Addis Ababa University (Since 1950)

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

Department of Microbial, Cellular and Molecular Biology

(Microbiology Stream)

Cultivation of Medicinal Mushroom: *Schizophyllum commune*

BY

WOINESHET ASSEFA

A Thesis Submitted To The School Of Graduate Studies In Partial Fulfillment Of The Requirements For The Degree Of Masters Of Science In Applied Microbiology

Addis Ababa, Ethiopia

June, 2014

Acknowledgements

I would like to express my deepest gratitude to my advisor Dr. Dawit Abate for his invaluable guidance and encouragement as well as critical comments and suggestions throughout this study.

My appreciations also go to Addis Ababa University, Department of Microbial, Cellular and Molecular Biology, for providing facilities and granting research fund to conduct this research.

I am also very thankful to Mycology laboratory technician, Zenebech Aytenew and my best friends Melete Berehe, Seniyet Dangi, Woinshet Alemu ,Yeshmebet Endale and Abate Tebebu for their unreserved contribution in one way or another for the success of my study.

I would like to extend my deepest gratitude to my husband, my kids and my family for their support and encouragement throughout my study.

Finally, my dedication goes to my late beloved brother Bekure Assefa always in my heart, whose love and support sustained me through.

Table Contents

List of Figures

Figure № **Title** **Pages**

List of tables

List of Abbreviations

BE …………………………………………… Biological Efficiency

JMP …………………………………………. John's Macintosh Project

MEA…………………………………………. Malt Extract Agar

PDA…………………………………………. Potatoes Dextrose Agar

SCP………………………………………….. Single Cell Protein

SDA………………………………………… Subordinate Dextrose Agar

SSC………………………………………… Solid-state Cultivation

SSF…………………………………………... Solid State Fermentation

USDA………………………………………… United States of Departmental of Agriculture

Abstract

Cultivation of edible and medicinal mushrooms on lignocellulosic wastes represents one of the most economically and cost effective organic recycling processes. Solid-state cultivation (SSC) was carried out to evaluate the suitability of using the logs of *Eucalyptus globulus*, *Cupressus lusitanica*, *Gravillea robusta*, *Acacia abyssinica*, coffee husk and cotton seed and the logs of Eucalyptus globulus, Cupressus lusitanica, Gravillea robusta and Acacia as substrates for cultivation of *S.commune*. The number of flushes, yield and biological efficiency of the *S.commune* grown on Eucalyptus, Cupressus, Gravillea and Acacia spp. were studied. *S.commune* recorded at least four flushes on all the substrates used and supported fruiting bodies formation on (Eucalyptus, Cupressus, Gravillea and Acacia). Flush 1 gave the highest yield in g/kg while flush 4 gave the lowest yield. Significant differences ($p<0.05$) in yield and % biological efficiency (B.E.) of the *S.commune* were recorded on each substrate. The results also showed that Eucalyptus spp. gave the highest B.E of (58.10%), followed by Cupressus (46.20%). Acacia gave relatively a low, B.E. of (32.55%). There was significant difference at ($p<0.05$) observed between each substrates on yield and B.E. Eucalyptus waste was more efficient than the others used substrate. The cultivation of *S.commune* on the logs of Eucalyptus, Cupressus, Gravillea and Acacia showed mycelial coverage and formations of small pinhead-like structures which didn't grow in to mushrooms. While, coffee husks and cotton seed didn't support the production of fruiting bodies and formations of this small pinhead-like structures except mycelia growth. The implications of this study are that, the suitability of Eucalyptus, Cupressus, Gravillea and Acacia with supplementary substrate to cultivate *S.commune*, for future utilization of its products for medicinal application.

Keywords: lignocellulosic wastes, mushroom cultivation

Introduction

Schizophyllum commune (Fries) is an edible mushroom which belongs to the phylum Basidiomycetes, order Agricales family Schizophllaceae (Adejoye .,*et al* 2007). With having more than 28,000 distinct sexes (Ouglals, 2006), it is probably the most widespread fungus in existence, being found on every continent except Antarctica, where there is no wood to be used as a substrate (Ohm *et al.*, 2010; Teoh *et al.*, 2011). The fungus usually grows abundantly during the rainy season and frequently appears on dead wood. Hence, it has a worldwide distribution on deciduous branches and fallen trees, and on lumber made from the same growing throughout the year above freezing temperatures (Paul, 2002).

The fungus genus name is called "split gill" making it named as split gill fungus due to the fact that the gills function to produce basidiospores on their surface and they appear to be split because they can dry out and rehydrate (thus open and close) many times over the course of a growing season. Thus it is sexually reproducing fungi where by the cap is shell-shaped, with the tissue concentrated at the point of attachment, resembling a stem. It is often wavy and lobed, with a rigid margin when old. It is tough, felty and hairy, and slippery when moist. It is grayish white and up to 4 cm in diameter. The gills are pale reddish or grey, very narrow with a longitudinal split edge which becomes enrolled when wet. In addition the fruiting bodies have great adaptation to for a climate with sporadic rains. Unlike other mushroom species, the mycelium only has to produce one set of fruiting bodies per year, which can then dry out and rehydrate and keep functioning, a great strategy for reproduction which is one of the major reasons responsible for the cosmopolitan distribution of the fungus.

With regards to the nutritional metabolism of the fungus, it is also called 'white rot' fungus that can degrade complex plant biomass, including lignin whereby, plant cell wall material is degraded into simple sugars, which are subsequently fermented into useful products such as organic acids. These compounds can be further converted by enzymes or catalysts into industrially useful chemicals. In addition, the fungus was reported as the common invader of rotten wood, which was able to colonize and cause severe infections in humans. However, this fungus exhibit other properties, such as tolerance to the fungicide benomyl, susceptibility to high concentrations of cyclohexamide, a dolipore-type septum and mating tests (Guarro, 1999).

The medicinal use of *Schizophyllum* species has been widely reported in recent years. *S.commune* has been extensively studied and its genetic background is well known (Wessels, 1993). It has been recently reported that *Schizophyllum* spp has numerous potential applications, such as thickener for cosmetic lotions, act as oxygen-impermeable films for food preservation, biological response modifier and a non-specific stimulator of immune system and enhance the effect of vaccines and anti-cancer therapies and also anti-tumor agent (Hao *et al.,* 2010). However many people who use this fungus were ignorant of its nutritional value since it serves as an important source of protein, vitamins, lipids and mineral elements for those who value the mushroom (Adejoye *et al* ., 2007). The other potential challenge in utilizing the benefits of this mushroom is that most of the *Schizophyllum* spp are seasonal and they are not available all year round. It is with this knowledge gap that this study was initiated to make the fungus available to consumers throughout the year. There has been growing interest in cultivating this mushroom with the intended objectives of using its nutritive and medicinal value. Optimization of the physical and cultural Eucalyptus parameters is one of the main investigation by which acceleration of mycelia growth of the mushroom under varying environmental conditions, such as solution pH, incubation temperature and agitation speed have been investigated (Teoh and Don., 2012). Furthermore, the pH of the growth medium played an important role by inducing morphological changes in the organism in which the pH change was observed during growth of the organism which affected product in the medium (Teoh, 2010).

In fact, the unfavorable reactions within alkaline or acidic condition could be attributed to the loss of nutritive value of protein and formation of potentially toxic substances such as lysinoalanine and thus influencing the growth of fungi were observed as well (Liu, 2008). In addition to this, temperature is probably one of the important environmental factor determining the fungal growth by which, most of the well-characterized microorganisms live best at temperature from 25-40°C, many thrive at high temperature and others might growth best (although slowly), at 0-15°C (Rousk, 2011). Biological efficiency is a term frequently used in the mushrooms industry to describe yield potentials of mushrooms from various agricultural by-products. The biological efficiency of cultivated mushrooms depends on the types of substrate used, the types of species/isolates employed (genetic nature), spawn type and prevailing mushroom growing conditions (Peyvast, 2008).

