again, because of the stirring of the compost at 4days intervals there was no statistical differences ($p>0.05$) between fungal population isolated from the different depths. The resident mycoflora in the rice amended with 1% $CaCO_3$ was predominated by six *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. niger, A. panamensis* and *A. penicilloides*) consisting 35- 68% of the total population (Figs 9&10). Generally fungal species belonging to 10 genera (*Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizoctonia, Rhodotorula, Trichoderma, Neosartorya, Yeasts*) including *Mycelia sterilia* were isolated on the two media. Again, the most frequently encountered were *A. flavus, A. niger, T. harzianum* and *Rhodotorula* some as were found in the rice straw (Figs 9&10).

Rice straw supplemented with 1% $CaCO_3$ and 10% rice bran

Amending the rice compost with 1% $CaCO_3$ and 10% rice bran marginally increased fungal population by less than 1log cycle (Figs 11&12) during 12days composting. The marginal difference in the three layers may be attributed to the turning of the compost heap at 4days intervals. The mycoflora in this rice straw supplemented with 1% $CaCO_3$ and 10% rice bran was predominated by 8 *Aspergillus* species (*A. flavus, A. fumigatus, A. panamensis, A. niger, A. penicilloides, A. sulphureus, A. candidus, A. alutaceus*) followed by *Penicillium* spp (*P. glabrum* and *P. citrinum*). The essential difference in the formulation of this substrate is the addition of 10% rice bran. Consequently two new *Aspergillus* species (*A. sulphureus* and *A. fumigatus*) were recorded and fungal species belonging to 12 genera (*Aspergillus, Cladosporium, Curvularia, Fusarium, Byssochlamys, Geotrichum, Penicillium, Rhizopus, Rhizoctonia, Rhodotorula, Trichoderma* and *Yeasts*) were isolated (Figs 13&14). Some thermophilic species *Byssochlamys* and *Geotrichum* were isolated for the first time in the amended rice compost. Again, *A. flavus, A. niger, A. fumigatus, Rhodotorula* and *Trichoderma* were the most predominated species isolated and show uniformity in the basal substrate for composting.

Rice straw amended with rice husk (1:1 w/w) and supplemented with 1% $CaCO_3$ and 10% Rice bran

The population of fungi resident in the mixture did not vary significantly ($p>0.05$) at the different depths (top, middle and bottom) owing to the turning of the compost at 4days intervals indicative of a very uniform mixture as was found for the previous compost of rice straw and its amendments (Figs 15&16). However, the fungal profile was different. Ten (10) *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. fumigatus, A. sulphureus, A.*

oryzae, A. ustus, A. niger, A. terreus and *A. versicolor*) predominated at all the depths sampled. This was followed by two *Penicillium* species (*P. italicum* and *P. citrinum*). Four new *Aspergillus* species namely, *A. oryzae, A. ustus, A. niger, A. terreus* and *A. versicolor* not found in the other composts were recorded for the first time. The genera of fungi resident in the mixture (rice straw and husk) were *Aspergillus, Cladosporium, Fusarium, Penicillium, Rhizopus, Rhodotorula, Rhizoctonia*, *Trichoderma* and *Yeasts* were akin to what was found in the other substrates. The most frequently encountered were *A. fumigatus* (>60%), *Rhodotorula* (<45-80%), *A. flavus* (12-30%), *T. harzianum* (>30%), *Rhizoctonia solani* (>44%) (Figs 17&18). The ability of *Pleurotus* mushroom to utilize these substrates for growth and successful yield will depend not only on the prevailing environmental and nutritional conditions but also on their competitive ability to withstand antibiosis activity of the resident mycoflora.

Influence of pH on radial and vegetative growth of two *Pleurotus* species

The best growth of *P. ostreatus* strain EM-1 on PDA was attained at a pH 5.4- 7.0 and was the same for *P. eous* strain P-31. Both species could not grow at pH 8.0 and beyond. Vegetative growth was best at pH 5.8- 6.6 for both strains. Narh *et al*. (2011) found that *P. ostreatus* grew best at pH 5.5- 6.5 and in Japan *Pleurotus* species performed best at 6.8—7.0 Stamets and Chilton, (1983). Recently, Obodai *et al*. (2011) found that the optimum pH range for growth of *P. ostreatus* was pH 6.0- 8.0. The pH of the substrates agrees with that reported in the literature.

Antibiosis activity of predominated mycoflora resident in compost

It is a well-established fact that some microorganisms are harmful to *Pleurotus* cultivation has been identified. *Trichoderma* species have been found to cause problems (Cailleux and Diop, 1978). *Monilia* sp., *Fusarium* spp., *Penicillium* spp., are harmful to *Pleurotus* cultivation and *Sclerotium. rolfsii* inhibited growth of *Pleurotus flabellatus* (Rajanathnam *et at.*1977). Jandaik *et al.* (1998) showed that *Penicillium cyclopium* infection of *Pleurotus sajor-caju* fruit bodies soon after emergence from substrate decreased yield by 50-75%. Studies by Obodai (1992) in Ghana showed that metabolites of *Trichoderma viride* were antagonistic to *P. ostreatus, P. sajor-caju* (from Mauritius and Hong Kong).

During the last decades representatives of the genus *Trichoderma* (*T. koningii, T. hamatatum, T. longibrachiatum, T. citreoviride, T. crassum, T. spirale* and *T. harzianum*) (Ospina-Geraldo *et al.*1999, Jandaik and Guleria, 1999; Castles *et al.*1998) have been isolated from mushroom compost. Aggressive colonization resulting in epidemic outbreaks was attributed originally to *T. harzianum* (Doyle, 1991; Seaby, 1987, 1989, 1996). Members of the genus *Aspergillus* and *Penicillium* have also been identified as antagonistic to mushroom cultivation. In section A of Results (Chapter Four), fungal population and their phenology profiles in the differently composted mushroom substrate for cultivation of *P. ostreatus* EM-1 and *P. eous* P-31 was investigated. *Aspergillus flavus*, *Rhodotorula* sp., *Penicillium glabrum, P. citrinum, A. niger* and *Trichoderma harzianum* persisted. *T. harzianum* was selected for the antibiosis studies because it is being recorded for the first time in mushroom compost in Ghana and also its involvement in the green mould disease in Oyster mushroom (*P. ostreatus*) in Sri Lanka caused by *T. harzianum.* This pathogen inhibited growth of mycelium and fruit bodies lowering the yield substantially (Jayalal and

Adikaram, 2007). *P. citrinum* forms a potent mycotoxin citrinin which could be lethal to humans and finally *A. flavus* produces aflatoxins (B_1, B_2, G_1, G_2) one of the most potent carcinogens produced by fungi.

The cultural filtrate of *A. flavus, P. citrinum, T. harzianum,* behaved differently in its effect on the *Pleurotus* species in liquid medium (Potato Dextrose Broth) and on agar (Potato Dextrose Agar). The culture filtrate of *A. flavus* at 1:1-1:5v/v dilution completely depressed radial growth of *P. ostreatus* and *P. eous* on agar. Dilution of 1:10v/v permitted feeble growth in 12 days (Tables 9 and 10).

Cultural filtrate of *P. citrinum* at 1:1-1:2 v/v dilution did not permit radial growth of *P. ostreatus* but allowed growth of *P. eous* at 1:2 -1:10v/v dilution was severaly depressed with very low dry matter accumulation with reduced diameter of colonies (Tables 11&12).

The culture filtrates of *T. harzianum* at all concentrations tested (1:1-1:10v/v dilution) completely prevented radial growth of both *P. ostreatus* and *P. eous* (Tables 13 and14).

Vegetative growth by dry matter accumulation in liquid medium was different. The dry weight obtained was commensurate to the dilution of the filtrate; the higher the dilution, the greater the growth but growth in the higher dilution (1:10v/v) never approximated the control (Tables 15- 20). At the highest concentration of 1:1v/v growth was depressed by 4-7 times. Although there are reported instances of growth in liquid medium differencing from what obtains on agar owing to aeration, it was clear that the three resident fungi were by and large antagonistic to the growth of *P. ostreatus* and *P. eous* with potency (in decreasing order *T. harzianum* > *A. flavus* > *P. citrinum*). There are therefore practical economic yield implications if contamination by particularly *T. harzianum* is allowed to reach epidemic populations in the substrate. Owing to time limitation, the antibiosis effect of the other

resident mycoflora on the two *Pleurotus* species could not be carried out to elucidate the possible antagonism of the other fungi although incidence may be minimal. Future studies will be carried out with this objective.

