CULTIVATION OF EDIBLE OYSTER MUSHROOMS *(Pleurotus ostreatus* AND *Pleurotus florida*) USING MILLET [*Eleusine coracana(L)*] STRAW AND BROAD BEAN [*Vicia faba(L)*] STALK AS GROWTH SUBSTRATES

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Cultivation of Edible Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*) Using Millet [*Eleusine coracana(L)*] Straw and Broad Bean [*Vicia faba(L*) Stalk as Growth Substrates

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By

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Haramaya University, Haramaya

APPROVAL SHEET

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this Thesis entitled Cultivation of Edible Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*) using Broad bean stalk and Millet straw as Growth Substrates prepared under my guidance by Ambachew Bogale. I recommend that it be submitted as fulfilling the thesis requirement.

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As a member of the Board of Examiners of the MSc. Thesis Open Defense Examination, I certify that I have read and evaluated the Thesis prepared by Ambachew Bogale and examined the candidate. I recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Biotechnology.

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DEDICATION

This thesis is dedicated to my best friends and family for their continual and unbound love, inspiration and support to complete this work.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

The author was born in Gondar in 1969 . He attended his primary education in South Gondar, Addis Zemen Elementary and Junior Secondary School, and his secondary education in Fasiledes Comprehensive Secondary School, Gondar. After the completion of his high school education, he joined the Department of Biology, Addis Ababa University, and graduated with B.Sc degree in Biology in 1996. Immediately after graduation, he has been employed by the MoE and serving as a Biology teacher at Hailemariam Mamo Preparatory School,Debre berhan,North Shoa until he joined Haramaya University for his graduate studies in Biotechnology in July, 2012.

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contributions towards my success by freely sharing my responsibilities at a time when I was in need of their help.

LIST OF ABBREVIATIONS AND ACRONYMS

AC	Ash Content
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BE	Biological Efficiency
BBS	Broad Bean Stalk
CFC	Crude Fat Content
CPC	Crude Protein Content
CRD	Completely Randomized Design
ESAT	Ethiopian Society of Appropriate Technology
FAO	Food and Agricultural Organization
FC	Fiber Content
HIV	Human Immunodeficiency Virus
MC	Moisture Content
MS	Millet Straw
PEM	Protein Energy Malnutrition
SAS	Statistical Analysis System

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CULTIVATION OF EDIBLE OYSTER MUSHROOMS (*Pleurotus ostreatus* AND *Pleurotus florida*) USING MILLET [*Eleucine coracana (L)*] STRAW AND BROAD BEAN [Vicia faba (L)] STALK AS GROWTH SUBSTRATES

Abstract

Most developing countries like Ethiopia are striving hard to solve acute protein deficiency in the diets of their increasing populations through cultivation of mushrooms in the urban areas without heavily depending on agricultural lands and capital. This study was conducted in Debre Berhan University, College of Natural and computational Sciences, with the aim of assessing the proximate chemical composition, biological efficiency and suitability of the selected substrates (Millet straw and Broad bean stalk) according to the methods described in AOAC (1995) for oyster mushroom cultivation. Accordingly, broad bean stalk and millet straw, and their different mix ratios were tested for their efficacy in oyster mushroom production. The spawns of oyster mushrooms were obtained from the Mushroom Research, Production and Training Laboratory of Haramaya University, College of Agriculture. The spawn production sorghum was separately inoculated with the pure cultures oyster mushroom and later on transferred to selected agricultural wastes (i.e. broad bean stalk and millet *straw) for the production of fruiting bodies. The oyster mushroom cultivation was undertaken under aseptic conditions and growth and development was monitored daily. Results of the study revealed that oyster mushroom can grow on broad bean stalk and millet straw with varying growth performances. The highest percentage of biological efficiency was obtained from S2(50%millet straw +50%broad bean stalk) for Pleurotus ostreatus (P.o) and from S5(100%broad bean stalk) for Pleurotus florida (P.f), while the least was from S5 (100%broad bean stalk) for Pleurotus ostreatus and from S1(75%millet straw+25%broad bean stalk) for Pleurotus florida. The maximum protein for Pleurotus ostreatus and Pleurotus florida was obtained from S4(100%millet straw) and the minimum for Pleurotus ostreatus from S3(25%millet straw+75%broad bean stalk) and for Pleurotus florida from S1(75%millet straw+25%broad bean stalk) respectively. The highest fiber for Pleurotus ostreatus and Pleurotus florida was gained from S4 (100%millet straw), and the least for Pleurotus ostreatus from S3 (25%millet straw+75%broad bean stalk) and for Pleurotus florida from S1 (75%millet straw+25%broad bean stalk) respectively. S4 (100%millet straw) was the most decomposable substrate than others. The nutritive values of the studied mushrooms were quite very high, and the selected growth substrates were reasonably acceptable.*

Key**words:** *Broad bean stalk, Millet straw, Nutritive values, Oyster mushroom, Pleurotus florida, Pleurotus ostreatus.*

1. INTRODUCTION

One of the world's biggest challenges is food insecurity. This problem is largely common in low- and middle-income countries which have poor food production systems and people suffering from serious problems of malnutrition. Such countries must find ways of improving food production so as to feed the vastly increasing human population (Diriba *et al*., 2013). Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of the vulnerable people (Imtiaj and Rahman, 2008).

More than 2000 mushroom species exist in nature, but only approximately 22 species are intensively cultivated and accepted as food; and only a few of them like *Agaricus bisporus*, *Pleurotus* spp., *Lentinula edodes*, *Volvariella volvacea*, etc. have attained the level of an item of commerce (Dursun *et al*., 2006). Wild edible mushrooms are becoming more and more important in our diet for their nutritional, organoleptic and pharmacological characteristics. Some of these wild edible ectomycorrhizal mushrooms, however, are seasonally consumed by certain groups of people, such as local residents of mushroom growing areas, enthusiasts, and gourmets (Dursun *et al*., 2006). Nowadays, they are undoubtedly consumed much more for their texture and flavor than for their nutritional and medicinal properties. In most countries there is a significant consumer acceptance of cultivated mushrooms. In general, it is well established that edible mushrooms have higher protein content than most vegetables and are rich in minerals, carbohydrates and vitamins except for their low fat content (Dursun *et al*., 2006).

Mushroom cultivation practice does not always require access to land (i.e., there is no need for larger space) and any significant capital investment, and hence it can be regarded as a viable and attractive activity for rural, peri-urban and urban dwellers. Mushroom cultivation is suitable for all job seeking groups including elders, disabled persons and youngsters. Although mushroom cultivation is labor intensive, this may not be a problem of tropical regions (Chang, 2007; FAO, 2009).

Mushrooms are a good cash crop; they are rather easy to grow and are brimming with proteins, vitamin B and minerals. They even have medicinal properties. Time between spawning and harvesting can be as short as three weeks. Furthermore, after the cultivation, we can still use the substrate as a good soil conditioner (Chang and Miles, 2004).

Mushroom cultivation drives towards full use of all materials in which nothing is left as waste; farming is done without any adverse impacts on the environment through sustainable utilization of lignocellulosic wastes available in abundance everywhere, usually as by-products from agriculture, forestry and households (Chang, 2007).

Edible mushroom species are highly nutritious, and often their nutritional value is compared favorably with that of meat, eggs, and milk. As a result, the cultivation of mushrooms is considered economically important for the food industry worldwide and has expanded in the past few years (Barros *et al*., 2007).

Mushroom cultivation offers benefits to market gardens when it is integrated into the existing production system by producing nutritious food at a profit, while using materials that would otherwise be considered “waste” (Beetz and Kustudia, 2004). This is because mushrooms contain many essential nutrients and they are found to solve dietary related health problems (Atikpo *et al*., 2008). As functional foods, they can be used as nutrient supplements to enhance immunity in the form of tablets. Their low starch and cholesterol content makes them suitable food items for diabetic and heart patients. One third of the iron in the mushrooms is in available form. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively. Biologically active compounds from the mushrooms possess antifungal, antibacterial, antioxidant and antiviral properties and have been used as insecticides and nematicides as well (Nanba, 1993).

Thus, because of such nutritional, medicinal, and ecological advantages, mushroom cultivation has attracted the attention of many people in the world. According to Beteez and Kustudia, (2004) cultivation of edible mushrooms may be the only currently economical biotechnology for lignocellulosic organic waste recycling that combines the production of protein food with the reduction of environmental pollution (Beteez and Kustudia, 2004). In fact, at a global level, mushroom production which had been approximately 80 tons in 1973 rose to 1400, 3052, 7728 and 10000 tons in 1983, 1991, 1995, and in the recent years, respectively (Erkel, 2004).

Mushroom cultivation and consumption culture is more developed in China, Japan, Korea, Thailand, America (Feeney and Beelman, 2004). However, it is least known in Africa, except in countries like Nigeria, Egypt, Kenya, Zimbabwe, and South Africa, which are relatively making good trials.

Mushrooms are eaten as meat substitutes and flavoring agents (Pathmashini, 2008). In addition to the nutritional and medical values, mushroom cultivation practices have paramount importance in food self-sufficiency attempts (Beetz and Kustudia, 2004). Especially for low-income countries like Ethiopia, mushrooms can generate additional employment and income through local, regional and national trade offering opportunities through processing enterprises (FAO, 2009).

Many literatures have revealed that it is possible to use various agro-industrial wastes for a range of purposes, including mushroom cultivation, in order to generate value added products and reduce environmental pollution (FAO, 1999). Despite the high diversity of wild edible mushrooms in Africa, including Ethiopia, very little is known on the extent to which they can be exploited in the country. Cultivation and production of mushrooms has not been practiced on commercial scales in most developing countries which has consequently affected commercial mushroom marketing which is yet to be embraced by most farmers (Dawit Abate, 1998; Olumide, 2007).

In developing countries, governmental and non-governmental organizations have not given attention to mushrooms as an important crop that can fetch farmers a substantial income to alleviate poverty (Olumide, 2007). Similarly, it is well accepted that mushrooms are not a luxury food but a national necessity to combat poverty and malnutrition. However, there is no sufficient mushroom cultivation practice in the country to fill the demands of people interested in mushroom consumption. Those very few mushroom farms in Ethiopia are restricted to the capital city. Some research based practices in some parts of the country are still at the stage of trials (Chang, 2007).