Based on the above mentioned facts, this work was carried out to evaluate the suitability of using sawdust of Gravillea, Acacia, coffee husk and cotton seed and the logs of Eucalyptus, Cupressus, Gravillea and Acacia spp as substrates for the production of *Schizophyllum commune* by using the plastic bag method of cultivation, Optimization of the culture parameters including P^H, temperature and agitation speed for maximum mycelia growth of *Schizophyllum commune,* to evaluate the growth parameters including measurement of the biological efficiency of the fungus, to identify and select the substrate mixture that provides the highest biological efficiency and thus its sustainable use for medicinal purpose.

2. Objectives of the study

2.1. General Objectives

- The general objective of this study was to cultivate the medicinal mushroom, *Schizophyllum commune* under the optimal conditions for the growth and fruiting of the mushroom.

2.2. Specific objectives

- The specific objectives of the current work were to evaluate the suitability of using logs and sawdust as substrates for the production of *Schizophyllum commune*
- To optimize the culture parameters including p^H, temperature and moisture content for maximum mycelia growth of *S.commune*
- To evaluate growth parameters including measurement of the biological efficiency of the fungus
- To determine the substrate mixture that provides the highest biological efficiency.

3. Literature review

3.1 Use of wild mushroom as food and medicine

Wild mushrooms with their delicate flavour and texture are recognized as a nutritious food and an important source of biologically active compounds with medicinal values. Generally, mushrooms are low in energy and high in dietary fibre (Emilia, *et al*., 2006), and an excellent source for antioxidants as they accumulate a variety of secondary metabolites, including phenolic compounds (Jose and Janardhanan, 2000; Cheung, *et al*., 2003). Antioxidants are substances needed in minute quantity which are capable of counteracting with free radicals to prevent oxidative damage. There are about 1200 species of mushroom used in 85 different countries for their gastronomic value and/or medicinal properties (Roman, *et al*., 2006).

Edible wild mushrooms are often regarded as being nutritionally high and with potential economic value. Many species with medicinal value are widely used in traditional medicine for a broad range of diseases (Roman, and Woodward, 2006). Some species are regarded as therapeutic food for their anti-carcinogenic, anti-cholesterolaemic and anti-viral properties.

3.2. World mushroom production

The use of mushrooms as food is probably as old as civilization itself (Chaube, 1995) and mushrooms have been treated as a special kind of food (Tripathi, 2005). The Chinese were the first to grow mushroom for human consumption. As early as 600 AD, varieties of *Auricularia* were being cultivated. Around 1000 AD, *Lentinula eddoes*, commonly known as shiitake, entered mushroom farming practices by early 17th century (Tripathi, 2005). Cultivation in France began with *Agaricus* (Ivors, 2003).Mushroom production quickly spread to England and other European countries, reaching the united state by the end of the 19th century (Flegg, *et al*., 1985).

In the last 25 years, worldwide mushroom production has increased over 30%, reaching approximately 2,961,493 tons in 2002 (USDA, 2006).China has become the top producing nation for all edible mushrooms, turning out over 40% of the world's supply (USDA, 2006).The U.S. is the next largest producer of mushroom, contributing about 13%, while the Netherland and France produce about 9.5% and 5% respectively (USDA, 2006). Overall U.S. production by volume has been steadily rising over the last decade. Operations are also diversifying, adding production of various edible mushrooms. Industry expansion, in both output and diversity, is largely due to improvements in cultivation technologies and the expansion of market demand (Yamanaka, 1997).

Cultivation methods for edible mushroom vary considerably around the world. Methods primarily depend on the type of mushroom. While the species of Agaricus, which include the white button mushroom, portebello and crimini, require composted substrate, white-rot fungi (*Schizophyllum commune*) can be cultivated on non-composted organic materials. The majority of the specialty mushrooms are the white-rot fungi; including shiitake (*Lentinula edodes*), wood ear (*Auricularia* spp.), paddy straw mushroom (*Volvariella volvacea*), oyster mushroom (*Pleurotus* spp.) and many others. Shiitake and wood ear mushroom are known to grow best on hardwoods, while paddy straws, like their name indicate, grow best on straw. Oyster mushrooms and *Schizophyllum commune* are renowned for their ability to grow well on a wide array of substrates (Yamanaka, 1997).

Technological developments in the mushroom industry in general have witnessed increasing production capacities, innovations in cultivation technologies, improvements to final mushroom goods, and utilization of mushrooms' natural qualities for environmental benefits. However, there is always the need to maintain current trends and to continue to seek out new opportunities. The challenge is to recognize opportunities such as increasing consumption capabilities with the increase in world population and to take advantage of this by promoting the consumption of mushrooms (Chang, 2006).

Production of mushrooms worldwide has been steadily increasing, mainly due to contribution from developing countries such as India, China and Vietnam. There is also increasing experimentally based evidence to support centuries of observation regarding the nutritional and medicinal benefits of mushrooms. The value of mushrooms has recently been promoted to tremendous level with medicinal mushrooms trial conducted for HIV/AIDS patients in Africa, generating encourages result (Chang, 2006).

Now a day, mushroom is being cultivated in more than 100 countries with an estimated annual product of around 6.6 million tons. The Europe shares about 55%, North America about 27% and Eastern Asia produces about 14% of world production. Remaining percentage of mushroom is produced in other parts of the world (Tripathi, 2005).

	Ranks	Mushrooms production quantity (tons)	Years
China	1	7,959,979	2012
Italy	2	5,158,810	2012
United States	3	785,000	2012
Netherland	4	388,450	2012
Poland	5	307,000	2012
Spain	6	220,000	2012
France	7	146,000	2012
Iran	8	116,574	2012
Canada	9	87,675	2012
United Kingdom	10	82,000	2012

Table: 1 Mushrooms production quantity (tons) - for most countries (FAOSTAT, 2012)

3.3. Wild *Schizophyllum commune* as medicinal value

Numerous types of mushrooms exist in nature; however, less than 25 species are widely accepted as therapeutic food and only a few have attained the level of an item of commerce (Mau *et al*., 2002). The authors further noted that, mushrooms are a good source of protein and minerals. And some investigators have even contended that the amino acid compositions of mushrooms are comparable to animal proteins (Nakano *et al*., 1996). Mushrooms are one of the many foods from the wild which are found in the diet of the various Naga tribes in Northeast India. Normally mushrooms are consumed fresh but the use of dried mushrooms during the off season is not uncommon. Of the varieties of mushrooms from the wild that are used by the Nagas as food source, *Schizophyllum commune* is used fresh as well as in the dry forms during offseason(Mau *et al*., 2002).

Different medicinal (anticarcinogenic, anticholesterol, immunostimulating) effects of *Schizophyllum commune* are known but little is known about its nutritive values (Nakano *et al*., 1996). Edible wild mushrooms are often regarded as being nutritionally high and with potential economic value. Many species with medicinal value are widely used in traditional medicine for a broad range of diseases (Roman, and Woodward, 2006). Some species like *Schizophyllum commune* are regarded as therapeutic food for their anti-carcinogenic, anti-cholesterolaemic and anti-viral properties.

3.4. Mushrooms cultivation in Ethiopia

Ethiopia has favorable climate, comparatively abundant land and labor, as well as reasonably good water resource that can creat ample opportunities for horticulture production (Kiflemariam, 2008). The range of altitude, temperature and soil variability of the country has created an enormous ecological diversity and a huge wealth of biological resources. However, the production and utilization of mushroom in Ethiopia is neglected. As a result this country is not benefited from mushrooms as the rest of the world (Kiflemariam, 2008).

Mushroom growing is one of the most science-based branches of agriculture and horticulture. It is a large, sophisticated, competitive and capital intensive industry. Together with these, lack of concept and skill on production technology, lack of research, extension and adaptation work, lack of appreciation about the food and dietary importance of mushrooms, low level of information supply both on production marketing aspect and the monotonous traditional diet and the conservative eating habit of the people may be a few of the impairments that constrained the introduction of the industry in to the country. Nevertheless, presently, there is a considerable level of awareness and interest among certain groups of the society for mushroom production. As part of its agricultural research and extension work, the Haramaya University has initiated a project on "Development and Transfer of Mushroom Production Technology to Rural Poor and Marginal Farmers of Ethiopia" in 2004/05 (Kiflemariam, 2008).