Colonization, primordia formation and correlation between BE and stipe length and pileus width

Colonization of substrate by *P. eous* was faster and primordia initiation and maturation on the various substrates was faster for *P. eous* than *P. ostreatus*. The amendment of rice straw with rice bran and rice husk did not seem to have significantly ($p>0.05$) enhanced the yield and Biological Efficiency of *P. eous* and *P. ostreatus* on the substrates used. It makes economic sense that production of *P. eous* on rice straw alone would be faster and yield better than 'wawa' which requires longer period of composting (up to 28days) for full and efficient yield and Biological Efficiency on this substrates (see Exp. 7).

The summary Table 72a and Scheffe Average Analysis of the spawn run period, mycelial growth rate (week), total yield and Biological Efficiency confirm that *P. eous* P-31 spawn run period to cover substrate in Experiment 1 (rice straw only) and Experiment 2 (rice straw amended with 1% $CaCO_3$ and 10% rice bran) was the fastest 3weeks irrespective of the composting period and was equally fast on uncomposted substrate. The remaining substrates took longer period of 4-6weeks (Table 72a). In the case of *P. ostreatus* strain EM-1the spawn run period in Experiments 1 and 2 was about 3 times (8 -9weeks) what was found for *P. eous* on the same substrate (Table 72b). The rest of the substrates were covered in 4-6weeks. The superior Biological Efficiency of *P. eous* P-31 on uncomposted rice straw and its amendments could be attributed to the ''battery enzymes'' induced by the substrates in *P. eous* responsible for the efficient utilization by *P. eous*. The main function of rice straw is to

provide a reservoir of cellulose, hemicellulose and lignin which is utilized during growth and fructification (Yildiz *et al.* 2002).

Presumably metabolic activity of *P. eous* strain P-31 enzymes on the rice straw compost made available sufficient amount of nutrients for better growth of *P. eous* more than *P. ostreatus.* The chemical analysis of the rice straw showed the availability of cellulose (29.71-38.82%), hemicellulose (20.81-24.99%), lignin (5.38-8.95%) and silica (11.74-19.18%) (Figs 38 to 39) (Appendices 4 and 5) substantiate this viewpoint. Recently Sharma *et al*. (2013) studied the growth and yield of *Pleurotus ostreatus* on different substrates. The fastest mycelia extension was observed in rice straw substrate followed by mixture of rice plus wheat straw, sugar cane bagasse, mixture of rice straw plus paper and sawdust respectively. Mycelia growth is a preliminary step which creates suitable internal conditions for fruits. Thus superior growth of *P. eous* on uncomposted medium over *P. ostreatus* is being recorded for the first time and is a vital factor in mushroom cultivation (Pokhrel *et al.* 2009). There was also positive correlation between stipe length (mm), pileus width (mm) and Biological Efficiency (Figs 19, 20, 23, 24, 27, 28, 33, 34, 35 and 36). The coefficient (R^2) values ranged from 0.777 to 0.962. Only the pileus in Experiment 4 obtained low $R^2 = 0.570$ for *P. ostreatus* EM-1 and $R^2 = 0.635$ (Exp. 3), $R^2 = 0.662$ (Exp. 4) for stipe length against Biological Efficiency (Figs 21, 22, 25, 26, 29, 30, 31, 32, 37 and 38). Biological Efficiency is therefore directly related to the sporophore development.

Chukwurah *et al*. (2013) showed that eight substrates formulated from dried maize straw and cobs mixed with palm kernel cake and sawdust with lime and water as additives for *P. ostreatus* obtained different growth rates and Biological Efficiencies. Higher mean values of the stipe length, pileus width were obtained from mushrooms grown in substrates which

composed of two different types of agricultural waste while lower values were obtained from those grown in substrates composed of single agricultural waste. This present finding contrasts that of Chukwurah *et al*. (2013). Single agricultural waste, uncomposted rice straw supported superior growth of *P. eous* and *P. ostreatus* grew better on composted medium but did not approximate that by *P. eous* on the same medium. The ratio of pileus width / stipe length was >1.0 (Appendices 6- 8). High coefficient of determination R^2 was obtained from correlation between Biological Efficiency and pileus width as well as stipe length in the present study depended on the type of substrate formulation in the present study and Biological Efficiency was highest in substrates made from rice straw.

Chemical and nutritional and mineral composition of lignocellulose and fruiting bodies

The use of lignocellulose biomass in cultivation of mushroom is not only a renewable resource but also the most abundant source of organic components in high amounts on earth. To date, intensive research and developmental studies on the effective utilization of lignocellulosic materials have been done (Taniguchi *et al*. 2005). One of the key problems hindering the effective utilization of this renewable source as a raw material for chemical reactions and feed is the low susceptibility of lignocellulose to hydrolysis, attributable to the crystalline structure of cellulose fibrils, surrounded by hemicellulose and the presence of the lignin seal which prevents penetration by degrading enzymes (Chalal and Chalal, 1999; Gong *et al*. 1999). Therefore, an ideal pre-treatment is needed to reduce lignin content and crystallinity of the cellulose, and increase surface area of these materials. Pre-treatment of the lignocellulosic substances vary widely. They include mechanical, physical, physio-chemical, chemical and biological pre-treatment (including composting) (Mtui, 2009). All pre-treatments of these substances are aimed to enhance digestibility, improve the rate of

enzyme hydrolysis and increase yields of the intended product (Moiser *et al*. 2005; Henderiks and Zeeman, 2009).

The chemical, nutritional and mineral composition of the seven different lignocellulose used as composts for the cultivation of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 were determined (Tables 73 to 80). Wawa sawdust amended with 1% $CaCO_3$ and 10% rice bran was added to compare with the newly formulated substrates for cultivation of the two *Pleurotus* species

The best substrate for the growth, yield and Biological Efficiency of *P. eous* were found on 0-8 days composted straw only (yield 221.5g; BE = 76.4%) whereas uncomposted rice straw amended with rice husk mixture (yield 211.0g; BE= 72.8%) was equally good. *P. eous* P-31 performed better by all criteria on all the substrates when used uncomposted or composted for a short period of 4days (Summary Table 72a). On the other hand, composting increased the yield and Biological Efficiency of *P. ostreatus* strain EM-1 on all the substrates (Table 72b). A wide array of enzymes such as laccase and xylanases (Ortega *et al*. 1992; Hatakka, 1994; Datta and Chakaravarty, 2001; Lo *et al.* 2001) produced by mushroom mycelia are capable of utilizing complex organic compounds such as found in the case of the two *Pleurotus* species under study in this thesis. However, *P. eous* was able to utilize effectively uncomposted substrates in these experiment while composting increased the BE of *P. ostreatus* strain EM-1 on the same substrates. The enzymatic activity of *P. eous* on the different substrates needs further investigation to elucidate this present finding.

It is known that the compost and mushroom species can influence the chemical composition and as a consequence, the nutritional value of the cultivated mushrooms (Manzi *et al*.

1999a&b); Patil *et al*. 2010). The proximate analysis of mushrooms cultivated on the best differently constituted composts is presented in Tables 79 and 80.