Currently, many research works focused on the cultivation of the three most commercially important mushrooms: the button mushroom (*Agaricus bisporus*), the shiitake mushroom (*Lentinula edodes*) and the oyster mushroom (*Pleurotus ostreatus*). The cultivation of these edible mushrooms has four important components: spawn production, substrate preparation, mushroom (fruiting-body) production and the management of mushroom houses (FAO, 2009).

Their cultivation generally requires the use of cellulosic materials or residues such as cereal straw, cotton stalks, various grasses and reed stems, maize and sorghum stover, sugarcane and sugarcane bagasse, banana residues, coffee pulp and coffee husk, cotton seed and sunflower seed hulls, peanut shells, rice husks, waste paper, wood sawdust and chips etc. Many of these are agro-industrial residues and by-products that can be recovered and upgraded to higher value and useful products as growth substrates (Dawit Abate, 1998).

Millet (Eleusine coracana) in and around Debre Tabor (South Gondar) and Broad bean (Vicia faba) in and around Debre Berhan (North Shoa) are staple food crops of the inhabitants. So the reason to choose millet straw and broad bean stalk is that they are sufficiently available in most of the farmer's farmlands and were collected for this research work. Thus, the overall objective of this study was to cultivate two edible mushroom species, i.e. *Pleurotus ostreatus* and *Pleurotus florida,* using broad bean stalk and millet straw as growth substrate.

The specific objectives of the study were:

- To determine the biological efficiency of *Pleurotus ostreatus* and *Pleurotus florida* grown on broad bean stalk and millet straw as well as their combinations
- To identify the optimal substrate mix ratio that is suitable for cultivation *of Pleurotus ostreatus* and *Pleurotus florida*
- To determine the proximate chemical composition of the selected mushroom species grown on the most effective substrate (substrate combination).

2. LITERATURE REVIEW

2. 1. History of Mushroom Cultivation

Geologically mushrooms existed on the earth even before man appeared on it, as evidenced from the fossil records of the lower cretaceous period. Thus anthropologically speaking, there is every possibility that man used the mushrooms as food when he was still a food gatherer and hunter on the chronology of Cultural Revolution (Bahl, 1983). Mushrooms offer tremendous applications as they can be used as food and medicine besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983).

Man has been hunting for the wild mushrooms since antiquity (Cooke, 1977). Thousands of years ago, fructifications of higher fungi have been used as a source of food (Mattila *et al*., 2001) due to their chemical composition which is attractive from the nutrition point of view. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors (Rai, 1994, 1997). Present use of mushrooms is totally different from traditional because, lot of research has been done on the chemical composition of mushrooms, mushrooms can be used as a diet to combat diseases.

The Egyptians considered mushrooms as a delicacy reserved for Pharaohs while the Romans ate mushrooms at feasts and believed that mushrooms provided strength for warriors in battle (Jahan *et al*, 2010) and in the far East mushrooms are venerated for their medicinal value (Chang and Miles, 2004).Mushrooms can be picked from the wild during the latter wettest part of the rainy season, where they are found growing on deeply decomposing organic matter. However not all mushrooms found growing in the wild are good for human consumption. Some are edible, but other species are poisonous making people skeptical about their consumption in general. Growing of safe known mushrooms therefore, presents a window of opportunity (Oei, 1996).

People have harvested mushrooms from the wild for thousands of years for food and medicines. Of the estimated 1.5 million species of fungi, about 10,000 produce the fruiting bodies we call mushrooms. While commercial harvesting of wild mushrooms continues today, most of the world's supply comes from commercial mushroom growers. The Chinese first cultivated shiitake (*Lentinula edodes*) mushrooms around 1100 AD, with domestication efforts beginning centuries earlier. White button mushrooms (*Agaricus* spp.), most familiar to Americans and Europeans, were first domesticated in France in 1650 (Sanmee *et al.,* 2003). Commercial production began in the United States in the 1880s. *Agaricus* is the leading mushroom crop worldwide and accounted for 99% of the 1997 United States' mushroom production. Oyster mushrooms (*Pleurotus* spp.) were more recently domesticated, and now rank second in world production. Shiitake mushrooms, which are very popular in Asian cultures, rank third. Many other edible mushrooms, such as straw and wood ear mushrooms, are gaining in popularity (Sanmee *et al.,* 2003).

2.2. Mushrooms and Mushroom Biology

2.2.1. Definition of a mushroom

The biological science that is concerned with fungi is called mycology. Mushroom biology is the branch of mycology that deals with any aspects of the scientific study of mushrooms. Mushroom science is mushroom cultivation and production (mushrooms themselves) and encompasses the principles of mushroom biology/ microbiology, bioconversion/ composting technology and environmental technology and provides food supplies through mushroom themselves (Chang and Buswell, 2008).

Mushrooms with other fungi are something special in the living world, being neither plants nor animals. They have been placed in a kingdom of their own called the kingdom of Myceteae. The word mushroom may mean different things to different people and countries. At present the specialized studies of mushrooms including the economic values and their products had reached a point for which a clear definition of the term "mushroom" was warranted. In a broader sense "a mushroom is a macro-fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand". Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium (Chang and Miles, 1992).

Thus, mushrooms need not be basidiomycetes, nor aerial, nor fleshy, nor edible. Mushrooms can be ascomycetes, grow underground, have a non-fleshy texture and need not be edible. This definition is not a perfect one but can be accepted as a workable term to estimate the number of mushrooms on the earth (Chang and Miles, 1992).

The most common type of mushroom (e.g. *Lentinula edodes*) is an umbrella shaped macro fungus possessing a pileus (cap) and a stipe (stem). Other species additionally have a volva (cup) e.g. *Volvariella volvacea* or an annulus (ring) e.g. *Agarius campestris* or both e.g. *Amanita muscaria*. Furthermore, some mushrooms are in the form of pliable cups; others round like golf balls. Some are in the shape of small clubs; some resemble coral; others are yellow or orange jelly-like globs; and some even very much resembles the human ear. In fact, there is a countless variety of forms (Chang and Miles, 1992).

The structure that we call a mushroom is in reality only the fruiting body of the fungus. The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cord-like strands that branch out through soil, compost, wood log or other lignocellulosic material on which the fungus may be growing. After a period of growth and under favorable conditions, the established (matured) mycelium could produce the fruit structure which we call the mushroom (Chang and Miles, 1992).

Accordingly mushrooms can be grouped into four categories: those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus;* mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., *Amanita phalloides;* and a miscellaneous category which includes a large number of mushrooms whose properties remain less well defined, which may tentatively be grouped together as 'other mushrooms'(Chang and Miles, 1992).

Certainly, this approach of classifying of mushrooms is not absolute and not mutually exclusive. Many kinds of mushrooms are not only edible, but also possess tonic and medicinal qualities. Mushrooms are devoid of leaves, and of chlorophyll-containing tissues. This renders them incapable of photosynthetic food production. Yet, they grow, and they produce new biomass. For their survival, for their growth, and for their metabolism, they rely on organic matter synthesized by the green plants around us, including organic products contained in agricultural crop residues. The organic materials, on which mushrooms derive their nutrition, are referred to as substrates (Chang and Miles, 1992). Mushrooms are a unique biota which assembles their food by secreting degrading enzymes and decompose the complex food materials present in the biomass where they grow, to generate simpler compounds, which they then absorb, and transform into their own peculiar tissues. Mushrooms lack true roots, they anchored into the substrates by their tightly interwoven thread-like hyphae, which also colonize the substrates, degrade their biochemical components, and siphon away the hydrolyzed organic compounds for their own nutrition (Chang and Miles, 1992).

2.2.2 The number of mushroom species

In 1990, the number of known species of fungi was about 69,000 (Hawks worth, 1991) while it was conservatively estimated that 1.5 million species of fungi actually existed. On average 700 species were described as new to science each year from 1920 to 1950. However, the annual total catalogued fungi reached around 1,400 in 1961, 1,500 in 1968 and averaged 1,700 each year for 1986 to 1990. Fungi are regarded as being the second largest group of organisms in the biosphere after the insects. Known fungal species constitute only about 5% of their species in the world. Thus, the large majority of fungi are still unknown (Miles and Chang, 1997). Out of about 69,000 described species of fungi, it has been suggested that around 14,000-15,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro fungi (mushrooms).

Of these, about 5,000 of the species are considered to possess varying degrees of edibility, and more than 2,000 species from 31 genera are regarded as prime edible mushrooms. But only 100 of them are experimentally grown, 50 economically cultivated, around 30 commercially cultivated, and only about 6 have reached an industrial scale of production in many countries (Miles and Chang, 1997). Furthermore, about 1,800 are medicinal ones. The number of poisonous mushrooms is relatively small (approximately 10%). Of these some 30 species are considered to be lethal (Miles and Chang, 1997).

2.3. Applied Mushroom Biology

The aims of the discipline of applied mushroom biology are to tackle the three basic problems: shortage of food, diminishing quality of human health and pollution of the environment, which human beings still face, and will continue to face, due to the continued increase of the world population. The 20th century began with a world population of 1.6 billion and ended with 6 billion inhabitants. The world's population is likely to reach 9.2 billion in 2050 from the current 6.7 billion with most of the growth occurring in developing countries (James, 1995).

The growing world population is increasing by about 80 million people per year. At present, about 800 million people in the world are living in poverty. On the other hand, it has been observed that over 70% of agricultural and of forest products have not been put to total productivity, and have been discarded as waste. Applied mushroom biology not only can convert these huge lignocellulosic biomass wastes into human food, but also can produce notable nutriceutical products, which have many health benefits. Another significant aspect of applied mushroom biology is using the biota in creating a pollution-free and beneficial environment (Chang and Miles, 1992). Thus, the three components of applied mushroom biology are closely associated with three aspects of wellbeing- food, health and pollution.

2.3.1 Mushroom cultivation

The cultivation of mushrooms can be both a relatively primitive farming activity, and a high technology industry. In each case, however, continuous production of successful crops requires both practical experience and scientific knowledge. Mushroom cultivation is both a science and an art. The science is developed through research; the art is perfected through curiosity and practical experience (Chang, 2008). Cultivated mushroom are generally saprophytes, utilizing substrate as primary or secondary decomposers (Stott and Caroline, 2004). Some species of mushrooms can grow on a wide range of materials and others cannot. After the mycelium has grown throughout the substrate, and when its specific requirements are met, the mushroom will fruit. Humidity, light, temperature, and carbon dioxide-to-oxygen ratio are conditions, which typically determine when a mushroom will fruit (Chang and Buswell, 2008).

