CULTIVATION OF THREE SELECTED *Pleurotus* SPECIES USING GROWTH MEDIA PREPARED FROM SUGARCANE BAGASSE AND DRIED LEAVES OF *Eichhornia crassipes* AND *Argemone ochroleuca*

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APPROVAL SHEET

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As thesis research advisors, we here by certify that we have read and evaluated this thesis prepared, under our guidance, by Tilahun Temesgen entitled "Cultivation of Three selected *Pleurotus* Species Using Growth Media Prepared from Sugarcane bagasse and Dried Leaves of *Eichhornia crassipes* and *Argemone ochroleuca*" We recommend that it be submitted as fulfilling the thesis requirement.

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DEDICATION

This piece of work is dedicated to my father Ato Temesgen Getachew and my mother Woizero Mulualem Girma, My little brother Haylamlak Temesgen as well as my sisters.

STATEMENT OF THE AUTHOR

By my signature below, I declare that this thesis is my genuine work and that all sources of materials used for the thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for MSc degree at Haramaya University, and it is deposited at the University Library to be made available to users under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born on December 14, 1994 at a place known as Wogdi in South Wello, Amhara Region. He completed his junior school at Gelebe Primary School and secondary education at Wogdi Secondary School. After successfully passing Ethiopian School Leaving Certificate Examination (ESLCE), he joined Hawassa University in 2013/14 and graduated with BSc degree in biology in June, 2016. Immediately after graduation, he was assigned and sponsored by Ministry of Education as Graduate Assistant and he joined Haramaya University for his graduate studies in Microbiology in September, 2016.

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ACRONYMS AND ABBREVATIONS

AOAC	Association of Analytical Chemist
BE	Biological Efficiency
CFa	Crude Fat
CFi	Crude Fiber
CP	Crude Protein
DFF	Days to Fruiting body formation
DMF	Days for Mycelium to fully colonize Substrate
DPF	Days to Pine head Formation
FAO	Food and Agriculture Organization
LAO	Leaves of *Argemone ochroleuca*
LEC	Leaves of *Eichhornia crassipes*
MoC	Moisture Content
PDA	Potato Dextrose Agar
SCB	Sugarcane Bagasse
TA	Total Ash
TC	Total Carbohydrate
TY	Total Yield

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ABSTRACT

Cultivation of edible mushrooms using agricultural invasive weeds could play a paramount role by producing foods with high nutritional value, and at the same time create environmentally friendly ways of food production. Thus, an experiment was conducted at Mushroom Research, Production and Training Laboratory of Haramaya University to cultivate three selected Pleurotus mushroom using three substrate and their combinations (dried leaves of Eichhornia crassipes (LEC) & Argemone ochroleuca (LAO) , and sugarcane bagasse (SCB) as a control). Phenological data, Yield and quality parameters were determined. From Phenological data, the fastest and slowest fruiting body formation (23.67 and 34.33 days) were recorded from Pleurotus ostreatus and Pleurotus sajor-caju grown on 100% SCB and 100% LAO respectively. For yield performance, highest and lowest yield (1697.00g &556.7g) were obtained for Pleurotus ostreatus and Pleurotus sajor-caju cultivated on 100% SCB and 100% LAO respectively. Highest value of biological efficiencies (71.67%) was obtained for Pleurotus ostreatus grown on 100% SCB. For quality parameters, highest percentage of crude protein (35.52% and 33.23 %) were obtained from Pleurotus ostreatus and Pleurotus florida grown on100% SCB alone, followed by Pleurotus ostreatus grown on 33% of (SCB, LAO and LEC) 31.66 %)). Lowest percentage of crude protein (14.38 %) was obtained for Pleurotus sajor-caju grown on100%LAO. Moisture content, crude fat, crude fiber, total ash and carbohydrate content obtained from this experiment generally ranged from 80.40 % - 88.53 %, 1.18 % - 1.97 %, 8.2 % - 12.82 %, 7.02 %- 8.28 % and 15.18 - 46.65 % respectively. A result from simple linear correlation analysis indicates that phenological parameters have no direct influence on total yield and biological efficiency. In general using the test substrate (LEC& LAO) in combination with SCB, provides comparable yield and quality parameters of the selected mushroom with that of the control substrate.