The fat / lipid content of *P. ostreatus* ranged from 11.1 – 17.8% and that of *P. eous* P-31 varied between 5.97-19.9%. This is far in excess of the reported lipid content of 1.6 to 5.0% on dry weight basis for *Pleurotus* species (Crisan and Sands, 1978; Zaki *et al.* 1993). Present data cannot explain the reason for the high fat content of the fruiting body. Crude protein of *P. ostreatus* varied from 24.07 - 33.15% on dry wt. basis and 21.63- 35.99% for *P. eous* P-31. Mattila *et al*. (2001) studied several species of mushrooms grown in Finland and reported that protein content of *P. ostreatus,* and *Lentinula edodes* were 24.6 and 21.4% on dry matter basis and considered *P. ostreatus* and *L. edodes* as good sources of protein for humans as compared to vegetables. From this present data, the same is true for *P. ostreatus* strain EM-1 and *P. eous* strain P-31. Mattila *et al*. (2001) reported that ash content of *P. ostreatus* and *L. edodes* in Finland were 8.0% and 5.8% on dry weight basis respectively. Hung and Nhi (2012) showed that ash content of *Ganoderma lucidum* and *P. ostreatus* were 1.4% and 9.0% on dry weight basis. These values are not significantly different from values of 6.53-10.54% on dry weight basis found for *P. eous* strain P-31in this present study. Total carbohydrates which include polysaccharides such as glucans, mono and disaccharides, sugar alcohols, glycogen and chitin varied in a range of 17.37-26.6% on dry weight basis for *P. ostreatus* strain EM-1 and 9.35-28.6% on dry wt. basis for *P. eous* strain P-31. Hung and Nhi (2012) recorded the following total carbohydrate content 52.5% (*Volvariella volvacea*) 65.1% (*P. ostreatus)* 65.1% (*L. edodes*), 82.3% (*G. lucidum*) 88.6% (*Auricularia polytricha*). Mattila *et al.* (2001) informed that carbohydrates content of *P. ostreatus* was 62.5% and in *L. edodes* was 69.9% on dry matter basis. Other studies (Crisan and Sands,

1978) reported that carbohydrate content of *L. edodes* varied from 67.5-78.0% on dry matter basis while Zaki *et al*. (1993) showed that total carbohydrate content in *P. ostreatus* varied from 46.0-81.8%. Findings in this present studies for *P. ostreatus* strain EM-1 differ from earlier published reports for *P. ostreatus* presumable because of the different substrates tested but data for the nutrient analysis for *P. eous* strain P-31 are being recorded for the first time for this strain in Ghana.

In earlier studies by Patil *et al*. (2010), soybean straw gave a maximum protein content (24.66%), fat (2.82%), and ash (6.71%) for *P. ostreatus*. They showed that protein content of *P. ostreatus* was significantly higher when grown on soybean straw than other substrates or their combination except for paddy rice straw alone or its combination. Similar results were reported for *P. sajor-caju* (Mane *et al*. 2007). The crude fibre (%) was maximum when *P. ostreatus* was grown on paddy rice straw (7.7%) followed by soybean paddy straw (7.68%) and the minimum was on soybean straw. alone (7.17%). Present data in this thesis show that the crude fibre content (%) on the seven substrates range from 5.56-15.26% for *P. ostreatus* EM-1 and 12.19-24, 54% for *P. eous* P-31. These values exceed the findings of Patil *et al* (2010).

Mineral Content

Minerals in the diet are essential for metabolic reactions, healthy bone formation, transmission of nerve impulses, and regulation of water and salt balances (Kalac and Svoboda, 2000). The mineral content of *P. eous* strain P-31 and *P. ostreatus strain* EM-1 harvested varied with different substrates and their combinations (Tables 77 and 78).

Calcium, Iron, Potassium, Magnesium, Sodium and Phosphorus were detected in the fruiting body of both *Pleurotus* species (Tables 77 and 78). The nutrient content of the mushrooms varied according to the substrate composition as also reported by Patrabansh and Madan (1997). The highest calcium content of *P. eous* strain P-31 was obtained in Experiment 1 (Rice straw only; 1.0769mg/kg) and the least (0.0327mg/kg) was recorded in Experiment 7 (wawa sawdust amended with 1% $CaCO_3$ and 10% rice bran). The highest concentration of calcium, 3950mg/kg (395mg/100g) was again recorded in Experiment 1 and the least concentration of 0.1017mg/kg (101.7mg/100g) was obtained in Experiment 7. Calcium content of *P. ostreatus* cultivated on single or soybean, paddy rice, wheat straw ranged from 240 to 330mg/100g (0.024 to 0.033mg/kg) (Patil *et al*. 2010)

Potassium content of *P. eous* ranged from 11.07mg/kg (Expt. 1) to 32.00mg/kg in Expt. 5. In *P. ostreatus* the highest level of Potassium was in fruit bodies from Expt. 2 (17.0mg/kg) and least (12.9mg/kg) in Expt. 5. The preponderance of potassium in the sporophore (fruiting body) tissue may be due to the absorption accumulation of this element from the substrate. Yildiz *et al*. (1998) also found a high accumulation (3.44-4.50% dry wt.) of potassium in the sporophore of *P. ostreatus* var *salignus* growing in four different straws. Potassium content in different *Pleurotus* species ranged from 182 to 395mg/100g (0.0182 to 0.0395mg/kg). Recommended Daily Intake (RDI) of potassium is 3100mg/day (Manzi *et al*. 1999).

Sodium concentration of *P. ostreatus* strain EM-1 and *P. eous* strain P-31 varied significantly with the different substrates in Experiments 1-7. The highest sodium concentration in *P. eous* was recorded in Experiment 5, 6 and 7 (14.00-14.90mg/kg) (Table 77) and the lowest was detected in Experiments 1-3 (6.00mg/kg). The highest sodium concentration in *P. ostreatus* strain EM-1 was recorded in Experiment 2 (8.00mg/kg) and the

lowest (0.3060mg/kg) in Experiment 7. The sodium concentration in the remaining sporophores nearly approximated what was obtained in the *P. eous* sporophores in Experiment 2 (6.005-7.500mg/kg) in Experiments 1, 3, 6 and 5). Sodium content of sporophore in Expt. 1, 3, 4 and 6 were not significantly ($p<0.05$) different (Table 77). The presence of high potassium content over sodium in diet suggests the effectiveness against hypertension. Patil *et al*. (2010) also found that concentration of sodium in *P. ostreatus* varied significantly with different substrates. A balance between high potassium and low sodium content in mushroom is obvious in this present study. Indeed, Patil *et al*. (2010) also reported a balance between high potassium and low sodium content in *Pleurotus ostreatus* grown on different substrates. Among different substrates paddy rice straw showed maximum K: Na ratio (7.79), followed by combination of soybean and wheat straw (7.69) while the least ratio was recorded on wheat straw (6.88) (Patil *et al*. 2010). Manzi *et al.* (1999) and Mattila *et al*. (2001) also reported high potassium and low sodium concentration in mushroom fruiting body.

Phosphorus content of sporophore of *P. ostreatus* varied from 5.22mg/kg (Expt. 1) to 14.5mg/kg (Expt. 5). In the case of *P. eous* the highest concentration was detected in sporophores from Expt. 3 (10.075mg/kg) and the least from Expt. 6 and 7 (6.08 and 6.32mg/kg respectively). Patil *et al.* (2010) showed that out of six substrates used for the cultivation of *P. ostreatus* the content of phosphorus ranged from 790-1000mg/100g (0.079 to 0.100mg/kg). Maximum phosphorus content of 1000mg/`100g (0.100mg/kg) was recorded on soybean straw. This was significantly higher than the others and was followed by sporophores cultivated on paddy rice straw (920mg/100g or 9200mg/kg) while the least was recorded with a combination of wheat and paddy rice straw (790mg/100g or

0.0790mg/kg). Since the recommended daily intake, RDI of phosphorus is 0.7g, *P. ostreatus* EM-1 and *P. eous* P-31 are high in phosphorus content to contribute to human nutrition as a good source of phosphorus (Calglarimak, 2007).

Ramirez *et al*. (1994) showed that rice straw contained a rich source of macro-minerals such as Potassium, Magnesium, Calcium, Sodium, Phosphorus and Sulphur as well as micro-elements such as Iron, Zinc, Copper, Molybdenum, Copper, Manganese and Selenium. Iron and Manganese were two elements which were present in high amounts in paddy soil and rice straw content varied widely but always above the requirements for domestic animals. Preliminary analysis of the soil and compost used for formulating the substrate for the cultivation of the two *Pleurotus* species showed that the soil and compost did not contain copper and Iron but contained levels of Zn, Mn, Pb, Ca, Mg, Na, K, N (Table 77). It is conjectured that the source of these elements in the sporophore of the two *Pleurotus* species investigated could have come from the rice straw compost and its amendments.