Mushroom species are often very particular about their substrate. The substrate is fermented, pasteurized, or sterilized in order to prevent or reduce competition from fungi other than the chosen species (Harris and Bob, 1994). The growing medium is then inoculated with spawn. Before any decision to cultivate a particular mushroom is made, it is important to determine if that species possess organoleptic qualities acceptable to the indigenous population or to the international market, and if suitable substrates for cultivation are plentiful (Rafats and Jerry, 1996).

Mushroom growth dynamics involve some technological elements, which are in consonance with those exhibited by our common agricultural crop plants. For example, there is a vegetative growth phase, when the mycelia grow profusely; and a reproductive (fruiting) growth phase, when the umbrella-like body that we call mushroom develops. After the vegetative (mycelial) phase has reached maturity, what the mushroom farmer needs next is the induction of fruiting (Harris and Bob, 1994).

Mushrooms belong to the genus fungi. Mushroom has been widely cultivated since the 1700's and presently more than 30 known species are cultivated as foods. Mushrooms are usually collected in the wild during the early periods of the rainy season. It is a practice mainly engaging women and children. Such mushrooms are used as meat substitute, sold fresh in local markets, or dried for use during the dry seasons when meat becomes very scarce (FAO, 2000).

Twelve mushroom species are commonly grown for food and/or medicinal purposes, across tropical and temperate zones, including the Common mushroom (*Agaricus*), Shiitake (*Lentinus),* Oyster (*Pleurotu*s), Straw (*Volvariella*), Lion's Head (*Hericium),* Ear (*Auricularis*), Ganoderma (*Reishi*), Maitake (*Grifola frondosa),* Winter (*Flammulina),* White jelly (*Tremella),* Nameko (*Pholiota),* and Shaggy Mane mushrooms (*Coprinus).* International mushroom markets are dominated by *Agaricus bisporus, Lentinula edodes* and *Pleurotus* spp*,* which represent three quarters of mushrooms cultivated globally (FAO, 2000).

2.3.2 Mushroom cultivation in some developing countries

Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocelluloses organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Obodai *et al*., 2003). Cultivated mushrooms should play a greater role in the endeavor to increase food protein. This is true especially in developing countries, since growth substrates for mushrooms are agricultural and industrial discards that are inedible for humans (Chang and Miles, 1984).

Mushroom cultivation has great scope in China, India and in some of other developing countries because of the cheap and easily available raw materials needed for this activity, coupled with faster means of communication and marketing (as a fresh commodity), and better purchasing power of the people. Using China as for example, in 1978, the production of edible mushrooms was only 60,000 tones. In 2006, China's mushroom production was over 14 million tones. Now there are more than 30 million people directly or indirectly engaged in mushroom production and businesses, and now China has become a leading mushroom producer and consumer in the world. It is hoped that the avocation of mushroom farming will become a very important cottage industry activity in the integrated rural development program, which will lead to the economic betterment of not only small farmers but also of landless laborers and other weak sections of communities (Chang, 1999; 2006; Delcaire, 1978).

The following statistics (Table 1) may serve to illustrate dramatic increases in the production of cultivated mushrooms during the period 1978 to 2002, with particular emphasis on China's contribution to total world production, given its current status as the leading mushroom producer.

Table 1. Total world mushroom production and China's contribution from1978-2006

Year	Total production	China's production (x1,000 tones)	China's contribution (%)

	(x1,000 tones)		
1978	1060	60.0	5.7
1983	1453	174.5	12.0
1990	3763	1083	28.8
1994	4909.3	2640	533.8
1997	6158.4	3918	63.6
2002	12250	8650	70.6

Source: Chang (1999; 2006) and Delcaire (1978).

In 1997, Asia contributed 74.4% of the total world mushroom tonnage, Europe, 16.3% and North America, 7.0%, both Africa and Latin America's shares were less than 1%. This is largely due to lack of know-how, lack of understanding that mushroom can play vital roles towards enhancing human health when used as dietary food supplements, lack of reliable sources of good quality mushroom spawn for supporting the efforts of local mushroom growers, lack of venture capital to support mushroom farming entrepreneurs, and absence of systematic government support towards promoting mushroom farming as a valuable non-traditional new food and cash crop (comparable to coffee, tea, cotton, tobacco, etc.) (Chang 1999, 2006, Delcaire 1978).

2.3.3. Mushroom cultivation in Ethiopia

According to Dawit Abate (1995), mushroom cultivation was a new activity in Ethiopia. Mushroom cultivation was a very recent practice and technology. Previously, mushroom consumption was confined to rural inhabitants and picked from farmlands, forests and around waste dump-sites when environmental conditions particularly humidity favor their sporocarp formation. Mushrooms are now cultivated and marketed in urban centers (Kumela, 2012). Dawit Abate (2008) reported that first scale mushroom farm was started in 1997 by the cultivation of the Oyster mushroom (*Pleurotus)* species. Later, the button (*Agaricus bisporus*) followed by Shiitake (*Lentinus edodes*) mushroom.

As well as being a method of bioconversion of non-edible plant biomass into nutritious food, mushrooms are a cash crop, the market for which is growing worldwide. While wild mushrooms were harvested in forests in Ethiopia during the rainy season, they were not a staple part of the diet and were not cultivated previously. Research on mushroom cultivation in Ethiopia was started in 1993 at the Department of Biology, Addis Ababa University. Some research based practices in some parts of the country are still at the stage of trials (Diriba *et al*., 2013). The local demand for mushrooms is steadily growing to about 36 tons per year (button 50%, oyster 40% and Shiitake 10%) at present. Technological development in the mushroom industry in general has been increasing production capacities, innovations in cultivation technologies, improvements to final mushroom goods, capitalizing the nutritional and medicinal properties of mushrooms, and utilizing the natural qualities of mushrooms for environmental benefits (Kumela, 2012).

Agricultural and agro industrial waste and other cellulose-rich materials available in the country were investigated for production of the three most commonly cultivated mushrooms: *Agaricus bisporus* (button mushroom), *Pleurotus ostreatus* (oyster mushroom) and *Lentinula edodes* (shiitake mushroom), (Kumela, 2012). Appropriate methods of spawn production, substrate preparation, composting and mushroom growing under existing environmental conditions have been achieved. The outcomes of the research project undertaken in the Department of Biology have provided the basis for the establishment of small-scale commercial mushroom enterprises in and around Addis Ababa (Dawit Abate, 1998).

The prevailing mild temperatures in Ethiopia, particularly in the highlands, are conducive to mushroom growing. Although the low level of relative humidity during most of the year is not optimal for cultivation, this is a problem that can be dealt with by using appropriate environmentally sustainable methods of moistening the air. The waste generated, the spent compost, is used as organic fertilizer for growing vegetables and tree seedlings. This has shown that sustainable and environmentally friendly small-scale mushroom production is feasible in Ethiopia (Dawit Abate, 1998).

2.3.4. Oyster mushrooms (*Pleurotus* species)

Pleurotus ostreatus is the scientific name for oyster mushroom and grown worldwide, and China is the major producer (OECD, 2005). To date, approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical with previously recognized species (Irie *et al*., 2001). The genus consists of a number of different species including *P.ostreatus, P. sajor-caju, P. florida, P. cystidiosus, P. cornucopiae, P. tuber-regium, P. citrinopileatus and P. flabellatus* (Fermor *et al*., 2000).

The oyster mushroom is the second most important mushroom in production in the world, accounting for 25% of total world production of cultivated mushrooms. It is the first mushroom to be introduced to the market in Ethiopia (Dawit Abate, 1998). *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). Another advantage of growing oyster mushrooms is that a high percentage of the substrate is converted to fruiting bodies, increasing potential profitability (Irie *et al*., 2001).

Having access to an economical growth medium suitable to the species chosen is an important factor in deciding which species to grow. The amount of processing necessary to prepare the substrate for the spawn is another consideration (Chang *et al*., 2003).The final choice of a species to grow depend upon the waste materials available for use as substrate, an appropriate facility, cost of necessary equipment, the level of skill required to manage the life cycle of the fungus, and the market already established for that species. Considering these criteria, oyster mushrooms (*Pleurotus* species) are probably best for most novices. They are relatively easy to grow and there is a growing market (Rafats and Jerry, 1996).

Mushroom cultivation is a profitable agri-business and Oyster mushroom (*Pleurotus ostreatus)* is an edible mushroom having an excellent taste and flavor. It belongs to the class *Basidiomycetes,* subclass *Hollobasidiomycetidae,* order *Agricales,* and family *Pleurotaceae. Pleurotus* species are classified mostly on the basis of morphological characters (Matsumoto *et al*., 2003). They grow wild in the forest and are cultivated in the temperate and sub tropical regions of the world (Ayodele and Akpaja, 2007). Oyster mushrooms were by far the easiest and least expensive mushroom to grow. They are fairly resistant to environmental changes and few other mushrooms demonstrate such adaptability, hardiness and productivity. They grow on a wide array of otherwise unused forest and agricultural wastes from farms, plantations and factories although a mixture of cotton- seed waste, wheat bran and chalk gave the best results. The substrate can be placed in boxes, plastic bags or even in common household items such as traditional clay pots. After harvesting, the decomposed substrate can be used for soil conditioning (Dawit Abate, 1998).

For small cultivators with limited budgets, oyster mushrooms are the clear choice for gaining entry to the mushroom market. Oyster mushrooms (*Pleurotus* spp*.)* are often recommended as a good choice for beginner mushroom cultivators because they are easier to grow than many of the other species, and they can be grown on a small scale with moderate initial investment (Kang, 2004). They can also be produced from a wide variety of high cellulose waste materials. Some of these materials do not require sterilization, but only pasteurization, which is less expensive (Kang, 2004).

Pleurotus species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency. They can be easily cultivated on different agro-industrial wastes but the yield potential may vary with the substrate used. Oyster mushrooms are a good source of dietary fiber and other valuable nutrients (Mane *et al*., 2007).