Despite the high diversity of wild edible mushrooms in Africa including Ethiopia, very little of it is known. 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 (Olumide, 2007).

In developing countries, governmental and non-governmental organizations have not given due 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 mushroom cultivation practice in the country to fill the demands of people interested in the 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. The current study was, therefore, initiated to assess the suitability of different locally available substrates and their combinations for cultivation of *P.ostreatus* and to estimate yields of cultivated mushrooms on different locally available cheap substrates(Olumide, 2007).

3.5. Potential uses of agro-industrial residue

For many agro-industrial wastes, there is a continuous effort to find ways of ecological utilization in order to avoid the current practice, which for the vast majority of agricultural wastes continues to be land disposal with or without any prior treatment of the waste. Various utilization processes which have appeared applicable to a variety of agro-industrial wastes may be indicated as: composting, drying and dehydration, by-product development (i.e, biomass) production of food ingredients and enzymes, methane generation and water reclamation (Israilides, 2003). However, the cultivation of edible mushrooms represents the only current, large-scale, controlled application of microbial technology for the profitable conversion of waste lignocellulosic residues from agriculture or forestry. From each kg cellulose and/or lignin containing dry waste, one can obtain one kg fresh mushrooms (Israilides, 2003). Moreover, 330 kg of dry waste becomes 1000 kg after moistening, and from this prepared substrate 200 to 300 kg of mushrooms can be obtained, i.e. bioconversion efficiency (BE) 20-30 % (Zervakis, 2000). Mushroom cultivation not only reduces disposal problems caused by residue accumulation, but also provides an economically acceptable alternative for the production of high quality food and fodder through mushroom production and high value-added metabolites like enzymes or polysaccharides, which may contribute significantly to the increase of the farmers' income (Zervakis, 2000).

Mushrooms are grown on some organic substrates, mostly waste materials from farms, plantations or agro factories. These otherwise useless by-products can therefore be recycled to produce value-added mushrooms. Currently, millions of tons of agricultural wastes are discarded, burned and neglected. In the process of mushroom growing, however, environmental pollution from such practices may be reduced (Bhawna, 2003). Examples of such agro-industrial wastes in abundance in the tropics are straw,corn cobs, grass, sawdust, sugarcane bagasse, cotton waste, oil palm waste, coffee husk , coconut husks, tree leaves, branches and logs. These all can be used alone or in combination to create mushroom growing substrate. With moderate effort and careful management, the very people hungry for food can get a new food source in the form of cultivated mushrooms. Development of agricultural technology has to pace with the food production forever increasing population and to degrade agricultural wastes without polluting the environment (Bhawna, 2003).

Several processes have been developed that utilize these as raw materials. Solid state fermentation (SSF) has emerged as an appropriate biotechnology for the management of these agro-industrial residues and for their value addition. These substrates are used under SSF for the production of bulk chemicals and value-added fine products such as ethanol, single cell protein(SCP), mushroom, enzymes, organic acids, amino acid, aroma compounds, biologically active secondary metabolites (Fan *et al*.,2000).

3.6. Chemical composition and function of supplementary substrates

3.6.1. Wheat bran

It is a byproduct of flour making industries. Wheat bran consists of a high amount of starch and other nutrients. Thus it is an important additional substrate in mushroom cultivation. It is also a common supplement in spawn preparation (Dawit, 1998).

3.6.2. Gypsum

Gypsum as substrate additive for mushroom cultivation acts as a buffer and helps to maintain proper pH level of the substrate.

3.7. Mushroom product from agricultural wastes

Mushroom is an attractive crop to cultivate in developing countries for many reasons. One of the most charming points would be that they are grown on agricultural wastes. It enables us to acquire substrate materials at low prices or even for free and to conserve our environment by recycling wastes. Presently three mushrooms namely *Pleurotus* species (Oyster Mushroom), *Volvariella volvaceae* (Straw Mushroom) and *Auricularia* species (Ear Mushroom) are under commercial cultivation (Chang & Miles, 1991). Commercial production of fresh edible mushrooms on agricultural wastes is a rapidly-growing industrial activity. During the period 1990-1994, world mushroom production increased by 30.5%, reaching about 4,909 thousand tons in 1994. The white button mushrooms have the highest consumer preference and account for about 90 percent of total mushroom production (Chang & Miles, 1991).

Compost or non-compost agricultural residues such as wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat barn, rich husk, sawdust and others can be used as substrate for growing mushroom. Cultivation methods for edible mushroom vary considerably around the world depending on the type of mushrooms. While the species of *Agaricus*, which include the white button mushroom, portebello and crimini, require composted substrate, white-rot fungi (*Schizophyllum commune*) can be cultivated on non-composted organic materials. Of the three cultivated species, oyster mushrooms (*Pleurotus* spp.) can utilize various kinds of substrate materials when compared to other mushrooms covering 16% of the total worldwide edible mushroom production. The worldwide survey on possible substrate for oyster mushroom, will illustrate oyster mushroom can be grown on "almost all types of available wastes." (Yamanaka, 1997) conclude that about two thousand (200) different wastes are available as oyster mushroom substrate.

Since mushroom cultivation practice does not always require access to land (that is, space conserving) and any significant capital investment, it is a viable and attractive activity for rural, peri-urban and urban dwellers. Mushroom cultivation is suitable for all job seeking groups including elders, disabled and youngsters. Although mushroom cultivation is labor intensive, this may not be a problem of tropical regions (FAO, 2009).

In addition, mushroom cultivation rives towards full use of all materials in which nothing left as waste, 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 (FAO, 2009). Currently, 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 (FAO, 2009).

3.8. Growth requirement and condition for mushroom production

The duration of different growth stages of cultivated mushrooms depends on the type of substrate and substrate formulation (composted or non composted or spent mushroom substrate and supplements) used, the type of species and/or the strain employed, spawn type and the level spawning rate applied, as well as on the prevailing mushroom growing conditions (Peyvast, 2008).

Schizophyllum commune mushroom grew well in acidic conditions and pH 5 was the most favorable (Nwokoye *et al*., 2010). Hamada, glucose peptone, Hennerberg, potato dextrose agar and yeast malt extract were favorable media for growing mycelia, while Lilly and glucose tryptone were unfavorable. Dextrin was the best and lactose was the less effective carbon source. The most suitable nitrogen sources were calcium nitrate, glycine, and potassium nitrate, whereas ammonium phosphate and histidine were the least effective for the mycelia growth of S. commune. A suitable temperature for mycelial growth was obtained at 30^0C (Nwokoye *et al*., 2010).

For fruiting body formation, CO_2, light and temperature are key environmental factors. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.006%), the stipe elongates and the growth of the caps will be prevented (Inamulhaq *et al.*, 2010). The requirements for light are different for the various stage of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place (Inamulhaq *et al.*, 2010).

3.9. Substrate optimization

Schizophylum.commune grows in nature on dead wood as saprophytes. Moisture content in substrate is a very important factor for the growth, development and yield of mushroom. Appropriate moisture content in substrate, suitable relative humidity and temperature in growing room (Green house) are necessary for good yield (Nerona and Latiza 1990). Different moisture levels in different substrates under different environmental conditions have been tested by researchers (Yoshida *et al.* 1993).

3.10. Classification and distribution of Schizophyllum commune mushrooms

Schizophyllum commune (Fries) is an edible mushroom which belongs to the phylum Basidiomycetes, order Agricales family Schizophllaceae (Adejoye *et al* 2007). Schizophyllum commune, a basidiomycete with a tetrapolar life cycle, has been used in a number of studies on differentiation and development. It possesses a relatively simple life cycle with several distinct developmental events and a usable genetic system (Ohsugi, 2001). Homokaryotic vegetative mycelia of the proper mating type fuse to form a binucleate dikaryotic mycelium. Under the appropriate environmental conditions, fruiting bodies (basidiocarps) are formed from the dikaryon. These yield haploid meiotic products (basidiospores) which germinate to complete the life cycle. This investigation concerns a change in enzyme activity associated with the formation of the fruiting body (Adejoye *et al* 2007).