Micro-elements as heavy metals in mushrooms

Some heavy metals such as copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb) and Zinc (Zn) were detected in the sporophore of both *P. ostreatus* strain EM-1 and *P. eous* strain P-31 albeit in small quantities.

Heavy metal concentration in mushroom is considerably higher than those in agricultural crop plants, vegetables and fruits. This connotes that mushrooms have a very effective mechanism which enables them to readily take up some heavy metals from the environment (Zhu *et al*. 2010) due to their dense mycelial system which infiltrates the substrates (Garcia *et al.* 1998 and 2005). The accumulation of heavy metal in mushrooms has been found to be

affected by environmental factors such as organic matter content, pH and metal concentration in the soil as well as fungal factors such as species of mushroom, morphological development of the carpophore, age of mycelium, intervals between fructifications and biological composition of the substrate (Radulescu *et al*. 2010).

Heavy metals like iron (Fe), copper (Cu), Manganese (Mn) and Zinc (Zn) detected in mushroom are essential metals since they play an important role in biological systems. Lead (Pb) is not essential and can be toxic in traces (Unak *et al*. 2007). The essential heavy metals can become toxic when there is excessive intake (Al-Khalifat and Al-Khashnnan, 2007; Gopalani *et al*. 2007). Five heavy metals were encountered and analysed in the sporophores of the two *Pleurotus* species namely Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb), Zinc (Zn) and in the substrates in which they were grown. The maximum Fe values were determined in the mushrooms were 0.350mg/kg (*P. ostreatus* strain EM-1) and 0.350mg/kg in *P. eous* strain P-31 (Tables 77.and 78). This is far below the limit of 15mg/kg set by WHO (1982). Iron deficiency anaemia affects one third of the world's population but excessive intake of iron is associated with an increased risk of colorectal cancer (Senesse *et al.* 2004).

Copper is an essential constituent of some metallo-enzymes and required in haemoglobin synthesis and in catalysis of metabolic growth (Silvestre *et al*. 2000). Copper concentration determined were either absent from the sporophore (Expt. 7 for *P. eous* and Expt. 3&4 for *P. ostreatus)* or low ranging 0.0018 to 0.0800mg/kg (Tables 77 and 78) which are far below the safe limit of 40.0mg/kg in foods set by WHO (1982). Copper levels reported in mushroom in pertinent literature are 4.71-51.0mg/kg (Tüzen *et al*. 1998), 13.4- 50.6mg/kg (Soylak *et al*. 2005) and 12-181mg/kg (Tüzen, 2003). Recently, Obodai *et al*. (2014) showed that there

was significant difference ($p \leq 0.05$) between the concentrations of Cu in the sporophore of *P. ostreatus* strain EM-1 cultivated on composted cassava peels (6.96±1.33mg/kg) than in the uncomposted cassava peels (3.7±0.08mg/kg).

Lead (Pb) is toxic even in trace levels (Dobaradaren *et al*. 2010). The impairment of human functions related to Pb toxicity includes abnormal size and haemoglobin content of erythrocytes, hyper stimulation of erythropoiesis and inhibition of haem synthesis (Vonugopal and Lucky, 1975). The maximum and minimum values of Pb concentrations determined in *P. ostreatus* strain EM-1 on seven differently formulated rice straw based compost were 0.0040mg/kg (Expt. 6) and 0.1600mg/kg (Expt. 1) respectively and nil (Expt. 7) to 0.2020mg/kg (Expt. 3) (Tables 77 and 78). The maximum and minimum values of Pb concentrations determined in *P. ostreatus* strain EM-1 by Obodai *et al*. (2014) were 8.34±2.17 and 3.75±0.24mg/kg on composted and uncomposted cassava peels respectively. These values far exceed values obtained in this present studies in *P. ostreatus* strain EM-1and *P. eous* strain P-31 on differently formulated and composted rice straw based substrates. However, the values were far below the 10.0mg/kg limit set by WHO (1982) for lead in raw plant materials. Lead levels reported in the literature in mushrooms are 0.75-7.77mg/kg (Tüzen *et al*. 1998), 0.40-2.80mg/kg (Svoboda and Kolac, 2003; Svoboda *et al*. 2000; Kalac and Svoboda, 2000) and 1.43-4.17mg/kg (Tüzen, 2003).

Manganese (Mn) is an essential metal as it plays an important role in biological systems such as its presence in metalloproteins (Unak *et al*. 2007). The highest and lowest Mn concentration determined in both mushrooms in this present studies were nil to 0.0598mg/kg (in *P. eous* strain P-31) and 0.0180 to 1.272mg/kg in *P. ostreatus* strain EM-1) (Tables 77 and 78). Obodai *et al*. (2014) reported that the highest and lowest Mn concentrations

determined in *P. ostreatus* strain EM-1 cultivated on composted and uncomposted cassava peels were 63.23±7.58 and 45.68±2.23mg/kg respectively. In both instances this present data and the report of Obodai *et al.* (2014), the values fall far below the toxicity limit of 400-1000mg/kg of Mn in plant set by WHO (1982). Interestingly, varying ranges of Mn values in mushrooms have been documented in the pertinent literatures as 14.5-63.6mg/kg (Isioglu *et al*. 2001), 12.9-93.3mg/kg (Tüzen, 2003), 14.2- 69.7mg/kg (Soylak *et al.* 2005).

Zinc (Zn) is also an essential mineral and is a component of a wide variety of enzymes and co-enzymes in which it performs the catalysis, structural and regulatory roles. According to Ma and Betts (2000) it contributes about 33ppm to adult body weight, is essential as constituent of many enzymes and is involved in a number of physiological function of protein synthesis and enzyme metabolism. The maximum and minimum concentrations of zinc in the fruit body of *P. eous* strain P-31 and *P. ostreatus* strain EM-1analysed in this present study were 0.013 to 0.190mg/kg (for *P. eous*) and 0.0110 to 0.250mg/kg (for *P. ostreatus*). The WHO recommended permissible limit of Zinc in foods is 60mg/kg (WHO, 1982). Value obtained in *Pleurotus* species in this thesis fall well below the permissible limit. However, Obodai, *et al*. (2014), found that the maximum and minimum concentrations of zinc in *P. ostreatus* strain EM-1 were 13.68±0.54 and 12.03±0.62mg/kg which also fall below the safe limit of Zinc in foods set by WHO. Clearly, differences in accumulation levels of heavy metals in mushrooms may be related to the species of mushroom, the nature and composition of the compost / substrate, the pH and other related physical and environmental parameters influencing growth of the fungus. In this present studies it was obvious that of all the heavy metals examined, Fe was accumulated excessively than Cu, Pb, Mn and Zn. The order of heavy metals accumulation in the harvested *P. ostreatus* strain

EM-1 was Fe> Zn > Mn > Pb > Cu and Fe > Mn > Zn > Pb > Cu for *P. eous* strain P-31. This is indicative of the fact that both *P. ostreatus* strain EM-1 and *P. eous* strain P-31 are good bio-accumulators of iron. Ramirez *et al*. (1994) stated that iron and manganese were two elements which were present in high amounts in rice straw ranged from 582 to 1,302mg/kg but found no correlation between extractable soil Fe and Mn content in rice straw.