With changes in the climatic patterns, it is becoming difficult to harvest wild mushrooms. The alternative then, is to grow mushrooms domestically. Quimio (1980) reported that the technology of artificial mushroom cultivation was stemmed from the realization that the incorporation of non-conventional crops in existing agricultural systems can help in improving the social as well as the economic status of small farmers. Mushrooms are a delicacy and are used in the preparation of many continental dishes. They have anticancerous, anticholesterol and antitumorous properties and are useful against diabetes, ulcer and lung diseases (Quimio, 1980). Moreover, cholesterol and the sterol known to be dreaded for heart patients, remain absent in mushrooms (Bhupinder and Ibitwar, 2007). FAO recognizes mushrooms as the right source of protein to fight protein malnutrition in the cereal dependent developing countries (FAO, 2004).

They also contain appreciable amounts of potassium, phosphorus, copper and iron but have low levels of calcium. Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976 cited in Bauh *et al*., 2010). Oyster mushroom has no starch, but it has low sugar content and high amount of fiber, hence it serves as the least fattening food (Oei, 1996). The growth of oyster mushroom requires high humidity(80-90%) and high temperature (25-30°C) for the vegetative growth called spawn running and lower temperature (18-25°C) for fruit body formation (Onyango *et al*., 2011). Like other mushrooms, oyster mushroom can also be grown on various agricultural wastes with the use of different technologies.

2.3.5. Growth of mushrooms

The raw materials to grow mushrooms (cellulose-rich agricultural and agro industrial wastes) are abundant in Africa .While environmental conditions such as temperature and humidity differ geographically; production techniques can be adapted to local conditions so that mushrooms can be grown throughout the yearl (Lal, 2005).

Since mushrooms lack chlorophyll, they cannot, like green plants, get their energy from the sun through photosynthesis. Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate. The degraded compounds are then absorbed by the hyphae and the mycelium enlarges, usually, laterally, and in some cases growing several meters in diameter within the substrate (Lal, 2005).

Environmental factors (temperature and light which are known to be critical) stimulate the second or reproductive growth stage. Cells of one mycelial strain fuse with cells of the opposite type to form a mycelium that contains both types of nuclei. The new mycelium continues to grow and eventually develops into a mature fruiting body, the gills of which are lined with spore bearing cells called basidia. Various mechanisms trigger the dispersal of spores, which in turn lodge in a substrate, become hyphae and begin the cycle anew (Dawit Abate, 1998).

2 .3.5.1 Mycelial growth

Immediately following inoculation, whitish mycelia begin to grow on the supplemented substrate or grain until colonization is completed. This is an active assimilation phase with high fungal metabolic rate. Enzymes are activated to break down complex substrate components (e.g. cellulose, hemicelluloses and lignin) into simpler molecules which can be absorbed by mycelia as nutrients for growth and propagation (Chen, 2001).

2 .3.5.2 Fruiting body production

Lentinus edodes, Flammulina velutipes, and *Pleurotus ostreatus* need relatively less moisture for fruiting compared with other cultivated mushrooms, such as *Pholiota nameko* (Chang and Hayes, 1978).

Aeration also plays an important role in fructification. The fruiting body formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room. The appearance of fruiting bodies or mushroom varies according to the species, but all have a vertical stalk (stipe) and a head (pileus or cap) (Stamets, 2000).

2.3.5.3 Life cycle of mushrooms

The life cycle of most mushrooms is the same or very similar but macroscopic and microscopic features are different; such morphological variations enable us to identify individual mushroom species (Dawit Abate, 1998). The general life cycle of mushrooms, start from a mature fruit body or basdiospore, when the condition is suitable, the basdiospores germinate and grow as threads (hyphae) in the substrate. Hyphae from two different compatible spores fuse and form cells containing two nuclei, one from each, and such hyphae are called dikaryotic. The dikaryotic hyphae grow extensively and later form diploid cells through fusion of the two nuclei. The genetic material undergoes division and the cell develops into a basidium, which forms the basdiospores at the same time as the fruit body matures. The life cycle continues through the fruit body disappear for most of the year (Dawit Abate, 1998).

2.4 Importance of Mushroom Cultivation

It has been well known that the 20th century has been an explosive time for the accumulation of knowledge. Modern technology for human civilization is expanding every day. However, human beings still face and will continue to face three basic problems: shortage of food;

pollution of the environment; and diminishing quality of human health, due to the continued increase of the world population (James, 1995).

World production of cultivated edible mushrooms is estimated to be almost 5 million tones, valued at about $9.8 billion per year, to which Africa contributes a very small proportion. Total global production of mushrooms has increased more than tenfold in the past 25 years and the market for mushrooms is growing. The production of mushrooms, therefore, has the potential to generate a significant cash income (Dawit Abate, 1998). Mushrooms can be cultivated on a variety of substrates, including agricultural and agro-industrial waste materials such as cereal straw, grass straw, cotton waste, corn cobs, bagasse (the biomass remaining after crushing of sugarcane or sorghum stalks for juice extraction), coffee waste, sawdust, animal dung, chicken manure and brewers' spent, which are abundant in sub-Saharan Africa, a predominantly agricultural region. Mushroom growing is a means of converting such non-edible biomass into food. The waste products of mushroom cultivation (spent compost) can also be used as organic fertilizer for growing vegetables and developing tree nurseries (Dawit Abate, 1998).

The area of land required for cultivation is small, as mushrooms do not need light for growth and they are commonly produced on shelves indoors. Thus mushrooms can be produced and harvested throughout the year with relatively little investment. The Department of Biology of Addis Ababa University had initiated a three year project on mushroom cultivation in 1993. The main objective of the project was to identify appropriate types of mushroom for cultivation in Ethiopia; evaluate the usefulness of different, readily available substrates; develop methods for spawn production; and construct suitable, simple growing houses for small scale mushroom cultivation (Dawit Abate, 1998).

2.5. Social Values and Economic Benefits of Mushrooms

Mushroom cultivation practices have paramount importance in food self-sufficiency attempts (Diriba et al., 2013), specially for low-income countries like Ethiopia. Mushroom growing is an activity that can create jobs and help to generate cash income .It is environmentally friendly and the by-products can be recycled or used for other agricultural purposes. Training and technical support to growers can significantly help the transfer and adoption of the technology of mushroom production. With the support of research and training, mushroom production could have a significant impact on poverty alleviation and food security in Africa (FAO, 2009).

Mushroom growing represents an employment opportunity for jobless people and cash generation for growers. Moreover, the development of a value chain comprising spawn producers, suppliers of substrates (raw materials), mushroom growers and supermarkets is gradually taking shape (Chang,2007). Some of the trainees of the Ethiopian Society for Appropriate Technology (ESAT) were employed by the emerging mushroom enterprises. Others are looking for financial support to establish themselves as mushroom growers. The mushroom industry can have even broader positive spill-over, generating complementary employment in areas such as accommodation, restaurant services, etc. The local mushroom industry can also be the main source of revenue for local government (Wasser, 2002a).

Edible mushrooms once called the “food of the gods” and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food. The extractable products from medicinal mushrooms, designed to supplement the human diet not as regular food, but as the enhancement of health and fitness, can be classified into the category of dietary supplements /mushroom nutriceuticals (Chang and Buswell, 1996). Dietary supplements are ingredients extracted from foods, herbs, mushrooms and other plants that are taken without further modification for their presumed health-enhancing benefits (Reid, 1989; Chang,2007; FAO, 2009).

There is an old Chinese saying which states that “MEDICINES AND FOODS HAVE A COMMON ORIGIN”. Mushrooms constitute a most rapidly growing new food category which the current health-oriented public is increasingly enjoying. (Reid, 1989; Chang, 2007; FAO, 2009). The extractable bioactive compounds from medicinal mushrooms would enhance human's immune systems and improve their quality of life (FAO, 2009).

Mushroom cultivation is a cash crop. The harvested fruiting bodies can be sold in local markets for additional family income or exported for an important source of foreign exchange that will definitely improve the economic standards of the people. Some warm mushrooms, e.g. *Volvariella volvacea* (Straw mushrooms) and *Pleurotus Sajor-caju* (Oyster mushrooms) are relatively fast growing organisms and can be harvested in 3 to 4 weeks after spawning. It is a short return agricultural business and can be of immediate benefit to the community (Chang, 2007).

2.5.1 Nutritional value

The food for human beings comes from three different sources, i.e. land, water, and air (microbes). Among microbes used as human food, the fungi comprise the largest and the most important group containing edible mushrooms (Singh, 2008). Healthy nutrition and diet are gaining importance, not only in the everyday life of human beings, but also in the treatment of chronic diseases. Medical practitioners of worldwide are recognizing that mushrooms are medicinal foods rich in nutrition (Stamets, 2005). Mushrooms have higher nutritional values than fish or beef (Fauzia *et al.,* 2003*)*. It is also suggested that a diet rich in mushrooms provides all the essential amino acids usually available in fruits and vegetables (Matila *et al*., 2002).

Mushrooms are low calorie food, rich in protein, very low in simple carbohydrates, rich in high molecular weight polysaccharides, high in antioxidants, and very low in fat. They lack cholesterol, starch, and vitamin C. They are a good source of vitamin B complex , riboflavin (B2), niacin (B3), pantothenic acid (B5), erg sterols (provitamin D2), other substances are found such as selenium, calcium, phosphorus and potassium in fair quantity along with copper, and iron (Onokpise *et al*., 2008). Also high potassium to sodium ratio present in mushrooms is desirable for the patients with hypertension, also high in dietary fiber important for immune function, for producing antioxidants that reduce free radicals, and helpful in excretion of waste and prevention of constipation (Onokpise *et al*., 2008).

In general mushrooms on dry weight bases, composes of 10%- 40% proteins, 2%-8% fat, 3%-28% carbohydrates, 3%-32% fibers, 8%-10% mineral (Stamets, 2005). The high nutritional value of mushrooms is due to the presence of 8 essential amino-acids, polyunsaturated fatty acids (linoleic and arachidonic acids) and reduced quantities of saturated fatty acids (Fortes *et al*., 2006). Factors such as growing site, type of substrates, developmental stages and part of the fungal samples usually influence the nutritional composition of the mushroom (Anthony, 2007).