The life cycle of most basidiomycetes encompasses two distinct phases: those of the monokaryon and the dikaryon. Initially, a meiotic haploid spore germinates, giving rise to a mycelium with uninucleate cells, the monokaryon. This mycelium can grow vegetative and, when it meets another monokaryon of the same species, hyphal fusions occur between the two mycelia (figure 1a). At that moment fertilization of the mycelium can occur. In most mushroom-forming basidiomycetes, fusion is followed by exchange of nuclei but not cytoplasm (May and Taylor 1988), resulting in a mycelium with binucleate cells, the dikaryon. Nuclei migrate from the contact zone through the whole receiving mycelium (Gladfelter & Berman 2009). The exact process of dikaryotization is unknown, but it must involve many nucleus duplications because the outcome of dikaryotization is that all cells of both receiving mycelia contain both nucleus types (figure 1b). Just like the monokaryon, the dikaryon can grow vegetatively, but it is also able to form sexual fruiting bodies (the mushrooms). In the fruiting bodies, the two nuclei fuse, directly after which meiotic spores are produced

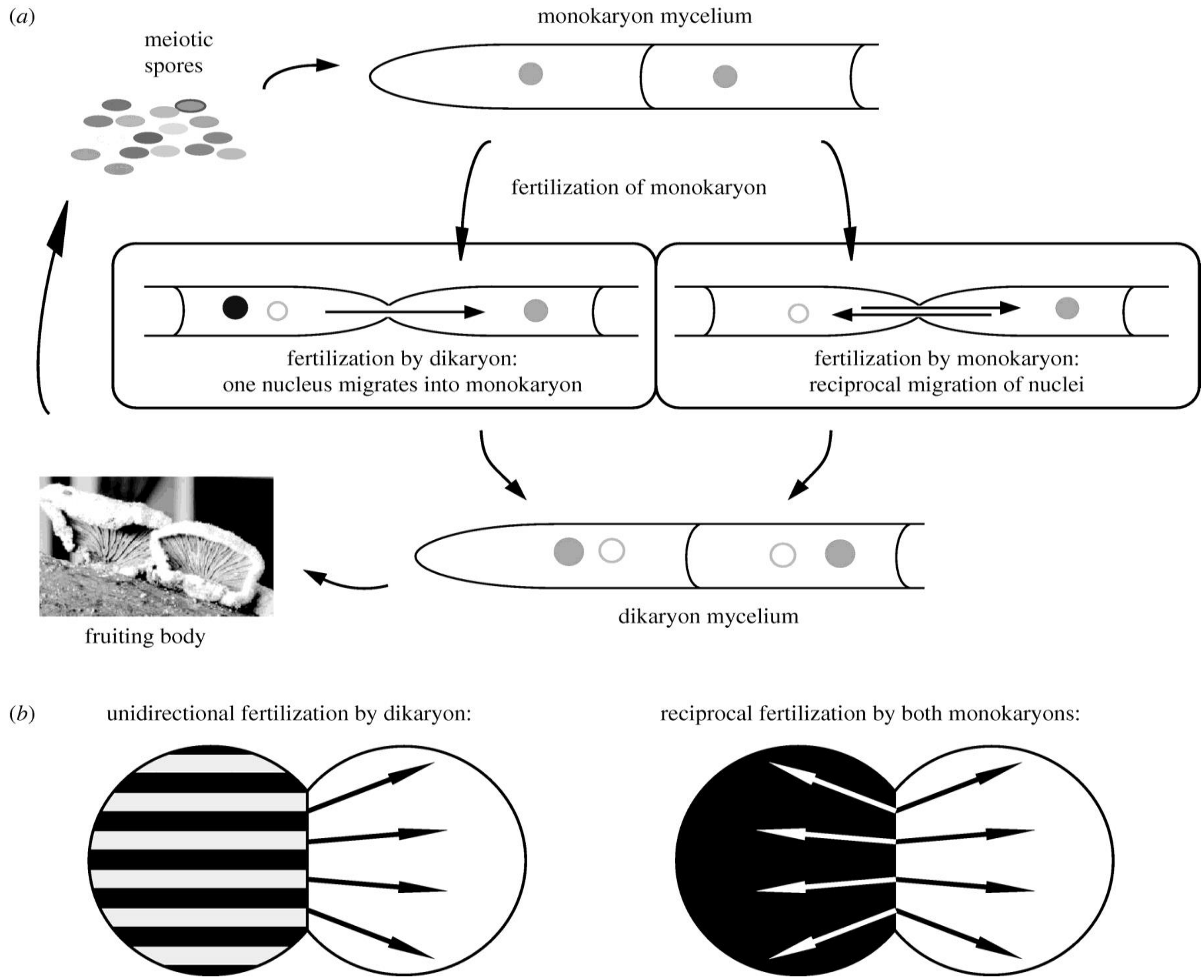


Fig.1. Life cycle of *Schizophyllum commune*

3.11. Cultivated Schizophyllum commune mushrooms

Cultivated mushroom are generally saprophytic, utilizing substrate as primary or secondary decomposers. *Schizophyllum commune* one of the most common mushrooms, is widely distributed worldwide and usually grows abundantly during the rainy season. This species frequently appears on dead wood and is a known wood decomposer of over 150 genera of flowering plants. They may be cultivated on a large number of substrates, according to local availability in different regions of the world. *Schizophyllum commune* is one of the four important wood inhabiting cultivated mushrooms in Japan, China together with *Lentinus edodes*, *Flammulina velutipes* and *Pholiota nameko* (Cooke, 2001).

3.12. Nutritional value of Schizophyllum commune and others mushroom

Mushrooms have been used as food in many parts of the world. Although mushrooms are often grouped with vegetable and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plant, animals and bacteria (Nwokoye *et al*., 2010). It is evidently clear that the growing interest in the cultivation of mushrooms can help in solving many problems of global importance such as protein shortage as well as improving the health and well being of people. Thus considering that mushrooms are valuable health foods which are low in calories and provide essential mineral (Nwokoye *et al*., 2010).

Mushrooms are highly nutritious and are important features of human diet worldwide. Edible mushroom are highly nutritious and can be compared with eggs, milk and meat. The content of essential amino acids in mushroom is high and close to the need of the human body (Oei, 2003). According to Carvalho *et al*., (2010) mushroom is considered as food with delicious taste and high nutritional value because their contents (g/100g) of protein (23.22), carbohydrate (63.17), phosphorus (104.13), fiber (34.00) and lipids (4.71). High protein contents of as much as 50 to 84% dry matter has been detected in the fruiting bodies and mycelia of oyster, *L. edodes*, *V. esculenta* and *T. clypetus.* Numerous types of mushrooms exist in nature; however, less than 25 species are widely accepted as food and only a few have attained the level of an item of commerce. Mushrooms are reported to be a good source of protein and minerals (Aletor, 1995), and some investigators have even contended that the amino acid compositions of mushrooms are comparable to animal proteins (Gruen and Wong, 1982).

Mushrooms are one of the many foods from the wild which are found in the diet of the various Naga tribes in Northeast India. Normally mushrooms are consumed fresh but the use of dried mushrooms during the offseason is not uncommon. Of the varieties of mushrooms from the wild that are used by the Nagas as food source, two species *Schizophykm commune* and *Lentinus edodes* are used fresh as well as in the dry forms during off seasons. Different biological (anti carcinogenic, anti-cholesterol, immuno-stimulating) effects of *Lentinus edodes* are known but little is known about its nutritive values (Olumide OJ, 2007).

S. commune is edible and the nutritional perspective of the mushroom was also documented in several previous studies. The islanders in Indonesia and Madagascar habitually chew carpophores of this mushroom. While the Yoruba people of South-Western Nigeria were reported to use *S. commune* to prepare delicious dishes among them (Longvah and Deosthale, 2001). *S. commune* was also one of the varieties of mushrooms that consumed by the Naga tribes in Northeast India as food source (Olumide OJ, 2007).