In a recent paper Obodai *et al*. (2014) reported on some phytochemical analysis on methanolic extracts of four oyster mushroom species (*Pleurotus pulmonarius, P. ostreatus, P. sapidus* and *P. citrinopileatus)* made up of 12 different strains. Magnesium content of the strains varied from 660.0mg/kg to 1993.7mg/kg, Fe content ranged from 349.0mg/kg to 1374.0mg/kg and could not be detected in *P. ostreatus* strain POA-7. Calcium content varied from 22.0mg/kg to 415.3mg/kg. Mn content varied from 10.7mg/kg to 48.3mg/kg and was below detection in the six strains of POA-10 and POA-6, *P. ostreatus* strain POA-7, *P. ostreatus* POA-10 and *P. ostreatus* POA-13. Cu content ranged from 15.3mg/kg to 23.7mg/kg but below detection in two strains *P. ostreatus* strain POA-6 and *P. ostreatus* strain POA-10. Zn content varied from 189.7mg/kg to 411.3mg/kg. Ni content varied from 175.0mg/kg to 296.7mg/kg. Cd content ranged between 57.7mgkg and 106.3mg/kg. Pb content varied from 13.0mg/kg to 230.7mg/kg. Cr content ranged between 17.7mg/kg and 124.0mg/kg. The levels of Mg, Ca, Fe, Mn, Cu and Zn detected in these *Pleurotus ostreatus* strain far exceeded what was recorded in *P. ostreatus* strain EM-1 and *P. eous* strain P-31 used in the present study. Presumably the differences can partly be attributed to the wawa sawdust compost used for growing the strains in the study of Obodai *et al*. (2014).

It was instructive to note that Sulphur (S) was not detected in the soil and rice straw used in these studies also and in sporophores of *P. ostreatus* strain EM-1 and *P. eous* strain P-31. Ramirez *et al*. (1994) detected 0.13-0.14% sulphur on dry matter basis) in Korean rice straw. Sulphur is found only in structures of poisonous mushrooms and was not detected in the sporophore of *Pleurotus ostreatus* var *salignus* by Yildiz *et al*. (1998).

Cultivation of *Pleurotus* on different substrates essentially needs understanding of the methods of cultivation as well as the chemical composition of both substrate and fruiting body (sporophore). This can be carried out in such a way that new formulations of lignocellulosic waste available in this country can be designed which does not affect the nutritional quality of the mushroom but improves it. Data from this thesis provides this wealth of information as well as the nutritional values of the spent compost. It is obvious that minerals both micro and macro-nutrients needed for the fruiting of the mushroom are similar to that of plants. Apart from P, K, Mg and S which are necessary nutrients for fungal growth (Wang *et al.* 2001) many nutrients like Na, Mg, Ca, are required for the fruiting body (Sueli *et al*. 2002). Potassium (K) is available for the fungus usually in the form of phosphate thus providing two essential minerals for metabolism (Miles and Chang, 1997). K is essential since it is a co-factor of several enzymatic reactions and is plentifully available in mushrooms (Chang *et al*. 1981); Wang *et al*. 2001). The widely studied micro-nutrients for the growth of many fungal species are Fe, Zn, Al, Mn, Cu, Cr, and Mo (Wang *et al*. 2001) some of which have been detected in the substrates or composts and fruit body of *P. ostreatus* strain EM-1 and *P. eous* strain P-31. Many may be present beyond detectable limit in the substrate in bound form (complex) making up co-enzymes, co-factor, activators of several enzymes etc. (Miles and Chang, 1997). The mineral content of lignocellulosic

biomass contain among others Ca, Mg, P, Si, K, Na, albeit usually little. The increase in protein content and Ca content of spent straw is because of decomposition of total carbohydrate, crude fibre, cellulose, hemicellulose which are utilized by the mushroom from the stage of inoculation (Patil *et al.* 2010). The increase in protein content of the spent rice straw is also because of the spread of mushroom mycelium in the substrate and secretion of extracellular enzymes by the mushroom. Thus the total carbohydrate, crude fibre, cellulose, hemicellulose, lignin etc. decreased in the spent straw (Table 82). This agrees with the findings of Patil *et al*. (2010).

Studies on the growth and yield and proximate analysis and nutrient levels in the two *Pleurotus* species show that *P. eous* strain P-31 performed better in most instances than *P. ostreatus* strain EM-1 in the bioconversion of the substrates used in Experiments 1-7 in Section E of the Results.

Although morphological features and colour characteristics are important in identification of the fungi, this may be deceptive in some instances. The current trend is the use of molecular and biochemical techniques to complement observed conventional taxonomic features. The morphological differences in the two species of *Pleurotus* is hard to separate by the layman and researcher alike except for use of size, length and colour of stipe and cap (pileus). While the pileus of *P. eous* strain P-31 is white that of *P. ostreatus* strain EM-1 is greyish brown to black. The stipe of *P. eous* is ‘‘chalky’’ while that of *P. ostreatus* is softer and malleable (Plates 12 and 13).

There were very clear differences in the superiority of *P. eous* P-31 performance over *P. ostreatus* EM-1in the bioconversion on the different formulation of rice lignocellulose waste and their supplementation and amendments as well as on ‘wawa’ sawdust compost

fermented for different periods. Although morphological features and colour characteristics are important in the identification of fungal species, the current trend is to use molecular level techniques to complement the conventional taxonomic features. It is believed that species identification within the genus *Pleurotus* is difficult because of the morphological and possible environmental effects on the species as well as the existence of discrete intersterility groups (OECD, 2005).

PCR performed with the ITS 1 and ITS 4 primers to characterise their ITS region grown in different substrates showed that the two *Pleurotus* species could not be separated as they both produced a characteristic band size of 650bp (Fig 41a). However, gel profile showing restrictive patterns generated by four restrictive enzymes Hha I, Hinf I and Rsa I and Hae III showed that only one restrictive enzyme Hae III was the most effective in segregating *P. ostreatus* EM-1 from *P. eous* P-31 (Fig 41b). Therefore one can conclude that the two *Pleurotus* species are different species with varying physiological capabilities of bioconversion of the test substrate and will need further gene sequencing to provide the molecular and physiological difference to complement the morphological features observed in these studies.

This is the first record of separation of these two species on molecular basis. It is clear that two distinct species of *Pleurotus* were used in this investigation with varying bioconversion abilities on different substrates. It remain to show why *P. eous* can utilize unfermented compost of rice straw and rice husk and their amendments more efficiently than *P. ostreatus* EM-1 using their DNA mapping and enzyme production / induction profiles.

Influence on Spent Mushroom Compost of *P. eous* P-31 (used as bio-fertilizer) on the growth and development of tomato, pepper and cowpea seedlings under greenhouse conditions

Spent Mushroom Compost is currently disposed off as waste and constitutes an environmental problem especially in Ghana. But there can be economic benefit from its use in agriculture as bio-fertilizer. Maher *et al*. (2000) in their report ''Managing Spent Compost'' stated inter alia: ''*We have examined a variety of possible end uses for Spent Mushroom Compost, SMC including its use as an alternative fuel. In the immediate future, we believe the predominant end use for SMC will be as an organic manure for field crop production and as a soil conditioner in the landscaping industry. Uses of this type are in line with both the Irish and European Union legislation regarding waste management. Our analysis suggests that tillage and horticulture offer the best promise for releasing the beneficial properties of SMC.*

We have tested SMC on field crops such as winter and spring wheat, lettuce, potatoes and on glasshouse crops such as potatoes. These experiments have shown that SMC increases soil organic matter and improves soil structure. SMC is very effective source of K and P and also provides trace elements. It makes a contribution to Nitrogen nutrition. Overall, our results indicate that SMC can be used with beneficial effect in field crop production''.

Since the study by Maher *et al*. (2000) many other workers have shown that SMC is rich in organic matter and constitutes an important source of macro and micronutrients for plants and microorganisms thereby increase the soil microflora, soil biological activity and enhance soil enzyme activity (Crecchio *et al*. 2001). It contains calcium carbonate which provides short term buffering of acid waters and elevates soil pH (Gonani *et al*. 2002;

Rupert, 1994). The SMC is able to bind mineral particles together promoting good soil structure and improving aeration and moisture retention. Use of SMC in agriculture reduces the quantity of biodegradable waste disposal in land fill sites and transforms them into economically useful agricultural products (Szmidt, 1994; Gonani *et al*. 2011)

Alsadon *et al*. (2006) conducted a greenhouse experiment to study the effect of leached-SMC on the growth of cucumber (*Cucumis sativus* cv. Super dominos) and showed that the number of flowers produced were not significantly different from the controls; but there was a significant difference in height of plants and number of fruits ($p<0.01$). They showed that amendment of sandy-loam soil with 15% and 25% SMC increased cucumber plants growth significantly (($p<0.01$). Fruit number was more in the SMC 25% and less in SMC 45% and that SMC at the rate of 35 and 45% were not effective or had negative effect on morphological growth parameters of plant height and fruit number. Recently, Spent Mushroom waste was used to improve growth and yield of maize (*Zea mays*) in Nigeria (Ogbonna *et al.* 2012) and tomato and *Piper nigrum* (Onal and Topcuoglu undated; Cayci e*t al*. 1998). These background information informed the choice of tomato, (*Lycopersicon esculentum*), pepper (*Capsicum annum*) and cowpea (*Vigna unguiculata*) as candidate crops to test the efficacy of the SMC from *P. eous* strain P-31 cultivation serving as compost or bio-fertilizer for greenhouse cultivation experiments.