According to Chang *et al*.(2007), about 800 million people in the world are living in poverty. On the other hand, it has been observed that over 70 % of agricultural wastes and forest products have not been put to total productivity, and have been wasted in processing. Mushrooms not only can convert these huge lignocelluloses biomass wastes into human food, but can also produce notable immune enhanced products, which have many health benefits (Chang *et al*., 2007).
In Ethiopia hunger and malnutrition are devastating problems, particularly for the poor and unprivileged society. About 50 percent of the population are living below the food poverty line and cannot meet their daily minimum nutritional requirement of 2200 calories (FAO, 1998). The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), Iodine; vitamin A deficiency disorders (Edris, 2004).

An ever increasing human population and diminishing farm sizes have resulted in declining soil fertility associated land productivity and increasing poverty levels. Elsewhere in the world, mushrooms are consumed widely. Wide spread malnutrition with ever increasing protein gap has necessitated the search for alternative source of protein because the production pluses has not kept pace with our requirement due to high population growth (Sanchez, 2009).

Animal protein is beyond the reach of most people in different countries (FAO, 1998). Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals with it became a new and alternative demand for poultry and animal protein in fresh mushrooms. In general mushrooms are highly nutritious, their taste and delightful aroma makes them one of the delicious preferred foods in restaurants throughout the world (Chang and Mshignei, 2000).

The desirability of a food product does not necessarily bear any correlation to its nutritional value. Instead, its appearance, taste, and aroma, sometimes can stimulate one's appetite (preference). In addition to nutritional value, mushrooms have some unique color, taste, aroma, and texture characteristics, which attract their consumption by humans (Wermer and Beelman, 2002).

2.5.2. Medicinal value

Different mushrooms were studied by the scientific community in the search for new therapeutic alternatives and the results proved their bioactive properties (Lindquist *et al*., 2005). Mushrooms are rich sources of nutraceuticals (Çag˘larirmak, 2007; Elmastas *et al.,* 2007; Ribeiro *et al*., 2007) responsible for their antioxidant (Mau *et al*., 2002; Lo and Cheung, 2005; Barros *et al*., 2007a), antitumor (Wasser and Weis, 1999), and antimicrobial properties (Smânia *et al.,* 1995; Hirasawa *et al.,* 1999; Hatvani, 2001; Barros *et al.,* 2007; Turkoglu *et al.,* 2007).

Some of the *Pleurotus* species have been shown to possess a number of medicinal properties, such as anti tumour, immune modulatory, antigenotoxic, antioxidant, anti inflammatory, hypocholesterolaemic, anti platelet aggregating, anti hyperglycemic, antimicrobial and antiviral activities (Rai and Arumugonathon, 2003).Besides their pharmacological features (Lindquist *et al.,* 2005), wild mushrooms are becoming more important in our diet due to their nutritional value, related to the high protein and low fat/energy contents (Diéz and Alvarez, 2001; Agahar-Murugkar and Subbulakshmi, 2005; Barros *et al.,* 2007).

Due to high amount of proteins, they can be used to bridge the protein malnutrition gap. Mushrooms as functional foods are used as nutrient supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients. One third of the iron in the mushrooms is in available form. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively (Kimenju *et al*.,2009). Biologically active compounds from the mushrooms possess antifungal, antibacterial, antioxidant and antiviral properties, and have been used as insecticides and nematicides as well. Thus the tremendous applications of mushrooms, reviews different aspects of mushrooms towards human health benefits such as food, medicine, minerals and drugs, (Nanba, 1993).

Nutriceuticals and dietary supplements: According to Chang and Buswell (1996), the recent upsurge of interest in traditional remedies for various physiological disorders and the recognition of numerous biological response modifiers in mushrooms have led to the coining of the term "mushroom nutriceuticals" (Chang and Buswell, 1996). A mushroom nutriceutical is a refined/partially defined mushroom extractive which is consumed in the form of capsules or tablets as a dietary supplement (not a food) and which has potential therapeutic applications. A regular intake may enhance the immune responses of the human body, thereby increasing resistance to disease and, in some cases, cause regression of a disease state. Differing from most pharmaceuticals, these biologically active compounds extracted from medicinal mushrooms have extraordinarily low toxicity, even at high doses. Long viewed as tonics, now it has been known that they can profoundly improve the quality of human health (Stamets and Yao, 1998).

Mushroom Biotechnology: enhance human health through mushroom derivatives. It has been pointed out that mushroom biotechnology is concerned with mushroom products and encompasses the principles of fermentation technology, mushroom biology/microbiology and bioprocess. Mushroom products have a generalized or tonic effect, which in some cases may act prophylactically by increasing resistance to disease in humans from the balancing of nutrients in the diet and the enhancing of the immune systems (Stamets and Yao, 1998).

2.5.3 Role of mushrooms against environmental pollution

Mushroom Bioremediation: benefit the environment through mushroom mycelia. This component of applied mushroom biology deals mainly with the aspects of benefits to the earth from the activities of mushroom mycelium. Environmental contamination can be ameliorated by the application of mushroom mycelial technologies. The use of bioconversion processes to transform the polluting substances into valuable foodstuffs, e.g., the proper treatment reutilization of spent substrates/composts in order to eliminate pollution problems (Beyer, 2005; Noble, 2005).

Substrate materials are usually by-products from industry, households and agriculture and are usually considered as wastes. Organic solid wastes are a kind of biomass which consists mainly of cellulose, hemicelluloses and lignin generally termed as lignocelluloses. Lignocellulosic wastes are available in abundance both in the rural and urban areas (Chang and Buswell, 2003).And these wastes, if carelessly disposed of in the surrounding environment by dumping or burning, will lead to environmental pollution and consequently cause health hazards. However, they are actually resources in the wrong place at a particular time and mushroom cultivation can harness this waste/resource for its own beneficial advantage because mushroom enzymes can break down lignin, cellulose and hemicelluloses present in these organic materials into simpler molecules for their growth and metabolism and then lead to further economic growth as well (Wasser, 2002b).

One of the most intriguing opportunities offered by mushroom mycelia in the area of bioconversion is the exploitation of their ability to degrade pollutants, many of which are highly carcinogenic, released into the environment as a consequence of human activity. The use of fungi/mushroom mycelia as tools for healing soil, what Stamets (2005) called "mycorestoration", which is the use of fungi/mushrooms to repair or restore the weakened or damaged biosystems of environment (Chang et al., 2007).Currently, according to Rai and Ahlawat (2002), mushrooms are regarded as the most profitable and environment-friendly method for recycling of the vast lignocellulosic waste substrates which could otherwise dropped into the environment and cause pollution (Rai and Ahlawat, 2002).

The previous and ongoing studies in different parts of the world summarize the significance of mushrooms in our drive towards alleviating poverty, enhancing human health, and arresting environmental degradation (Bhalla *et al.,* 1999) as follows:

Mushrooms can convert lignocellulosic waste materials into a wide diversity of products, which have multi-beneficial effects to human beings, e.g., as food, health tonic, and medicine, as feed, as fertilizers, and for protecting and regenerating the environment (Bhalla *et al.*, 1999). In addition, mushroom cultivation can positively generate equitable economic growth. The tropical regions, particularly, have a wet and warm climate and have an abundant supply of agricultural wastes. These materials are resistant to natural biological degradation because they contain mainly cellulose, hemicelluloses and lignin. Mycelia of mushrooms can excrete enzyme complexes which can directly attack/degrade these components of lignocellulosic materials. Therefore, mushrooms can use these wastes as nutrients for their growth and in the process become food and medicine for human consumption (Bhalla *et al.,* 1999).

Mushrooms are relatively fast growing organisms. Some tropical mushrooms can be harvested and consumed within 10 days after spawning. By the use of different varieties, mushrooms can be cultivated year round. They can be cultivated by using primitive farming techniques in rural areas or by using highly industrialized technologies in the urban and periurban communities

(Bhalla *et al.,* 1999). Mushroom cultivation can be labor intensive. Thus the activity can generate new jobs, especially in tropical or less developed countries. While land availability is usually a limiting factor in most types of primary production, mushroom cultivation requires relatively little space. Actually they can be stacked using shelf like culture systems (Bhalla *et al.,* 1999).

Mushrooms have been accepted as human food from times immemorial, and can immediately supply additional protein to human food. Edible mushrooms should be treated as healthy vegetables. After improving the cultivation techniques, they should be cultivated as widely and as cheaply as other common vegetables, which will thus be beneficial to the general public. In view of their pleasing flavor, their high protein, and tonic and medicinal values, mushrooms no doubt represent one of the world's greatest untapped resources of nutritious and palatable food for the future (Bhalla *et al.,* 1999).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Debre Berhan University, in the Mushroom Production and Training Laboratory within the College of Natural and Computational Sciences. Debre Berhan University is located in North Shoa zone of Amhara region, about 120 kms northeast of Addis Ababa. The town has latitude and a longitude of $9^0 41'$N, $39^0 32'$ E and an elevation of 2,840 meters. It has biremodal annual rainfall which is about 1,080mm, consisting of long term rainfall from June-September, and short term rainfall from December-April (Ethiopian meteorological agency, 2004).

3.2. Sources of Experimental Materials

3.2.1. Spawn source

Pleurotus ostreatus and *Pleurotus florida* spawns were obtained from Mushroom Research, Production and Training Laboratory of Haramaya University,College of Agriculture.

3.2.2. Source of substrates

Millet [*Eleusine coracana (L*)] straw was collected from various farmers farmland around Debre-Tabor, South Gondar. Broad bean [*Vicia faba (L*)] stalk was collected from the farmland of some farmers around Debre-Birhan town, North Shoa.

3.2.3. Mushroom cultivation

3.2.3.1. Preparation of substrate for spawning

In this study, two different substrates, i.e. millet [*Eleusine coracana (L)*] straw and broad bean[*Vicia faba (L)*] stalk and three levels of their mixtures were used as growth substrates of *Pleurotus ostreatus* and *Pleurotus florida.* The substrates were spread on different dishes and dried for about one week with a regular turning interval of 3 days. After drying, the substrates were sterilized for 2 hours at 121^0C in a dry fire-heated drum to avoid contaminants. The sterilized substrates were then kept in a clean room and allowed to cool down for 12 hours (Atikpo *et al*., 2008). After cooling, they were placed in ten (10) sterile transparent plastic bags, of which five (5) were used for growth of *Pleurotus ostreatus* and the other five (5) for growth of *Pleurotus florida* according to the mix ratios indicated in Table 2.