3.13. Medicinal properties of Schizophyllum commune and others mushroom

Most of the medicinal extracts from mushrooms are different forms of polysaccharides and all of them are strengtheners of the immune system with little or no side effects. Recent studies on various white rot fungi possess a number of beneficial medicinal properties such as antitumor, immune-modulators, anti-genotoxic, anti-oxidant, anti-inflammatory, anti-allergic, hypocholestrolaemic, antihypertensive, anti-hyperglycemic, antimicrobial and antiviral activities. These activities have been reported for varies extracts and isolated compounds, such as polysaccharides, polysaccharide-protein complexes, proteoglycans, protein and DNA from oyster mushroom fermentation broth, mycelia or fruiting bodies. In particular, polysaccharides appear to be potent antitumor and immune-modulating substances, besides possessing other beneficial activities (Khan *et al*., 2011).

Some mushrooms are used for the treatments of gastric ulcer, duodenal ulcer and chronic gastritis. A good example is *Hericium erinacius*. Some mushrooms such as *Tremella fulciformis* are used for curing leukemia, coughing, phlegma and asthma of patients suffering from chronic bronchitis (Oei, 2003). Various bioactive compounds isolated from culture extract of Ethiopian higher fungi showed other biological properties such as anti-protozoa, anti-helmentic, phytotoxic and brine shrimp lethality activities (Dawit, 1998).

For example, a sizofiran, antitumor polysaccharides extracted from the culture broth of *Schizophyllum commune* is an effective immuno-therapeutic agent for cervical carcinoma because it stimulates a rapid recovery of the immunological status impaired by radio-therapy (Oei, 2003). Mushrooms have long been appreciated for their flavor and texture. Now they are recognized as a nutritious food as well as an important source of biologically active compounds of medicinal value (Breene, 1990). Mau *et al.* (2002) had reported in their studies that medicinal mushrooms are commonly used for pharmaceutical purposes and as health foods. Hence, searching for new biological activities and other medicinal substances from mushrooms and to study the medicinal values of these mushrooms has become a matter of great significance. The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and has found variable therapeutic activities such as anti-carcinogenic, anti-inflammatory, immuno-suppressor and antibiotic effects (Asfors and Ley, 1993).

At present, there are at least 270 species of mushrooms that are known to have various therapeutic effects (Mau *et al.*, 2002)). *Schizophyllum commune* was the mushroom selected to be studied for its biological activity. Previous studies showed that a β 1-3, β 1-6D-glucan called Schizophyllan isolated from *S. commune* was found to be medically active in several therapeutics effects such as antitumor, anticancer and immuno-modulating activities (Kidd, 2000).

Schizophyllan is a non-ionic, water-soluble homopolysaccharide consisting of a linear chain of β-d-(1-3)-glucopyranosyl groups and β-d-(1-6)-glucopyranosyl groups produced by fermentation from filamentous fungi *S. commune* ATCC 38548. This polysaccharide has attracted attention in recent years in pharmaceutical industry as immune-modulatory, anti-neoplastic and antiviral activities that are higher than other glucans. However, schizophyllan was found rather ineffective against gastric cancer, but extended survival time in patients with head and neck cancer (Kimura *et al.*, 2002).

3.14. Uses of spent

The lignocellulosic substrate used for mushroom production and which is left after harvesting of the mushrooms can be used as compost for soil conditioning. It should be noted that this compost besides being rich in nitrogenous material contains partly degraded lignocelluloses components, when combined with animal dung or human excreta in a biogas digest would yield not only biogas but also a good quality organic nitrogenous fertilizer in the form of sludge. The sludge from the biogas plant as a nitrogenous fertilizer is far more beneficial than the compost from which it has been derived. Part of the biogas that is produced in the vicinity of the mushroom house can also be conveniently used for pasteurization of the mushroom bed material (Chang, 2006).

Spent after mushroom cultivation on different substrates such as cottonseed waste, cereal straw and bagasse serve as animal feed supplements (Dawit, 1998).The degradation of this lignocellulosic material promoted by the action of lignocellulolytic enzymes excreted by

Pleurotus. This spent substrate desirable for diverse uses, such Analyses of the banana leaf straw after its use in the culture of *P. ostreatus* (spent substrate) has shown as animal feed, mulch in agriculture and substrate for growing fungi. *Agaricus blazei* is a secondary decompositor and cannot grow directly on cellulose and lignin present in the straw, requiring a previous degradation of the substrate through a composting process (Oei 2003).

Mushroom spent used as organic fertilizer. The spent is made of degraded and undegraded plant polymers. Spent from already composted substrates such as *Agaricus bisporus* could be applied directly to fertilize the gardens and horticulture plants (Dawit, 1998).

4. Material and Methods

4.1. Sample collection

The young fruit bodies of *Schizophyllum commune* was collected from decaying wood at the Addis Ababa University, College of Natural and Computational Sciences park area located at an altitude of 2444 meters above sea level with the mean annual rainfall of 1196 mm and located 9^{o}01N and 038^{0}45E. The stipes of the young mushrooms was serving as a source of pure culture of *S .commune* mushroom.

4.1.1. Pure culture establishment

A large healthy mushroom should be chosen in the later button. It was cleaned with 75% alcohol. The mushroom was split in half by hand longitudinally and some inside tissue taken from the upper part of the stipe. It was placed centrally on the surface of the medium with a sterilized needle. As soon as we transfer the tissue, the test tube was closed and dated before it is returned to the incubator at 25°C. Within two or three days some white, delicate mycelia was produced from the small piece of the tissue. They grow upwards encircling the inner wall of the test tube. About ten days later the mycelium was growing rapidly and covers the surface of the agar medium. Then it was ready and transferred to spawn substrate to make spawn (Chang, 1991).

4.2. Preparation of culture media on culture of *Schizophyllum commune*

The mushroom species, pure culture of *S .commune* obtained from the stipe maintained on MEA. A 20g of MEA was added to 500ml of distilled water in 1liter of flask. Then the medium was heated to dissolve agar. It was autoclaved at 121^{O}C for 15min. This was inoculated with *S. commun*e culture and incubated at 25 ^{O}C. After 20 days of incubation, it was inoculated with the sorghum bottle for spawn preparation.

4.2.1. Spawn making for Schizophyllum *Commune*

Different substrates (sorghum, sawdust, coffee parchment, wheat bran and wheat grain) were used in spawn making with the addition of wheat bran (10%) and gypsum (2 %) as supplement. Spawn was made by soaking about 15 kg (88%) of sorghum or coffee parchment in water. The excess water was drained off and (10 %) wheat bran and (2%) gypsum was added. The ingredients are thoroughly mixed; moisture is adjusted to 55-60% by using oven dry methods with some modification of determination of moister contents according to Fan *et al* (2000).

Using the principle of loss of weight upon drying the standard for moisture measurement the balance automatically weighs a sample, dries it, measures the weight loss due to drying and calculates the moisture content of the seeds. The analysis was automatically terminated when drying is complete and the dry weight was stable. The final result was shown on the digital display.

Sawdust, wheat bran and wheat grain were used in the same ways without soaking in water, simply by moisturizing and by adding the same amount of supplement (wheat bran (10%) and gypsum (2 %)). The mixture was distributed equally in to 500 ml glass bottle and autoclaved at 121^{O}C for 30 min. After cooling, each bottle was inoculated with fungal culture. After 20 days of incubation, at 25^{o}C, the spawn was ready to be used for inoculation of the solid substrate (Fan *et al.*, 2000).

4.3. Substrate Optimization

Moisture content of substrate was adjusted to 55-60% (oven dry method)

$$\text{SMC (\% wb)} = \frac{\text{wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100$$

International Seed-Testing Association [ISTA] 2005). Substrate was adjusted to 5 P^{H} 1g was measured out from each substrate before inoculation soaked in10ml distilled water contained in a clean glass beaker. The substrates were allowed to stand for 10 minutes while stirring after 2 minutes intervals. It was standardized with buffering solutions.