Tomato ((*Lycopersicon esculentum*)

Results obtained in this thesis showed that SMC at 10% mixture with soil was the best in stimulating plant height. The height of tomato plants growing in 5-25% SMC were similar and not significantly different ($p>0.05$) but did not approximate that growing in 10% SMC after 8 weeks (Fig. 44A). The poorest growth was in plants growing in unamended soil.

Similar trends were obtained for leaf area, number of leaves per plant and dry weight of shoot and root. Chlorophyll content in the leaves however decreased with increasing percentage of SMC in soil but were still statistically ($p<0.05$) superior to what existed in the unamended soil (Fig. 44D.). The Spent Mushroom Compost of *P. eous* used in the present studies contained dry matter (90.8±1.79%), crude protein (7.76±0.48%), cellulose (32.34±1.97%), lignin (7.26±1.94%); Neutral detergent fibre (53.3±6.07%), Acid detergent fibre (50.42±4.64%) (Table 82) as well as a miscellany of mineral elements such as Calcium, Potassium, Magnesium, Sodium and some heavy metals e.g. Copper, Iron, Manganese, Lead and Zinc (Table 82). According to some researchers (Szmidt and Chong, 1995; Rhoads and Olson, 1995; Guo and Chorover, 2004), Spent Mushroom Compost is a rich source of nitrogen, carbon, and abundance of the inorganic cations K^+, Na^+, $Ca^{2+,}$ Mg^{2+} and anions Cl^-, sulphite SO_4^{2-} and nitrates (NO_3^-) which makes SMC well suited for supporting plant growth. But high concentrations such as recorded in this thesis increased concentration of SMC beyond 20% which may be inhibitory and may also limit or inhibit growth beyond a threshold value.

Pepper (*Capsicum annum*)

Pepper plant responded differently from tomato. The data on plant height, leaf area and number of leaves per plant followed a near sigmoid curve (Fig .45). There were no statistical differences ($p>0.05$) between the performance of the plants in terms of height, leaf area and no. of leaves per plant as concentration increased from 5-20% SMC. However, 30% SMC severely depressed height, no. of leaves and leaf area but was superior to the plants growing in unamended soil. Presumably the high concentration of the mineral elements beyond a threshold of 20% SMC depressed the parameter measured. Clearly, the control was inferior

by all criteria to the soil amended with 5-30% SMC (Fig 45).Total Chlorophyll content in the pepper plants sown in unamended soil was the lowest (11-16 CCI) as compared to (18-22 CCI) in the soil amended with 5-30% SMC (Fig 44). The changes in total chlorophyll content in pots containing 5-30% SMC were erratic over the 8weeks observation period. In future, the contribution of chlorophylls a and b to the total chlorophyll level will be examined. This is because photosynthesis takes place in phases light and dark reactions in the photosystem I and II in the chloroplast. Reaction sites could be influenced by the level of chlorophylls a or b and total growth of the plant will be affected by efficiency of the coordinated reactions in photosystems I and II.

Cowpea (*Vigna unguiculata*)

Again compost mixtures with soil 5-100% SMC proportionately increase plant height, leaf area, no. of leaves, number of axillary branches, no. of floral buds, no. of flowers, no. of pods setting, weight of the pods and weight of seeds. At higher proportions of 25-100% SMC there was an adverse effect indicative of the fact that the mineral content and other nutrient have become inhibitory in their effect just as was recorded for tomato and pepper (Figs 44 &.46). The effect on the chlorophyll apparatus was severer. High concentration of SMC reduced chlorophyll content while 5-10% gave the highest total chlorophyll content in 4-5 weeks. It can be conjectured that the difference may be due to the chlorophyll a and b variations owing to the added inhibitory levels of mineral elements in the compost soil mixture.

According to Guo and Chorover (2004) high concentrations of mineral elements may be inhibitory and may limit or inhibit growth beyond a threshold concentration (Table 45) shows how increasing %SMC beyond 15% drastically reduced shoot and root weight of

cowpeas in this study. The plant seems to efficiently assimilate nitrogen. Biological activity of N_2 fixation is related to dry matter yield (Prevost and Prevost, 2006; Antoun and Prevost, 2005). Nodule formation was highest in 5% SMC mixture with (Total of 89) and this declined with increasing proportion of SMC mixture with soil (5-30% SMC) but completely depressed nodule formation in the plants growing in SMC only (Experiment L). This is the first record of Spent Mushroom Compost preventing nodule formation in cowpea at higher concentrations. Using the criteria of Nodule Index (Rebah *et al*. 2002), the best nodule size and weight of nodule was formed in the growing medium of 5% and 10% SMC (18 and 12 respectively (Table 88). Using a selective medium prescribed by Prevost and Antoun (2006) *Rhizobium* population in nodules was found to increase with increasing percentage of SMC up to 30% but none was formed on the plants growing in soil only. The nodules were all pink- red and thus viable except in the unamended soil. This is the first record of stimulation of nodule formation in soil by spent mushroom compost / soil mixture. Spent mushroom compost of *P. eous* strain P-31 at low percentages up to 30% SMC (Plate 21; Tables 87 and 88) improve nodulation.

Preliminary data from this thesis shows the availability of rice lignocellulose on sustainable basis in Ghana (Map 1 and Table 3). The mushroom cultivators must now be trained to use the available rice straw and husk for *Pleurotus* cultivation after which the spent mushroom compost can be used for crop improvement. This is an environmentally friendly way of disposing agricultural waste for profit and should be pursued vigorously.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Mushrooms cultivation exploits the natural ability of fungi to bio-convert solid waste generated by industry and agriculture into food (Tripothi and Yadar, 1992; Chiu *et al*. 2001). In nature, several mushroom species including *Pleurotus*, *Volvariella, Lentinula, Schizophyllum*, have been isolated from decomposing wood of different tree species. The development of commercial production of *Pleurotus* species has led to the development of the bags system on the cultivation of *Pleurotus ostreatus* in Ghana with success. Substrates tried include several agricultural lignocellulosic wastes. However sawdust has been the most exploited. Invariably, sawdust undergoes a long period of 28 days of composting during which resident microorganisms compete to exclude the less vigorous competitors. Microorganisms colonizing the compost during composting process regarded as active agents, which finally determined chemical composition of the substrate; thus making it possible for mushroom growth (Fermon *et al.* 1985). Thus during fermentation and pasteurization, proper conditions both physical and chemical for mushroom growth are created (Oei, 1991). Although wawa sawdust has been used extensively for *Pleurotus* cultivation in Ghana, it takes 28 days to prepare the substrate for sterilization and inoculation of the spawns. Economically, it would be faster and cheaper to have compost which requires a shorter composting period and a good medium for high bioconversion (Biological Efficiency) of the substrate by *Pleurotus* without compromising on the nutritional and medicinal values of the fruiting body. The diversity of fungi present in the six newly formulated rice straw based composts (Experiments 1-6) were sampled for diversity of fungi in the entire composting period of 12 days. Wawa sawdust was included as the seventh experiment to serve as a check. The compost mycoflora included members of

the genera *Aspergillus, Cladosporium, Curvularia, Penicillium, Rhizopus, Rhodotorula, Rhizoctonia, Geotrichum*, *Neosartorya, Fusarium and Trichoderma*. *Aspergillus* species predominated over the other genera followed by *Penicillium*. The species which predominated after composting for up to 12 days were *Aspergillus flavus, A. niger, Penicillium citrinum* and *Trichoderma harzianum*. *A. flavus, P. citrinum* and *T. harzianum* metabolites were found to be antagonistic to *P. ostreatus* strain EM-1 and *P. eous* strain P-31 in culture and may pose a problem if proper sterilization of compost is not achieved before spawning.