Table 2. Arrangement of growth substrates for cultivation of the two selected species of mushrooms

Code	Proportion of growth substrates (Substrate mix ratio)
S1	75%MS+25% BBS
S2	50% MS+50% BBS
S3	25%MS+75% BBS
S4	100% MS
S5	100% BBS

S = Substrate, MS = Millet Straw, BBS = Broad Bean Stalk

The mixing of the two substrates with different mix ratios was to obtain these growth substrates sufficiently any time when needed, to minimize their scarcity, and to find out which mix ratio could provide a better yield. The two substrates (Millet Straw and Broad Bean Stalk) were mixed on clean separate plastic containers and then soaked in water containing 2% formalin for about 24 hours. After 24 hours of soaking in water, the excess water was removed from the moist substrate by decanting and manually squeezing by hand. At this stage when the water stopped dripping, the substrate was ready for spawning. Subsequently, a total of 30 plastic bags, each with the dimension of 20x30 cm, were filled with the 5 different substrate mix ratios for two species spawning, and each bag contained 500 g (dry weight) of the substrates. Then the bags were labeled and arranged according to the substrate mix ratio and the mushroom species they contain.

3.2.3.2. Spawning and spawn running

After the substrate preparation, 50g (equal to 10% of the weight of the substrate) of the edible mushroom spawn was inoculated into each bag and thoroughly mixed with the substrate kept in the polyethylene bags using sterile spoons under the laminar flow hood. Then the open ends of the bags were tied by rubber bands and a number of small holes were made using a sterile needle to allow air exchange.

3.3. Incubation, control of the environment and cropping

All spawned bags were transferred to a clean and disinfected incubation room and incubated in a complete darkness for 2 to 3 weeks at ambient temperatures (24-30 ^{0}C) on shelves 15cm apart (Dawit Abate, 1998). A dark environment and room temperature of around 24-26 ^{0}C was maintained for both species during incubation to enhance the quick colonization of the substrate. After full colonization of the bags, they were transferred to the cropping room, whose environment was kept illuminated by sunlight through the improvised windows and a temperature and humidity of 28^{0}C and 75-85%, respectively, through adequate watering or by sprinkling the bags with water twice a day. The required humidity and temperature ranges were maintained using a heater and by spraying water on the walls and floors of the cropping room. Formation of a complete mushroom occurs one week after the colonized bags have being transferred into the cropping room (Oei, 2005).

3.4. Experimental Design

The experiment was laid in a 2 x 5 factorial arrangement involving five growth substrates (Millet straw, Broad bean stalk and their three different mixtures) and two edible mushrooms namely: *Pleurotus ostreatus* and *Pleurotus florida* arranged in a Completely Randomized Design (CRD) with three replications. After 34 days of cultivation, fully matured mushrooms of both species grown on each substrate were collected and analyzed for their proximate composition (moisture, crude protein, crude fat, crude fiber, ash and carbohydrate content) and biological efficiency.

3.5. Data Collection

Biological efficiency (yield of mushroom/bag) (%) and Proximate compositions of the edible mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*) were determined according to the methods described in AOAC (1995).

3.5.1. Determination of biological efficiency (Yield of mushroom / bag) (%)

The biological efficiency (BE) of a mushroom was calculated by using the formula recommended by Chang and Miles (1989), Peng *et al.*(2000) as follows:

Where, W2= fresh weight of the harvested mushroom

W1= Dry weight of substrate before inoculation

3.5.2. Determination of moisture content (%)

The moisture content (MC) of the harvested mushroom was determined by the gravimetric method (AOAC, 1995).

One gram of a mushroom grown on each substrate mix ratio was measured separately into a porcelain dish. Then it was dried in an oven at 105^0C for 3 hours, cooled in a desiccator and reweighed. The cooled sample was returned to the oven for further drying. Drying, cooling and weighing were done repeatedly at 1 hr interval until no further reduction in the weight (constant weight) was obtained.

The weight of moisture loss was determined and expressed as percentage of the sample analyzed.
The percentage moisture content (%MC) was generally calculated using the following formula:

Where; MC= Moisture Content

3.5.3. Determination of crude protein content

The crude protein content of the sample was determined by the Kjeldahl method (James, 1995 and Chang, 2003) in which the nitrogen content was priorly determined and then multiplied with 6.25 to obtain the crude protein content. The sampled mushroom species were dried and ground using mortar and pestle and analyzed for crude protein content (CPC). Sample weight (1g) was added into a Kjeldahl digestion flask. Catalyst mixture (Na_2SO_4 was mixed with $CuSO_4$ in the ratio of (10:1) of 1g has also been added into digestion flask followed by the addition of 10ml of concentrated H_2SO_4. Hereafter, the digestion flask was placed in the digester and the temperature was brought to 350^0C. The mixture was then heated until a clear solution was obtained and allowed to cool appropriately.

After cooling of the solution, 30ml of distilled water was added. Then 25 ml of 45% NaOH solution was added into the digestion flask. The contents were distilled immediately by inserting the digestion tube line into the receiver flask that contained 25 ml of 4% boricacid solution in which 3 drops of a mixture of indicators (Methyl red and bromocresol green) were added and about 150 ml of distillate was collected. Finally the distillate was titrated by a standard acid (0.1N HCl).
Percentage of nitrogen was converted to % crude protein by multiplying with 6.25 (AOAC,

1995).

Where, Ws= Weight of sample in g on dry matter basis.
Therefore, % Crude Protein Content (CPC) = 6.25 x % of Nitrogen.

3.5.4. Determination of ash content

Two grams of each processed mushroom sample collected from each substrate mix ratio were dried at 120 ^{0}C for 1 hour in drying oven. Sample dish was removed from the oven and carbonized by a blue flame of Bunsen burner. Ash content was determined by subjecting the carbonized sample at 550^{0}C for 8 hours in a muffle furnace until ashing is complete. At this temperature all the organic matter have been burned off as CO_2, Oxides of Nitrogen and water vapor and the remaining matter was recorded as ash content (AOAC, 1995).

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Where, W1= Weight of empty crucible, W2 = Weight of crucible + ash and
W= Weight of sample

3.5.5. Determination of fat content

The soxhlet solvent extraction method of James (1995) was employed for determination of the fat content. Two grams of each processed mushroom sample was separately wrapped in a porous filter paper and put in a thimble. The thimble was then placed in a soxhlet reflux flask and mounted into a weighed extraction flask containing 200ml of petroleum ether. The upper end of the reflux flask was converted to a condenser. When heated, the solvent was condensed into the reflux flask and allowed to cover the sample until the flask was filled up and siphoned over carrying the oil (fat) extract down to the boiling flask. The process was allowed to go on repeatedly for about 4 hours before the defatted sample was removed and used for crude fat content analysis.

The solvent was recovered while the flask with its oil extract was dried in the oven at 60 ^{0}C for 30 minutes, cooled in a desiccator and re-weighed to obtain the weight of the oil extract (fat), which was then expressed as % of the sample.

The percentage (%) of fat content was calculated using the following formula:

Where, W2 = Weight or mass of flask and fat (oil) extract,

W1 = mass of the extraction flask.

3.5.6. Determination of fiber content

Fiber content was determined through digestion of 2g of each dried, ground (using a mortar and pestle) and fat free sample by boiling in a weak solution of 1.25% H_2SO_4 for 30 minutes. The sample was boiled again in a weak solution of 1.25% NaOH for 30 minutes. Then each species residue was washed with 25-30ml of near boiling water and filtered on to ashless filter paper after each washing and drying. The dry residue was then transferred to ashing dish and ignited at 550^{0}C in a muffled furnace (AOAC, 1990).

The fiber content in % was calculated using the following formula:

Where, W3 = Weight of crucible with dry residue before ashing

W2 = Weight of crucible with ash after ignition

2W1 = Weight of sample used in g on dry matter basis.

3.5.7. Determination of Carbohydrate content

The available carbohydrate content of the mushroom samples was determined by the following equation (Raghuramulu *et al*., 2003):

3.6. Data Analysis

The data collected on proximate composition and biological efficiency of the edible mushrooms were subjected to Analysis of Variance (ANOVA) (Gomez, 1984) with three replications using Statistical Analysis System (SAS) Version 9.0. Means were compared using Duncan`s Multiple Range Test (DMRT) at $p<0.05$.

4. RESULTS AND DISCUSSION

4.1.The Effect of Growth Substrates on Proximate Composition of *Pleurotus ostreatus* and *Pleurotus florid*

Pleurotus ostreatus and *Pleurotus florida* were grown on millet straw and broad bean stalk and their mix ratios. The effects of these growth substrates and their mix ratios on proximate composition of the selected mushrooms were evaluated. The results are summarized in Table 3 below.

4.1.1. Moisture content

The results indicated that the moisture content of *Pleurotus ostreatus* grown on two types of substrates and their combinations ranged from 90.84 to 92.69% (Table 3). Asshown from the same table, there was a statistically significant difference between moisture contents of *Pleurotus ostreatus* grown on different mix-ratios except between those grown on S1 and S2. The maximum (92.69%) and minimum (90.84%) moisture content were however obtained when *P. ostreatus* was grown on S3 and S5, respectively. The present results of the moisture content of different substrates inoculated by *P. ostreatus* disagree with those of Crisan and Sands (1978) who reported that the moisture contents of *Pleurotus ostreatus*-inoculated substrates were in the range of 73.70-90.80% with statistically significant difference among substrates. The difference in the results of this study and those of Crisan and Sands (1978) might be due to differences in environmental factors such as temperature and humidity that prevailed in the study sites which ultimately affected the moisture content of the selected substrates.