4.4. Measurement of growth

The growth was measured qualitatively and quantitatively through:-

a. Colony diameter measuring on Petridish
b. Mycelia invasion (visual observation of mycelia growth)
c. Biological efficiency (BE)

4.5. Biological efficiency

With proper management, 4-5 flushes mushroom can be harvested and the biological efficiency measured. Biological efficiency (BE), which is defined as the ratio of weight of fresh fruiting bodies to the weight of substrate multiplied by 100 (Fan *et al*., 2003). It indicates the ability of the fructification of fungus utilizing the substrate.

$$BE = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate}} \times 100$$

4.6. Experiment on fruit body production

4.6.1. Based on wood logs

The process of inoculation was done by introducing the spawn of S. *commune* fungus grown on sorghum into *Eucalyptus globulus, Cupressus lusitanica, Acacia abyssinica* and *Gravillea robusta*. This inoculum, which is also called spawn, was produced under laboratory conditions. First, drill holes into the substrate log, using a drill bit and slightly larger for sawdust spawn to a depth of 7/16 inch. Holes spaced about 4 to 6 inches apart within rows and rows was 2 inches apart. The holes in adjacent rows offset to create a diamond pattern. Next, grain spawn was inserted into the holes using a special injecting tool (spatula) then covered by plastic and lasted for 12 weeks. The use of covering with plastic was to keep from dehydration and contamination with other microorganisms. Logs watered lightly once or twice to maintain surface moisture per week.

4.6.2. Cultivation using sawdust in plastic bags

The sawdust of *Eucalyptus globulus*, *Cupressus lusitanica*, *Acacia abyssinica*, *Gravillea robusta and* also Coffee husks and Cotton seed used to grow *S.commune.* Ingredients used as supplements (12 percent dry weight) such as gypsum and wheat bran were added. Once the proper ratio of ingredients (12% to 88%) was selected, they were combined in a mixer with water to raise the moisture content of the mix to about 60 percent. Then it was inoculated with the spawn prepared and incubated at green house.

4.7. Data analysis

The data on the biological efficiency value obtained from the species of the fungus (*S. commune*) were subjected to analyses of variance (one-way ANOVA) at the 5% level using the statistical Soft Ware JMP IN version 5.0.1 (John’s Macintosh Project).Analyses were performed for all data with triplicates for each and were reported as the mean ± SD.

5. Results and Discussion

5.1. Results

5.1.1. Culture production and mycelia growth rate on different agar media

The result revealed that *S. commune* showed relatively maximum average mycelia growth rate per day in diameter on MEA (malt extract agar) with (7.50mm/day) (Table 1). It showed 4.53mm/day on the SDA media and 3.80mm/day) and on PDA (Table 1). The lowest mycelia growth rate in diameter was observed on PDA of the three agar media used (Table 1).

Table 2: Average mycelia growth rate per days in diameters (mm) of the *S.commune* on different agar media.

Species	Culture media		
S. commune	MEA	SDA	PDA
	7.50 ± 0.32^{a}	4.53 ± 0.38^{b}	3.80 ± 0.60^{c}

All values are means of triplicates± SD. Levels not connected by same letter under the same row are significantly different ($p \leq 0.05$)

5.1.2. Spawn making

Fully mycelia invasion of the *S. commune* was observed on the different substrates after 20 days of incubation. Sorghum supports the fastest mycelia growth/colonization than the rest. It was ready to be used for the inoculation of the solid substrate (fig.2).

Fig.2. *S. commune* spawn grown on sorghum after 20 days of incubation.

5.1.3. Mushroom yield and biological efficiency (BE) on different substrates

The crop of the *S. commune* mushroom (12 weeks of cropping) was harvested for four flushes and their mushroom yields on the different substrates are given in (Table 3). The highest total weight of mushroom harvested on 2.00 kg dry substrate was recorded from Eucalyptus (1162g) followed by Cupressus which yield 924 g on the same amount of dry substrate. However on Gravillea and Acacia it produced relatively minimum yield but it did not produce any fruiting bodies or yield except mycelial growth on coffee husks and cotton seed (Table 3).

The fresh weight of mushrooms compared to the dry substrates on which they grew differed significantly between each substrate ($p < 0.05$). Eucalyptus gave the highest biological efficiency (B.E.) of (58.10%) followed by Cupressus (46.20%). Acacia gave relatively a low, B.E. of (32.55%).

Table 3: Yield (g) and biological efficiency of the *S.Commune* on different substrates (gram)

Substrates	flushes					
	1st	2nd	3rd	4th	Total	B.E in %
Eucalyptus	500±0.55[a]	350±0.54[a]	200±0.55[a]	112±0.50[e]	1162±0.64[a]	58.10±0.60[a]
Cupressus	332±0.50[b]	280±0.80[b]	215±0.51[b]	97±0.31[f]	924±0.61[b]	46.20±0.51[b]
Gravillea	286±0.64[c]	197±0.36[g]	160±0.61[c]	58±0.76[g]	701±0.73[c]	35.05±0.50[e]
Acacia	255±0.90[d]	204±0.57[d]	147±0.53[d]	45±0.24[h]	651±0.57[d]	32.55±0.57[d]
Coffee husks	-	-	-	-	-	-
Cotton seed	-	-	-	-	-	-

All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different ($p \leq 0.05$)

5.1.4. Mushroom yield on logs of Eucalyptus, Cupressus, Gravillea and Acacia species

The cultivation of *S.commune* on the logs of Eucalyptus, Cupressus, Gravillea and Acacia revealed that, mycelial coverage and small pinhead-like structures was observed (fig.3). Nevertheless, these pinhead-like structures didn't grow into normal mushrooms.

A

B


.C


.D

Fig.3. The mycelia growth of *S.commune* grown on logs of (A=Eucalyptus, B=Cupressus, C=Gravillea and D=Acacia) species

5.1.5. Flushing patterns of the *S. commune* on different substrates

Four flushes were recorded on each of the four different substrates (Table3). There was an expected progressive decline in yield over the course of the flushes on the four different substrates (Table3). From the results it seemed that substrates did not affect this pattern.

5.2. Discussion

The first stage in any mushroom cultivation process is to obtain a pure culture of the specific mushroom strain. Mushrooms grow on a variety of culture media and on different agar formulas, both natural and synthetic, depending on the organism to be cultivated and the purpose of the cultivation (chang *et al*., 1993). In this study, *S.commune* showed different mycelia growth rate on the different substrates used. Malt extract agar gave the fastest mycelia growth rate followed by SDA. While, the slowest mycelia growth rate was seen in PDA (potatoes dextrose agar) media. Statistically, there is a significant variation in their mycelia growth rate on the growth substrates ($p \leq 0.05$). This may be due to the difference in the type of substrate used and the variations in the prevailing mushroom growing conditions. Similarly, Atikpo *et al*. (2008) have reported that the genetic nature of the mushroom species/strains and their extensive enzyme systems determine their mycelia colonization on different substrates.

In the present study the yield and biological efficiency of the *S.commune* cultivated on the different substrates supplemented with the supplementary substrate wheat bran and gypsum were investigated. The results revealed that, the highest yield was obtained from <u>Eucalyptus</u> followed by <u>Cupressus</u>. <u>Gravillea</u> and <u>Acacia</u> produce relatively lower yield and biological efficiency. Statistically, there is a significant variation in their yield and biological efficiency on each substrate.

This may be due to the difference in the type of substrate used, variation in extracellular enzymes production and the prevailing mushroom growing conditions. The duration of different growth stages of cultivated mushrooms depends on the type of substrate and substrate formulation (composted or non composted or spent mushroom substrate and supplements) used, the type of species and/or the strain employed, spawn type and the level spawning rate applied, as well as on the prevailing mushroom growing conditions (Peyvast, 2008).

While coffee husks and cotton seed didn't support the production of fruiting bodies except mycelia growth. This may be due to the unfavorability between the nature of the substrate and the mushrooms isolates and the prevailing mushroom growing conditions. However, when the two substrates that gave the highest yield (Eucalyptus and Cupressus) were compared, Eucalyptus was more efficient than Cupressus by producing 12% biological efficiency. The overall B.E. % for Eucalyptus was superior to those recorded for *S.commune* grown on sawdust which ranged from 6.3 to 57.74% (Obodai and Vowotor, 2002).