Key words: Biological efficiency, Invasive Weeds, *Pleurotus* Mushroom, Proximate Analysis, Spawn

1. INTRODUCTION

Food insecurity to the ever growing world population is a major challenge, which is largely common in low and middle-income countries that mainly have poor food production systems and suffer from serious malnutrition. According to Eswaran and Ramabadran, (2000), in developing countries recycling and management of the organic wastes has become another issue, but these can be efficiently utilized in cultivation of mushroom which will ultimately reduce the malnutrition problems in these countries and will also reduce environmental pollution. Das and Mukherjee, (2007); Akinmusire *et al*., (2011) also stated that mushroom cultivation play a paramount role in managing organic wastes whose disposal have become a problem and are causing massive pollution to the environment as a result of dumping of agricultural wastes. So, the widespread malnutrition problem coupled with increasing protein requirement in many developing countries has necessitated the search for new and alternative means of food production system to meet the protein requirement of the country, and one of the alternative ways is the production of mushroom which is noted for its high protein content (Hasan *et al.*, 2010).

Mushrooms are macroscopic fungi with a distinctive spore-bearing fruiting body typically produced aboveground (on soil) or on a substrate (Stevenson and Lentz, 2007). They are fleshy, spore-bearing reproductive structures of fungi. Most species of mushrooms belong to the Basidiomycota and Ascomycota. According to (Dike *et al.,* 2011) the life cycle of a mushroom may be traced from - a spore which under favorable conditions germinates to form a mass of branched hyphae of mycelia which colonize a substrate. Mushrooms go through two stages, the vegetative stage and the reproductive phase. The vegetative stage ceases when the hyphae fully colonize its substrate. The reproductive phase starts when the hyphae develop primordia. The mushroom is a fruit that results from fully matured primordia of the fungi (Dike *et al.,* 2011).

Mushrooms include edible, medicinal and poisonous species and edible mushrooms are nature’s gift as they are protein rich foods for human beings. For a long time, wild edible mushrooms have played an important role as a human food (Chang and Miles, 2004).

Edible mushrooms once called the “food of the gods” and still treated as a garnish or delicacy food item that can be taken regularly as part of the human diet or be treated as healthy food or as functional food. Mushrooms are delicacy food items praised for their characteristic texture when biting and enjoyable flavor. They have received overwhelming attention from food and pharmaceutical researchers due their bioactive constituents (Mariga *et al*., 2014). The natural substrates of the mushrooms include logs of woods, decomposed agro- and animal wastes, and soil where nutrients are available through external digestion and absorption by the mycelium. Due to their nutritional, medicinal and ecological advantages, mushrooms have attracted the attention of many people in the world (Imtiaj and Rahman, 2008).

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). Mushroom cultivation offers benefit, 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” (Beteez and Kustudia, 2004). 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). Currently, many research works focused on the cultivation of the three most commercially important mushrooms: the white button mushroom (*Agaricus bisporus*), the shiitake mushroom (*Lentinula edodes*) and the oyster mushroom (*Pleurotus* mushroom).

The oyster mushrooms (*Pleurotus* species) are in the third place after the white button and shiitake among the world mushroom production (Gyorfi *et al*., 2007). *Pleurotus* species is one of most extensively studied white-rot fungi for its exceptional ligninolytic properties (Bellettini, M.B. *et al*., 2016). *Pleurotus* species are commercially important edible mushrooms commonly known as the oyster mushroom. Cultivation of oyster mushroom has recently increased tremendously throughout the world because of their abilities to grow at a wide range of agro-based residues.

They have immense abilities to utilize various lignocellulose substrates with the aid of extracellular enzymes capable of degrading complex organic material (Martinez-Carrera, 2002). The wide range of plant waste that have been reported include sawdust, paddy straw, sugarcane baggage, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana, water hyacinth, duck weed, rice straw etc. and does not require costly processing method and enrichment material (Mondal 2010; Stanley, *et al* (2011). These white-rot fungi are useful decomposers of various agricultural wastes (Kurt and Buyukalaca, 2010).They are efficient colonizers and bio converters of lignocellulosic agro-industrial residues into pleasant human food with medicinal properties, with the productivity of the conversion being expressed by biological efficiency (Singh and Singh, 2005). They can utilize almost all agricultural wastes as substrates including weed (Taye *et al*., 2009). The disposal of these plants through burning causes environmental pollution as they release high level of CO_2 as well as resulting in unnecessary wastage of large amount of organic materials (Croan, 2000). Such plant materials can be used as substrate for primary decomposers such as white-rot fungi and which have lignocellulosic-degrading enzymes (polyphenol oxidases, peroxidases etc.) causing significant phenolic removal (Olivieri *et al*., 2006).