Table 3. The proximate composition of *Pleurotus ostreatus* and *Pleurotus florida* grown on different substrate mix-ratios

S	Proximate Composition (%)											
	FiC		FaC		AsC		ProC		MoiC		CarC	
	Po	Pf	Po	Pf	Po	Pf	Po	Pf	Po	Pf	Po	Pf
S1	13.11b	9.64c	4.93b	4.14b	9.85c	7.56b	28.76c	25.63b	92.16b	95.26a	48.81c	42.23 c
S2	10.60d	10.15b	4.73c	3.37c	8.06e	6.89c	32.30b	30.00a	92.03b	90.52c	47.72c	40.94c
S3	9.66e	10.12b	4.91b	3.60c	8.62d	7.02c	27.68c	27.13b	92.69a	90.86c	43.56d	38.73 d
S4	15.15a	12.15a	6.14a	5.05a	10.52b	7.91a	35.72a	31.56a	91.24c	94.41b	58.77a	51.07a
S5	11.81c	11.89a	3.77d	4.69a	11.36a	7.13c	34.86a	31.14a	90.84d	93.73b	52.64b	48.57 b
CV	0.31	2.28	0.54	5.18	0.19	1.75	2.11	2.98	0.22	0.43	1.19	2.39
LSD	0.07	0.46	0.05	0.41	0.03	0.24	1.27	1.63	0.38	0.76	1.13	1.99

CV = coefficient of variation, LSD=least significant difference, S = Substrate, Po = *Pleurotus ostreatus*, Pf = *Pleurotus florida*, FiC = Fiber content, FaC = Fat content, AsC = Ash content, ProC = Protein content, MoiC = Moisture content, CarC = Carbohydrate content; values with different superscripts on the same column (uppercase) are significantly different ($p<0.05$)

Similarly, the moisture content of *P. florida* was shown to vary with different growth substrates and mix ratios. The results of this study indicated that the moisture content of *Pleurotus florida* ranged from 90.52 to 95.26% (Table 3). . The maximum (95.26%) and minimum (90.52%) moisture contents were obtained when *P.florida* was grown on substrates S1 and S2, respectively. As shown from Table 3, *P. florida* grown on S1 increased the moisture content by 4.7, 4.4, 0.9 and 1.5% over those shown when *P. florida* was grown on substrates mix ratios S2, S3, S4 and S5, respectively.

The results of this study are in agreement with those of Hamid *et al*. (1996), who reported that the moisture content of the mushrooms ranged from 80-95%. The similarity of the results might be due to the similarity in the type of substrates and substrate combinations used for growth of *P. florida*.

4.1.2. Protein Content

As indicated in Table 3, the protein content of *Pleurotus ostreatus* was highly significantly ($p<0.01$) affected by the type of growth substrate. The percent crude protein content generally ranged from 27.68 - 35.72. *P. ostreatus* grown on S4 and S5 gave significantly higher crude protein than that grown on other substrates ($P< 0.05$). The lowest crude protein content was obtained from *P. ostreatus* grown on S3.

The results of this study were similar with Chang *et al*. (2003) who reported that the crude protein content of *P. ostreatus* grown on agricultural wastes was in the range of 26.9-37.2%. This similarity could be due to the ability of *P. ostreatus* mushroom to degrade and use the nitrogen content of the selected growth substrates properly and effectively.

The crude protein content of *Pleurotus florida* was also highly significantly ($p<0.01$) affected by the type and combination of growth substrates (Table 3). Protein content of *P.florida* grown on S4 and S5 was significantly higher than that of *P.florida* grown on S1, S2 and S3.

Even though, there is no statistical difference between S2 and S5, the crude protein content of *P.florida* grown on S4 was higher than that of *P.florida* grown on other substrates; and this was followed by the one grown on S5. The lowest crude protein content (25.63%) was obtained from S1.

This result is similar with Crisan and Sands (1978) who reported the crude protein content of *P. florida* as 27%. On the other hand, the results of this study disagree with those of Breene (1990) who reported a crude protein content ranging from 19-39% for Oyster mushrooms.

Therefore, both the similarity and dissimilarity in the result of this study with others concerning the crude protein content could be attributed to the number of factors such as the environmental factors, the mushrooms stage of development and the available nitrogen content of the growth substrates used. Generally, the study indicated that the protein contents of *P. florida* were highly significantly ($p<0.01$) affected by the type of different growth substrates. The lowest crude protein contents i.e. 27.68 and 25.63% were obtained from *P. florida* grown on S3 and S1, respectively.

4.1.3. Total Ash

The ash content of *Pleurotus ostreatus* grown in different mix ratios of millet straw and broad bean stalk showed statistically significant difference ($P\leq0.01$). As can be seen from Table 3, the total ash content of *P. ostreatus* ranges from 8.06 to 11.36 %. The maximum (11.36%) and the minimum (8.06%) ash contents were obtained when *P.ostreatus* was grown on S5 and S2, respectively.

This result is in agreement with that of Oei (2003) who reported 8.8% total ash content for *P. ostreatus* grown on agricultural wastes, but higher than that of Dawit Abate (1998) who reported 7.2% total ash for *Pleurotus* species grown on lignocellulosic wastes. This result was gained due to the ability of the selected mushroom species to degrade and use those substrates effectively. According to Anthony (2007), a number of factors such as the site of growth, the type of substrate used, the developmental stage of the fungal species usually influence nutritional composition of the mushrooms.

The study also showed that *Pleurotus florida* grown on different substrates demonstrated a highly significant ($p<0.01$) difference in ash content (Table 3). The total ash content in *P.florida* grown on different substrates ranged from 6.89 to 7.91 %. The maximum (7.91%) and minimum (6.89%) total ash content of *P.florida* were obtained from S4 and S2, respectively. This result disagrees with Oei (2003) who reported a total ash content of 8.8% for *Pleurotus* species. But this finding is in line with Dawit Abate (1998) who reported the ash content of *Pleurotus* species as 7.2%. The dissimilarity might be due to the carbon-oxygen ratio within the substrates and their types that were used to grow *P. florida.*

Statistical analysis of the data also showed that *Pleurotus ostreatus* and *Pleurotus florida grown* on different substrates significantly varied ($p<0.01$) from one another in ash content. In this study the total ash content of *P. ostreatus* and *P. florida* grown on different substrates ranged from 8.06 to 11.36% and 6.89 to 7.91 %, respectively. The maximum (11.36%) and minimum (8.06%) total ash contents of *P.ostreatus* were obtained from S5 and S2 respectively.

At the same time, the maximum (7.91%) and the minimum (6.89%) total ash contents of *P. florida* were obtained from S4 and S2 respectively. The ash content obtained for *P.ostreatus* which ranged from 8.06-11.36% was in line with the 6.1-9.8% reported by Rai and Crison (1978) but different from Dawit (1998) and Oei (2003) who reported an ash content of 7.2% and 8.8% for *Pleurotus* species respectively. On the other hand, the results obtained from *P. florida* were in agreement with Rai and Crison (1978) who reported 6.1-9.8% ash content and with Dawit (1998) and Oea (2003)who reported ash content of 7.2% and 8.8%, respectively, for *Pleurotus* species.

4.1.4. Crude Fat

A highly significant difference ($p<0.01$) was observed in the fat content of *Pleurotus ostreatus* grown on different substrates (Table 3). The crude fat content of *P.ostreatus* grown on S4 was significantly higher than that of *P. ostreatus* grown on other substrates. The highest (6.14%) and the lowest (3.77%) of crude fat were obtained when *P.ostreatus* was grown on S4 and S5, respectively. *P. ostreatus* grown on S5 had a crude fat content reduction of 2.37%, 1.16%, 1.14% and 0.96% over that recorded from S4, S1, S3 and S2, respectively.

These results disagree with those of Crisan and Sand (1978) and Anthony (2007) who reported that the crude fat content ranged from 1.6-2.2% and 0.6-3.1%, respectively, for *P.ostreatus.* The dissimilarity might be due to the type of substrate and substrate combinations used to grow the selected *P. ostreatus*.

The crude fat content was also significantly varied ($p<0.01$) when *Pleurotus florida* was grown on different substrates (Table 3). *P.florida* grown on S4 resulted in a significantly higher crude fat content than on any other substrate. Even though, there was no statistically significant difference between the crude fat content of *P.florida grown* on S4 and S5, the second highest fat content was recorded from *P.florida* grown on S5. The maximum crude fat content was obtained from *P.florida* grown on S4 (5.05%) while the lowest crude fat content was obtained on S2 (3.37%). *P.florida* grown on S2 had a crude fat content reduction of 0.77, 0.23, 1.68% and 1.32% from S1, S3, S4 and S5, respectively. These results disagree with those of Crisan and Sands (1978) and Anthony (2007) who reported a crude fat content ranging from 1.6-2.2%, and 0.6-3.1%, respectively for *Pleurotus* species.

As can be seen from Table 3, the crude fat contents were highly significantly ($p<0.01$) affected by the type of substrate. The highest and the lowest crude fat contents (6.14 and 3.77%) were obtained when P. *ostreatus* was grown on S4 and S5, respectively. On the other hand, in *P.*

florida, the highest and the lowest crude fat contents (5.05 and 3.37%) were obtained on S4 and S2 respectively.

4.1.5. Crude Fiber

The crude fiber content of *Pleurotus ostreatus* is shown in Table 3. Statistical analysis of the data indicated that there was a highly significant (p<0.01) difference among the crude fiber contents of *P. ostreatus* grown on different substrate mix ratios. In the present study, the maximum (15.15%) fiber content was obtained from *P. ostreatus* grown on S4 followed by S1 (13.11b %), while the lowest fiber content was obtained on S3 (9.66%). *P. ostreatus* grown on S4 showed in 2.04, 4.55, 5.49 and 3.34% increase in crude fiber content over S1, S2, S3, and S5 respectively (Table3).

This result is in agreement with that of Obodai (1992) who reported 7.5-16.5% crude fiber content for *Pleurotus* species. This similarity might be due to the ability of *P. ostreatus* to degrade and use the substrates properly.