The cultivation of *S.commune* on the logs of Eucalyptus, Cupressus, Gravillea and Acacia didn't support the formations of fruiting bodies/mushrooms. But mycelial coverage and small pinhead-like structures was observed. This may be due to the compact strong structure of the logs which may not be easily utilized efficiently by this mushroom or due to the inability of this mushroom to produce the corresponding necessary extracellular enzymes to degrade these logs efficiently.

Approximately, (91 to 96 %) of the total fresh weight was obtained in the first three consecutive flushes, with the last flushes producing (4 to 9%) in all the substrates used. Similarly, Obodai and Vowotor (2002) have demonstrated that regardless of the mushroom species of the substrate (composted or non-composted) used to grow mushrooms, the pattern of gradually lessening mean yield per flush remains the same for any cultivated edible mushroom. This has been attributed to the finding that the quantity of mushrooms harvested in each flush is directly proportional to the nutrients disappearing from the substrate. The assimilable nutrient sources (carbon and nitrogen) in the organic waste substrate were absorbed by mycelia translocated and mobilized to supply the fruit bodies (Stamets and Chilton, 1983).

The major part of mushroom production in the all substrate investigated was obtained in the first three flushes, the economic flushes could be limited to three flushes; the fourth flushes can be ignored. Shortening cropping period by promoting rapid intensive early flushes could be of advantage in order to obtain maximum yield in a short time, which could ultimately lower the cost of production.

6. Conclusion and Recommendation

6.1. Conclusion

- The highest yield of cultivated *S.commune* was produced on Eucalyptus followed by Cupressus.
- Eucalyptus and Cupressus as a substrate for *S.commune* proved to be a better substrate than Gravillea and Acacia in terms of mushroom productivity. Coffee husks and cotton seed didn't support the production of fruiting bodies.

6.2. Recommendation

Schizophylum.commune more efficiently utilizes Eucalyptus and Cupressus as a substrate than the rest and should be further evaluated. Further study must be carried out using a combination of different agro-industrial wastes to optimize Eucalyptus and Cupressus as a good substrate not only for *S. commune* but also for other edible mushrooms (*Lentinula edodes* and *Agaricus bisporus)*.

However, the effects of different supplements and spawn rate aimed at more mushroom fruit body yield in as short a time as possible remains to be investigated.

7. REFERNCES

Adejoye, O. D. Adebayo-Tayo, B.C. Ogunjobi, A.A. and Afolabi O.O. (2007). Physicochemical Studies on *Schizophyllum commune* (Fries) a Nigerian Edible Fungus. World Applied Sciences Journal **2** : 73-76

Aletor, V. A. (1995) Compositional studies on edible tropical species of mushrooms. *J. F.Che.* **54:** 265-268.

Asfors, K.E. and Ley, K. (1993). Sulfated polysaccharides in inflammation. *J. Lab.Clin.Med.* **121**: 201-202.

Atikpo, M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L. and Awumbilla, B. (2008). Sustainable mushroom production in Africa: A case study in Ghana. *Afr. J. Biotechnol* **7**: 249-253.

Bhawna, A.K. (2003). Biological efficiency of different *Pleurotus* sp. on the leaf stalk biomass from coconut palms. *Mushroom Res.* **12**: 97-100.

Breene, W.M. (1990). Nutritional and medicinal value of specialty mushrooms. *J. F.Prot*. **53:** 883-894.

Carvalho, C.S.M., Campos, C.S. and Andrade, M.C.N. (2010). Mushrooms of the *Pleurotus* genus. A review of cultivation techniques. *Interciencia.* **35**: 177-182.

Chang, S.T. (2006). The World Mushroom Industry: Trends and Technological Development. *I. J. Med Mush.* **8**: 297-314.

Chang, S.T.and Miles, P.G. (1991). *Edible Mushroom and Their Cultivation*. Academic Press, London.Pp: 265-275.

Chaube, H.S. (1995). Nutritional and Medicinal Values of Mushrooms. **In:** *Mushroom Production Technologies*, pp. 132-141, (Miles, P.G.ed) Distt. Nainital, India.

Cheung, L.M., Cheung, P.C.K. and Ooi, V.E.C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *F.Chem*. **81**: 249-255.

Cooke, W. (2001). The genus *Schizophyllum*. *J.Mycol*. **53:**575-99.

Dawit Abate. (1998). Mushroom cultivation: A practical approach. Berhanena Selam printing press, Addis Ababa. pp. 16-21

Emilia, B., Grazyna, J. and Zofia L. (2006). Edible mushroom as a source of valuable nutritive constituents. *Ac.Sci.Pol.Technol.Ali.* **5:** 5-20.

Fan, L., Pandey, A. and Soccol, C.R. (2000).Solid state cultivation-an efficient method to use toxic agro-industrial residues. *J.Basic Microbiol*. **40**: 187-197.

FAO, (2009). Make money by growing mushroom. Rural Infrastructure and Agro-Industries Division Diversification booklet. No7. United Nations,pp.1-53

FAOSTAT, (2012). Large time-series and cross sectional data relating to hunger, food and agriculture for 245 countries and territories. United States Department of Food and Agriculture, Foreign Agricultural Service, Washington.

Flegg, P.B., Spencer, D.M. and wood, D.A. (1985). The biology and technology of the cultivated mushroom, wily, New York.

Gladfelter A. and Berman J. (2009).Dancing genomes: fungal nuclear positioning. *Nat. Rev. Microbiol*. **7**, 875–886

Gruen, E. H. and Wong, M. W. (1982) Distribution of cellular amino acids, protein and total inorganic nitrogen during fruit body development in *Flammulina veluptipes. C.J.Bot.* **60:** 1330-1341

Guarro, J., Gene J. and Stchigel, A.M. (1999). Developments in fungal taxonomy. *Clin. Microbiol. Rev*.**12**: 454-500.

Hao, L.M., Xing, X.H., Li, Z. Zhang J.C. and Sun J.X. *et al*., (2010). Optimization of effect factors for mycelia growth and exopolysaccharide production by *Schizophyllum commune*. *Appl. Biochem. Biotechnol*., **160**: 621-631.

Heitman J., Kronstad J. W., Taylor J. W. and Casselton L. A. (2007). In*Sex in fungi: molecular determination and evolutionary implications.* Washington,

Inamulhaq, M., Nasir, A., Khan, M.A., Khan, N.A., Binyamin, J.R. and Irshad, G. (2010). Use of medicinal plants in different composts for yield improvement of varies strain of oyster mushroom. *Pakistan. J. Bot*. **42:** 3275-3283.

Ivors, k. (2003).Tracing the root of cultivated mushroom. Mycological society of San Francisco, San Francisco.

Israilides, C. (2003). ‘Phyto-remediation of agro-industrial wastes for the production of polysaccharides and mushrooms. Chania, Greece.

Jose, N. and Janardhanan, K.K. (2000). Antioxidant and antitumour activity of *Pleurotus florida*. *Cur. Sci.* **79**: 941-943.

Khan, A., Rahman, M.M., Tania, M. and Ahmed, N.U.S. (2011). *Pleurotus sajor*-cajo and *Pleurotus florida* mushrooms improve some extent of the antioxidant systems in the liver of hypercholesterolemia rats. *The open Nutraceuticals J*. **4**: 20-24

Kidd, P.M. (2000). The use of mushroom glucans and proteoglycans in cancer treatment. *Alt. Medi.Rev*. **5:** 4-27.

Kiflemariam Yehuala (2008). *Potential and constraints of mushroom production in Ethiopia*. MSc. Thesis, Bahir Dar University, Faculty of Agriculture and Environmental science

Kimura, Y., Taniguchi, M. and Baba, K. (2002). Antitumor and antimetastatic effects on liver of triterpenoid fractions of *Ganoderma lucidum:* mechanism of action and isolation of an active substance. *Journal of Anticancer Research.* **22:** 3309-3318

Liu, J., X. Guan, D. Zhu and J. Sun, (2008). Optimization of the enzymatic pretreatment in oat bran protein extraction by particle swarm optimization algorithms for response surface modeling. LWT-Food Sci. Technol., **41**: 1913-1918.