The best pH of the substrate for radial growth and vegetative growth of both *Pleurotus* species was pH 5.8-6.6 which agrees with the information in the pertinent literature for Ghana.

Growth yield and Biological Efficiency, mycelial density, total no. of fruiting bodies on all seven media were significantly ($p \leq 0.05$) and was better in *P. eous* strain P-31 than *P. ostreatus* strain EM-1. Correlation coefficient R^2 value for stipe length, cap width against Biological Efficiency were high ranging from R^2= 0.96- 0.77 except in two media. The best substrate for the growth and Biological Efficiency of *P. eous* strain P-31 was found 0-4 days composted raw straw only (yield 221.5g; BE= 76.4%) whereas uncomposted rice straw amended with rice husk (yield 211.0g; BE=72.8%) was equally good. *P. eous* generally performed better by all criteria on all the substrates when used uncomposted or composted for a short period of 4 days. Longer period of composting of 8-12 days was more suitable for *P. ostreatus* strain EM-1. The proximate analysis and mineral contents for *P. eous* contained higher concentrations of these parameters than *P. ostreatus* in most instances. The level of

nutrients and proximate analysis was influenced by the type of substrate and period of composting.

Calcium, Sodium, Iron, Potassium, Magnesium and Phosphorus were detected in the fruit bodies of both *Pleurotus* species but varied according to the substrate composition. There was a preponderance of potassium over calcium in the sporophores tissue of both *Pleurotus* species which suggests effectiveness against hypertension and the balance between high potassium and low sodium in mushroom was evident. Some heavy metals Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb) and Zinc (Zn) were detected in the sporophore of both *Pleurotus* species but were below the allowable limits set by WHO (1982) sources of microelement and macro-element in fruiting body could have come from the rice straw compost and its amendments.

The two *Pleurotus* species were separated by their gel profile after PCR performed with ITS1 and ITS4 primers. Restrictive patterns generated by four restrictive enzymes Hha, Hinf I, Rsa I and Hae III showed that only Hae III was the most effective in segregating the two *Pleurotus* species.

The greenhouse studies using varying percentages of spent mushroom compost (0-100%) of *P. eous* strain P-31 showed that the effect of the compost was different for each test crop. Low percentage of the compost soil mixture up to 20-25% improved plant height, leaf area, no. of leaves / plant and total chlorophyll of pepper, tomato and cowpea. SMC concentration beyond 30% SMC depressed growth. In the case of cowpea rhizobia population and nodulation was increased by 5% SMC and declined with increasing % SMC and none was found in plants grown in unamended SMC only. The highest Nodule Index was at 5% SMC

(18) followed by 10% SMC (12) and thereafter declined to Nodule Index 2 at 30% SMC. There are prospects for practical application of these finding reported in the thesis.

RECOMMENDATIONS

- The best substrate for growth and BE of *P. eous* strain P-31 was 0-8 days composted rice straw only (BE=76.4%, yield 221.5g) and uncomposted raw rice straw: husk mixture yielded 211.0g with a BE=72.8%. *P. eous* therefore performed better on uncomposted or short days composted rice straw than *P. ostreatus* strain EM-1 on the same substrate which needed longer period of composting > 12 days to utilize the substrate. Economically, uncomposted and short period composting will facilitate and fast-track mushroom crop production better than the wawa sawdust currently in use.
- The amendment of the rice straw / rice husk compost did not significantly ($p<0.05$) increase yield and BE of the compost. Therefore this will be a cut down in production cost.
- The nutritional and mineral values of the fruit body of *P. eous* and *P. ostreatus* were not compromised and nutrient and mineral contents were acceptable and within the acceptable WHO (1982) stipulated limits.
- The use of spent mushroom compost from cultivation of *Pleurotus* species as biological fertilizer has been shown to be feasible for pepper, tomato and cowpea. The fact that higher concentration of SMC in soil become inhibitory shows the increased preponderance of nutrients and mineral elements may be responsible for this. The field application trials should be pursued vigorously so that the technique could be demonstrated to the farmers and at the same time test the economic

feasibility of these findings. If this is done successfully, data from thesis would have been a springboard to improving agricultural productivity and also alleviate poverty of the rural farmer and the mushroom cultivator alike.

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APPENDICES

Appendix 1: Influence of spent mushroom compost (SMC) on the growth and development of tomato seedlings under greenhouse conditions at 30±2◦C for 12 weeks period

Proportion of SMC (%)	**Plant height (cm) Mean ±SE**	**Leaf area (cm^2) Mean ±SE**	**No. of leaves Mean ±SE**	**Chlorophyll content (CCI) Mean ±SE**	**No. of floral buds Mean ±SE**	**No. of flowers Mean ±SE**	**No. of fruits Mean ±SE**	**Weight of fruits Mean ±SE**	**Stem girth (mm) Mean ±SE**
0 (control)	21.3±3.0	80.3±31.6	4.9±2.4	16.2±4.0	4.2±0.7	2.8±0.6	0.0±0.0	0.0±0.0	3.2±1.0
5	42.1±5.0	311.8±60.0	7.8±2.4	34.1±0.9	39.0±2.4	17.0±0.9	3.0±0.2	5.4±1.0	5.3±1.6
10	50.3±7.2	378.8±81.2	7.9±2.5	29.7±2.0	51.4±3.2	27.8±2.4	4.0±0.3	6.0±1.1	5.7±1.7
15	45.8±6.3	372.4±100.6	8.4±2.8	26.3±1.2	45.8±3.2	24.6±2.4	5.0±0.4	7.7±2.8	5.8±1.7
20	40.4±6.1	348.0±96.4	7.4±2.4	21.3±1.2	29.8±3.3	16.2±1.9	5.0±0.4	9.5±2.6	5.2±1.6
25	41.0±6.3	267.5±71.0	7.4±2.4	24.5±1.5	38.8±2.9	17.0±0.9	5.0±0.4	16.2±4.7	5.7±1.7
30	41.6±5.6	330.4±72. 3	7.8±2.7	24.2±1.7	42.6±3.4	20.0±2.4	8.0±0.6	34.2±7.7	5.4±1.6

Appendix 2: Influence of spent mushroom compost (SMC) on the growth and development of pepper seedlings under greenhouse conditions at 30±2°C for 12 weeks period

Proportion of SMC (%)	Plant height (cm) Mean ±SE	Leaf area (cm^2) Mean ±SE	No. of leaves Mean ±SE	Chlorophyll content (CCI) Mean ±SE	No. of axillary branches Mean ±SE	No. of floral buds Mean ±SE	No. of flowers Mean ±SE	No. of fruits Mean ±SE	Weight (g) of fruits Mean ±SE	Stem girth (mm) Mean ±SE
0 (Soil)	12.2±1.9	14.5±3.3	13.4±1.5	13.0±0.6	2.8±0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.7±0.5
5	29.8±5.3	64.3±15.0	52.6±8.6	20.1±0.9	7.4±0.8	5±0.2	0.0±0.0	0.0±0.0	0.0±0.0	8.1±0.7
10	30.8±6.3	53.5±7.4	55.9±8.5	19.8±0.4	8.4±0.6	15±0.8	3.0±0.4	3.6±0.6	4.0±0.6	8.5±0.7
15	30.1±6.5	66.2±12.9	52.2±9.7	20.3±0.7	7.8±1.2	18.0±1.5	9.0±0.4	4.8±1.0	12.8±0.5	8.5±0.7
20	28.2±6.0	49.1±10.3	50.9±9.5	18.7±0.2	7.4±1.2	21.0±3.5	12.0±0.4	6.4±1.0	19.2±0.6	7.6±0.4
25	26.4±5.2	38.8±7.2	47.6±8.5	19.0±0.6	7.8±0.7	24.0±1.9	16.4±0.8	8.6±1.7	23.0±1.4	6.5±0.3
30	20.4±3.6	33.2±5.9	34.9±5.9	20.0±0.8	6.8±1.2	32±3.6	19.4±1.3	10.8±1.2	31.9±3.4	5.9±0.4