The crude fiber content of *Pleurotus florida* is also shown in Table 3. As in the fiber content of *P. ostreatus*, analysis of variance indicated that the fiber content of *P. florida* was highly significantly (p<0.01) affected by variation in growth substrates (Table 3). The highest crude fiber content (12.15%) was obtained when *P. florida was* grown on S4 followed by S5 (11.89%). But when observed on S4 and S5, the results of the crude fiber content were not statistically different from one another. The lowest crude fiber content was obtained when *P. florida* was grown on S1 (9.64%). As can be seen fom Table 3, growth of *P. florida* on S4 has improved crude fiber content by 2.51, 2.00, 2.03 and 0.26% over *P. florida* grown on S1, S2, S3, and S5, respectively. The present study revealed that the crude fiber content of *P.florida* varied from 12.15-9.64% (Table 3). This result was similar with that of Obodai (1992)and Crisan and Sands (1978) who reported 7.5-16.5% and 11.5% crude fiber content in *P. florida*, respectively. These findings could have been attributed to the ability of *P. florida* to degrade and use the selected substrates and substrate combinations efficiently.

As in the case of *P. ostreatus*, analysis of variance indicated that the fiber content of *P. florida* was highly significantly *(p<0.01)* affected by application of different growth substrates. The highest crude fiber contents (15.15 and 12.15%) were obtained when *P. ostreatus* and *P. florida* were grown on S4. The lowest crude fiber content (9.66 and 9.64%) was obtained from *P. ostreatus* grown on S3 and *P. florida* grown on S1. The difference in crude fiber content was due to the variations in substrate and substrate combinations used for cultivation of the selected *Pleurotus* species.

4.1.6. Carbohydrate Content

The carbohydrate content of *Pleurotus ostreatus* grown on two types of substrates and substrate combinations was also evaluated. The results are shown in Table 3. As can be seen from the table, the carbohydrate contents of *P. ostreatus* grown on different substrate mix ratios ranges from 43.56 to 58.77%. The highest (58.77%) and the lowest (43.56%) values correspond to carbohydrate contents of *P. ostreatus* grown on S4 and S3, respectively. The second highest carbohydrate content was obtained when *P. ostreatus* was grown on S5. The results of this study are in agreement with those of Bernas *et al*. (2006) who reported that the total carbohydrate content for *Pleurotus* species range from 16-85%. It is also in agreement with Bano and Rajarathnam (1982) who reported 45-77% total carbohydrate content for *P. ostreatus*. This result was obtained because of the ability of the selected mushroom species to degrade the substrate and use it efficiently.

The carbohydrate contents of *P. florida* grown on different substrates showed highly significant (p<0.01) difference (Table 3). As shown from the table, the carbohydrate content of *P.florida* grown on different substrates ranges from 38.73 to 51.07%. The lowest and the highest percentages of carbohydrate content in the range were obtained from *P. florida* grown on S3 and S4, respectively. These results were similar to those of *P.ostreatus* grown on substrate 1 and 2 and they are in agreement with those of Bernas *et al.* (2006) who reported that the total carbohydrate contents for *Pleurotus* species range from16-85%. The similarity could be due to the ability of the selected mushrooms to degrade and use those substrates and substrate combinations effectively.

4.2 The interaction effect of substrates and varieties of mushrooms on different parameters (proximate compositions)

The number of factors such as the site of growth, the type of substrate used, the developmental stage of the fungal species usually influence nutritional composition of the mushrooms (Anthony, 2007). When different varieties of Oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus florida*) were grown on various substrates (broad bean stalk and millet straw) and their different mix ratios, the proximate composition of the mushrooms would show variation.

In factorial experiment, the interaction effect was shown in table 4, and there was highly significant difference among substrate types with the interaction of varieties of mushrooms in their proximate composition. The mean separation result showed that, the interaction of S4 with that of V1 has recorded maximum fiber, crude fat, protein and carbohydrate content than the other interaction effect, and also the interaction of S5 with V1 has resulted the highest total ash and protein content than the other interactions. Additionally, the interaction of S1 with V2 has resulted the highest moisture content than the other interactions.

On the other hand, the interaction of S1 with V2 has resulted the least crude fiber content and protein content. Simultaneously, the interaction of S2 with V2 has resulted the least crude fat, total ash and moisture content than other interactions. Therefore, this result indicates that the proximate composition of mushrooms varies due to the interaction of the mushroom varieties with the substrate types and substrate mix ratios on which they were grown.

4.3. The Effect of Growth Substrates on Biological efficiency of *Pleurotus ostreatus* and *Pleurotus florida*

The effect of substrate type and substrate mix-ratio on the biological efficiency (yield) of *Pleurotus ostreatus* and *Pleurotus florida* was evaluated and the results are shown in Table 4.

Table 4. The biological efficiency of *Pleurotus ostreatus* and *Pleurotus florida* cultivated on millet straw and broad bean stalk with their various mix ratios.

Substrate (S)	*Pleurotus ostreatus*			*Pleurotus florida*		
	Fresh weight (W2)	Dry weight (W1)in g	BE (%)	Fresh weight (W2)	Dry weight (W1)in g	BE (%)
S1	331.75	500	66.35c	278.55	500	55.71e
S2	373.05	500	74.61a	292.15	500	58.43c
S3	343.55	500	68.71b	299.8	500	59.96b
S4	272.8	500	54.56d	283.3	500	56.66d
S5	218.08	500	43.61e	320.05	500	64.01a
CV			2.70			1.26
LSD			3.14			1.40

S=Substrate, W1=Dry weight, W2=Fresh weight. BE=Biological Efficiency, CV=Coefficient Variation, LSD=Least Significant difference

As can be seen from table 5, for *P. ostreatus*, the highest biological efficiency (yield) (74.61%) was obtained from S2. The second highest value of biological effiency for *P.ostreatus* was obtained on S3 and S1 respectively. The BE of *P.ostreatus* grown on S2 had an 8.3, 5.9, 20.1 and 31.0 increase over the BE of *P.ostreatus* grown on S1, S3, S4 and S5, respectively. The lowest BE (yield) of *P.ostreatus* was obtained from S5. The findings of this study were in agreement with those of Patra and Pani (1995) who reported that the biological efficiency of *Pleurotus* species grown on most of agro-industrial residues was ranging from 50-75%. This result might be obtained due to the type of substrates used and environmental factors of the area in which the selected mushroom species were grown.

In contrast the highest biological efficiency of *Pleurotus florida* was obtained on S5 (64.01%). The BE of *Pleurotus florida* was highly significantly ($p<0.01$) affected by the substrate type and combination of substrates (Table 5). The lowest (55.71%) biological efficiency (yield) of *P.florida* was obtained from S1. The BE of *P.florida* grown on S5 had an 8.3, 5.6, 4.1 and 7.4% increase over those obtained when grown on S1, S2, S3 and S4, respectively.

From this study, it was observed that the highest yield was obtained from *Pleurotus ostreatus* that was grown on S2 followed by S3 and S1, and *Pleurotus florida* grown on S5 followed by S3 and S2 respectively. To the contrary, the minimum yield of *Pleurotus ostreatus* was obtained from S5 followed by S4. In the meantime, the minimum yield of *Pleurotus florida* was obtained from S1 followed by S4.

Therefore, this study shows that, in terms of the mushroom variety, *Pleurotus ostreatus* was the most nutritious mushroom when compared with *Pleurotus florida*. In terms of substrate mix, S2 provides the highest yield of *Pleurotus ostreatus*, and S5 provides the highest yield of *Pleurotus florida*. Thus, to cultivate *Pleurotus ostreatus*, S2 and S3, and to cultivate *Pleurotus florida*, S5 and S3 are the most decomposable and suitable substrate mixes to provide the highest yield in comparison with the other substrates used.

These results are similar with those of Patra and Pani (1995) who reported that the biological efficiency of *Pleurotus* species grown on most of agro-industrial residues was ranging from 50-75%. The observed findings might be the result of the type of substrates used and the environmental factors of the area in which the selected mushroom species were growing.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary and Conclusion

Cultivation of selected *Pleurotus* species was carried out to obtain edible mushroom production from substrate and substrate combinations of millet straw and broad bean stalk at five different treatments with different ratios in a completely Randomized Design (CRD) with three replications. The type of mushroom species, and the growth substrates significantly ($p<0.05$) affected crude fiber content, crude fat content, total ash content, crude protein content, moisture content and carbohydrate contents of the selected mushrooms.

Throughout this investigation, the maximum crude fiber was obtained from *Pleurotus ostreatus* and *Pleurotus florida* grown on S4, the maximum crude fat was obtained from *Pleurotus ostreatus* and *Pleurotus florida* both grown on S4. The maximum total ash content was recorded from *Pleurotus ostreatus* grown on S5 and *Pleurotus florida* grown on S4. The highest crude protein was recorded from *Pleurotus ostreatus* and *Pleurotus florida* both grown on S4. The maximum moisture content was obtained from *Pleurotus ostreatus* grown on S3 and *Pleurotus florida* grown on S1. The highest Biological efficiency was gained from

Pleurotus ostreatus grown on S2 and *Pleurotus florida* grown on S5, and then the maximum carbohydrate was recorded from *Pleurotus ostreatus* and *Pleurotus florida* both grown on S4. This shows that, in this study, *Pleurotus ostreatus* was the most nutritious when grown on these substrate and substrate mixes. Concerning the substrate types, S4 alone is the most decomposable and suitable substrate in comparison with the others for cultivation of the selected *Pleurotus* species.

In conclusion, the type of mushroom species, the growth substrates or substrate combinations can affect nutritional value of mushrooms. Despite their differences in the nutritional composition, the overall nutritional potential of the mushrooms was quite good. Moreover, the agricultural wastes such as Broad Bean Stalk and Millet Straw can be used for the cultivation of *Pleurotus* species. If other suitable substrates for mushroom cultivation are not available, Broad Bean [*Vicia faba (L)*] Stalk and Millet [*Eleusine coracana (L)*] Straw are good opportunity and easily available for those people who can't get other optional substrates.

5.2. Recommendations

Based on the findings of the study, the following recommendations are made:

- Further study should be made to develop more refined techniques to improve the yield of *Pleurotus ostreatus.*
- Efforts should be made to create awareness among the Ethiopian population on the importance and feasibility of cultivation of *Pleurotus ostreatus* using broad bean stalk and millet straw.
- Improved methods of substrate preparation and pretreatment techniques should be developed to enhance the degradability of millet straw and use it as an inexpensive substrate for the cultivation of edible *Pleurotus* species.
- Further studies should be made to determine the amino acid and mineral composition of *Pleurotus ostreatus* grown on agro-industrial wastes.
- Finally, further detailed studies must be made to investigate methods of production of

quality spawn.

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APPENDEX

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