Mau, J.L., Lin, H.C. and Chen, C.C. (2002). Antioxidant properties of several medicinal mushrooms. *J.Agric. F. Chem*. **50:** 6072-6077.

May G. and Taylor J. W. (1988). Patterns of mating and mitochondrial DNA inheritance in the agaric basidiomycete *Coprinus cinereus*. *Gen*. **118**: 213–220.

Miyazaki, K., Mizutani, H., Katauchi, H., Fukumaka, K., Fujisaki, S. and Okamura, H. (1995). Activated (HLA-DR+) T-lymphocyte subsets in cervical carcinoma and effects of radiotherapy and immunotherapy with sizofiram on cell-mediated immunity and survival. *J. Gynaecol. Oncol*. **56:**412-420.

Nakano, T., Oka, K. and Wagner. F (1996). Intratumoral administration of sizofiran activates Langerhans cells and T-cell infiltration in cervical cancer. *J.Cli. Immu.Immunopathol*.**79:**79-86.

Nwokoye, A.I., Kuforiji, O.O. and Oni, P.I. (2010). Studies on mycelia growth requirements of *Pleurotus ostreatus* (Fr.) singer. *Intern. J. Basic. Appl. Sci*. **10**: 70-89.

Oei, p. (2003). *Mushroom cultivation, appropriate Technology for Mushroom Growers*. Backhungs Publishers, Leiden

Ohm, R.A., de-Jong, J.F. Lugones, L.G. Aerts A. and Kothe E. *et al.*, (2010). Genome sequence of the model mushroom *Schizophyllum commune*. Nat. Biotechnol., **28**: 957-965.

Okamura-Matsui, T., Takemura, K., Sera, M., Takeno, T., Noda, H., Fukuda, S. and Ohsugi, M. (2001). Characteristics of a cheese-like food produced by fermentation of the mushroom *Schizophyllum commune*. *Journal of Bioscience and Bioengineering*. **92:** 30-32.

Olumide OJ (2007). Economic analysis of mushroom marketing as a copping strategy for poverty reduction in Ondo State, Nigeria. *Afr. Crop Sci. Conf. Proc*. pp. 1255-1260.

ouglas, A., (2006). Male, female and or How does nature define the sexes. Am. Biol. Teach. **68**: 372-375

Paul Stamets (2002). Mycomedicinals. An Informational Treatise on Mushrooms. **3**:40-42

Peyvast, G. (2008). Lawn Clippings for Cultivation of Oyster Mushroom. *Int. J. Veg. Sci*. **14**: 98-103.

Quimio T.H., Chang S.T. and Royse, D.J. (1990). Technical guidance for mushroom growing in the tropics. FAO Publication, pp: 1-31

Rau, U., Gura, E., Olszewski, E. and Wagner, F. (1992). Enhanced glucans formation of filamentous fungi by effective mixing, oxygen limitation and fed-batch processing. *J.Indus. Microbiol*.**9:**19-26.

Roman, D.M., Boa, E. and Woodward, S. (2006). Wild gathered fungi for health and rural livelihoods. *P. N. Soc*. **65**: 190-197.

Rousk, J. and Baath, E. (2011). Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiol. Ecol., **78**: 17-30.

Sciences, Department of Plant Sciences, Bahir Dar

Stamets, P. and Chilton, J. (1983). The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home. Agarikon Press, Olympia, Washington, pp. 61-107.

Teoh Y. and Don M. M., (2012.) Optimization of Parameters for Mycelia Growth by *Schizophyllum commune* and a Kinetic Model Study of its Growth Morphology. *J.Appl. Sci*. **12**: 1100-1105

Teoh, Y.P., Don M.M. and Ujang, S. (2011). Nutrient improvement using statistical optimization for growth of *Schizophyllum commune* and its antifungal activity against wood degrading fungi of rubberwood. *Biotechnol*. *Prog*, **28**: 332-341.

Teoh,Y.P. and Mashitah, M.D. (2010). Cellulase production by *Pycnoporus sanguineus* on oil palm residues through pretreatment and optimization study. *J. Applied Sci*. **10**: 1036-1043.

Tripathi, D.P. (2005). Mushroom Cultivation. Panchsheel Park, New Delhi, India.

USDA, (2006).*Tropical products: World Market and Trade*. United States Department of Agriculture, Foreign Agricultural Service, Washington.

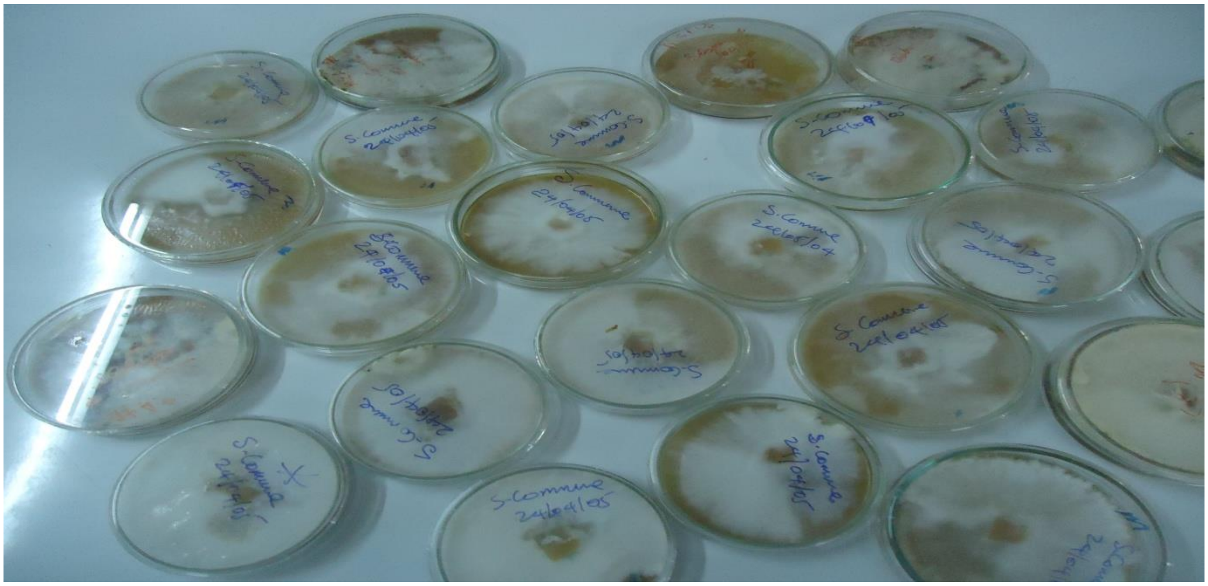
Weasels, G.H. (1993). Gene expression during fruiting in S*chizophyllum commune*. Mycological Research **96**, 609-620.

Yamanaka, k. (1997).Production of cultivated edible mushroom. *I.F.R*. **13***:*327-333.

Yoshida, N., Takahashi, T., Nagao, T. & Chen, J.(1993) Effect of edible mushroom (*Pleurotus ostreatus*) cultivation on *in vitro* digestibility of wheat straw and sawdust substrate. J. *Japanese Soc*. *Grassland Sci*. **39**: 177-182.

Zervakis, G. (2000). Management of agro-industrial wastes through the cultivation of edible mushrooms. **In**: *Waste Management*, pp.111-119, (Tripathi, D.P. and Zervakis, G.eds) Milan, Italy.

Appendices



Appendix 1: Culture media of *S.commune*

A

B

C

Appendix 2: Important substrate during the cultivation of the *S.commune*

A=Coffee parchment B= sawdust C=sorghum

Appendix 3: Sorghum inoculated with pure culture of S.commune during spawn making

Appendix 5: Substrate inoculated with the spawn prepared.

A= Eucalyptus

B=Cupressus

Appendix 6: Fruiting bodies of the *S.commune* cultivated on different substrates

Appendix7: Wateringof*S.ommune*cultivatedondifferentsubstrates

Appendix:8. Fruiting body of *Schizophyllum commune*

Appendix 9: Dry weight of *S.commune* harvested from different substrates