Appendix 3: Influence of spent mushroom compost (SMC) on the growth and development of cowpea seedlings under greenhouse conditions at 30±2°C for 12 weeks period

Proportion of SMC (%)	Average plant height (cm) ±SE	Average leaf area (mm^2) ±SE	Average no. of leaves ±SE	Average chlorophyll content (CCI) ±SE	Average no. of axillary branches ±SE	Average no. of floral buds ±SE	Average no. of flowers ±SE	Average no. of pods ±SE	Average weight of pods ±SE	Average weight of beans/seeds ±SE
0 (control)	31.8±9.0	170.8±64.6	8.8±1.5	51.9±4.3	0.0±0.0	7.3±2.6	6.4±2.5	2.9±1.4	2.8±1.1	2.3±0.8
5	50.4±10.9	263.7±82.7	13.4±1.7	70.5±8.0	3.0±1.4	12.6±4.4	11.7±4.6	7.1±4.1	11.0±2.0	10.2±3.7
10	42.2±7.6	293.6±99.9	13.4±1.7	67.5±6.0	7.7±3.4	14.3±5.3	11.4±4.3	8.9±3.8	12.8±4.3	12.1±3.8
15	40.8±7.9	276.1±90.5	11.4±1.1	59.5±2.6	9.4±3.5	13.0±4.1	11.1±4.2	10.6±6.2	14.0±2.4	13.8±4.2
20	47.9±13.0	331.9±123.4	11.9±1.9	57.4±8.0	6±2.3	8.9±3.7	8.3±3.2	4.7±2.1	5.2±1.7	4.1±1.7
25	47.8±11.2	207.1±69.3	12.6±2.3	35.8±4.2	2.3±1.5	7.6±3.5	8.4±3.1	4.3±2.0	4.0±1.5	3.6±1.3
30	30.2±7.8	166.9±55.5	11.6±2.2	19.3±2.4	0.0±0.0	7.6±3.5	8.0±2.9	3.4±1.5	3.5±1.9	2.6±1.1
100	28.4±6.7	83.6±21.7	10.0±1.7	16.9±5.7	0.0±0.0	4.7±2.1	7.1±2.6	2.1±1.4	2.1±1.4	1.8±0.7

Appendix 4: Chemical analysis of unfermented and fermented rice straw only compost

Period of composting (day(s)	% Gross Dry Matter	% Fine Dry Matter	% Gross Moisture	% Hemicellulose (DMB)	% Cellulose (DMB)	% Lignin (DMB) %	% Crude Protein (DMB)	Silica (DMB
0	20.83	93.28	79.17	24.75	38.82	5.62	4.50	11.74
4	27.84	92.40	72.16	24.79	34.36	5.96	5.03	14.29
8	26.63	92.32	73.37	20.81	36.64	6.35	5.36	14.01
12	33.70	93.23	66.30	23.94	36.31	6.68	4.41	13.82

Appendix 5: Chemical analysis of unfermented and fermented rice straw compost amended with 1% $CaCO_3$ and 10% rice bran

Period of composting (day(s)	% Gross Dry Matter	% Fine Dry Matter	% Gross Moisture	% Hemicellulose (DMB)	% Cellulose (DMB)	% Lignin (DMB) %	% Crude Protein (DMB)	Silica (DMB
0	21.85	91.02	78.15	21.42	29.71	8.95	4.67	19.18
4	24.88	91.44	75.17	25.85	33.05	5.79	5.00	16.46
8	24.52	91.33	75.48	23.63	33.90	5.38	5.01	16.14
12	30.64	91.09	69.36	24.99	32.41	7.82	4.49	15.82

Appendix 6: Cap to stipe ratio of *P. eous* and *P. ostreatus* on unamended and amended rice straw substrate

Period of composting (days)	Rice Straw Only		Rice straw amended with 1% $CaCO_3$ and 10% rice bran	
	P. eous P-31	*P. ostreatus* EM-1	*P. eous* P-31	*P. ostreatus* EM-1
0	1.08	1.36	1.19	1.40
4	1.15	1.07	1.11	1.21
8	1.23	1.07	1.03	1.36
12	1.12	1.29	1.12	1.32

Appendix 7: Cap to stipe ratio of *P. eous* and *P. ostreatus* on sawdust substrate amended with 1% $CaCO_3$ and 10% rice bran

Period of composting (days)	*P. eous* P-31	*P. ostreatus* EM-1
0	1.28	1.02
4	1.09	1.12
8	1.08	1.10
12	1.09	1.12

Appendix 8: Cap to stipe ratio of *P. eous* and *P. ostreatus* grown on amended rice straw and rice husk

Period of composting (days)	Compost Treatment (% Rice Bran)	Rice straw amended with different proportions of rice bran prior to bagging		Rice straw and husk mixture amended with different proportions of rice bran prior to bagging	
		P. eous P-31	*P. ostreatus* EM-1	*P. eous* P-31	*P. ostreatus* EM-1
0	D_5	1.37	1.03	1.14	1.08
	D_{10}	1.48	1.08	1.19	1.15
	D_{15}	1.59	1.19	1.17	1.19
4	C_5	1.37	1.27	1.24	1.25
	C_{10}	1.34	1.19	1.21	1.15
	C_{15}	1.36	1.19	1.33	1.02
8	B_5	1.59	1.09	1.15	1.18
	B_{10}	1.42	1.22	1.11	1.13
	B_{15}	1.28	1.20	1.11	1.21
12	A_5	1.37	1.15	1.07	1.05
	A_{10}	1.39	1.23	1.07	1.06
	A_{15}	1.27	1.21	1.10	1.02

Appendix 9: Influence of spent mushroom compost (SMC) on chlorophyll content of leaf extract of cowpea seedlings

Treatment (%SMC)	Chlorophyll A (mg/l)	Chlorophyll B (mg/l)	Total Chlorophyll (mg/l)
0	9.65	20.46	30.89
5	9.33	23.15	33.33
10	12.32	6.75	19.43
15	20.38	10.64	31.59
20	18.65	9.03	28.18
25	22.57	12.30	35.46
30	18.30	9.63	28.43
100	11.95	5.56	17.81

Spectrophotometry Method by Bansal *et al*. (1999)

Appendix 10: Effect of *Aspergillus flavus* culture filtrate on growth of *Pleurotus eous* strain P-31 on Potato Dextrose Agar (PDA)

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Rep 1	9.082	1	6	.024
Rep 2	8.574	1	6	.026
Rep 3	9.080	1	6	.024

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Rep 1	Between Groups	2812.500	1	2812.500	7.947	.030
	Within Groups	2123.500	6	353.917		
	Total	4936.000	7			
Rep 2	Between Groups	3872.000	1	3872.000	11.126	.016
	Within Groups	2088.000	6	348.000		
	Total	5960.000	7			
Rep 3	Between Groups	3160.125	1	3160.125	8.135	.029
	Within Groups	2330.750	6	388.458		
	Total	5490.875	7			

Appendix 11: Statistical analysis of fruit bodies of *Pleurotus eous* strain P-31 grown on rice straw lignocellulose amended with different percentages of rice bran prior to bagging

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Weight (g) of FB Flush 1	7.119	11	48	.000
Weight (g) of FB Flush 2	2.835	11	48	.006

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Weight (g) of FB Flush 1	7.119	11	48	.000
Weight (g) of FB Flush 2	2.835	11	48	.006
Weight (g) of FB Flush 3	.839	11	48	.603

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight (g) of FB Flush 1	Between Groups	15639.783	11	1421.798	2.824	.006
	Within Groups	24168.900	48	503.519		
	Total	39808.683	59			
Weight (g) of FB Flush 2	Between Groups	3077.028	11	279.730	1.431	.190
	Within Groups	9385.048	48	195.522		
	Total	12462.077	59			
Weight (g) of FB Flush 3	Between Groups	2981.446	11	271.041	2.017	.047
	Within Groups	6449.100	48	134.356		
	Total	9430.546	59			