In Ethiopia, close to 35 invasive alien plant species are posing negative impacts on native biodiversity, agricultural lands, rangelands, national parks, waterways, lakes, rivers, power dams, roadsides, urban green spaces with great economy and social consequences (Rezene and Taye 2014). At present water hyacinths (Eichhornia *crassipses*) have been ranked as one of the world's worst invasive weeds (Bhattacharya *et al.,* 2015 Villamagna and Murphy 2016) causing problems for millions of users of water resources. Water hyacinth has been reported to invade two major areas of Ethiopia: the Nile basin and Awash basin extending down to the rift-valley region (Habtamu, 2013).

Mexican poppy (*Argemone ochroleuca* subsp. ochroleuca): another invasive weed which is naturalized throughout large parts of Australia and is common in the northern and eastern parts of the country. This species is found in a wide range of environments (i.e. from temperate to tropical and from arid to humid), however it is most common in semi-arid, sub-tropical and warmer temperate regions. It is a weed of roadsides, railway lines, sandy stream beds, river

flats, waste areas, disturbed sites, gardens, pastures, crops and fallows (Sanaa and Moussa 2012).

Sugarcane bagasse, the sugar cane residue after sugar extraction is one of the most plentiful lignocellulosic wastes. According to Luz *et al.,* (2007), bagasse consists of cellulose 43.8% hemicellulose 28.6%, lignin 23.5%, ash 1.3%, and other components 2.8%. About 54 million tons of bagasse is produced annually throughout the world.

Despite the massive research efforts that are underway to manage these invasive weeds with emphasis on biological control using pathogens and insects (Taye *et al*., 2009), immediate and complete control of the weeds could not be expected. Therefore, an attempt to convert the weed biomass into protein rich food through mushroom cultivation is prudent. So, by taking into account the above problems, the present study was conducted by incorporating the following objectives:

General Objectives

The overall objective of this study was to cultivate three edible oyster mushroom species: *Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida* using growth media prepared from sugarcane bagasse, and dried Leaves of *Eichhornia crassipes* (water hyacinth) and *Argemone ochroleuca* (Pale Mexican pricklypoppy).

Specific Objectives

- ✓ To determine the phenological characteristics (days for *Pleurotus* mycelium to fully colonize a substrate, Days to pinhead formation and fruiting body formation) that was observed on the selected *Pleurotus* species (*Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida)* with in each substrate and substrate combination.

- ✓ To determine the biological efficiency and total yield of selected Oyster mushroom species (*Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida)* cultivated in

three substrates and their combination (sugarcane bagasse, and dried Leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca.*

- ✓ To determine the proximate composition of the three mushroom species cultivated using substrate or combination of substrates of sugarcane bagasse and dried Leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca.*

- ✓ To identify the substrate and substrate combinations that is suitable for cultivation of *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida.*

2. LITERATURE REVIEW

2.1. Definition of Mushrooms

The word mushroom has many meanings in different parts of the world. Many researchers have tried to explain or more still define the word mushroom. Most of the mushrooms belong to the ascomycetes and Basidiomycota with most of them being in the *Basidiomycota* group (Mensah, 2015). Chang (2007) also stated that mushrooms are not only basidiomycetes; they can also be ascomycetes, grow underground, have a non-fleshy texture and could be inedible. All the poisonous and the non-poisonous fungi that can be seen with the naked eye and can be picked with the hand are described as mushrooms. According to Mensah (2015), a mushroom is a fungus that has a stem, a cap and gills or pores on the underside of the cap. Cho and Kang (2004) defined mushroom as a macro fungus with a distinctive fruiting body which can be either epigeous (growing on or close to the ground) or hypogenous (growing underground). The various types and shapes of mushrooms that can be picked from the wild include the most common type of umbrella shape with a pileus (cap) and a stipe (stem) i.e. *Lentinula edodes* (Chang, 2007). There are other species that have different shapes such as volva (cup) in *Volvariella volvacea* or an annulus (ring) in *Agarius campestris* and some even, like the human ear such as *Pleurotus ostreatus* (Chang, 2007).

2.2. Classification of Mushrooms

Classification is the arrangement of things or organisms into classes according to common features shared by the organism. Mushroom classification is therefore the arrangement of mushrooms based on their common characteristics. Mushrooms can be classified by their tropic pattern as saprophytes, parasites or mycorrhizae (Cho and Kang, 2004). The saprophytes are decomposers growing on organic matters like wood, leaves and straw in nature. The parasites on the other hand grow, feed and are sheltered on or in a different organism while contributing nothing to the survival of their host, while mycorrhizas form a symbiotic association of their mycelia with the roots of certain plants. There are three groups of mushrooms according to their economic importance; these are edible mushrooms, toxic mushrooms and medicinal mushrooms (Mensah, 2015).

Edible mushrooms are mushrooms that have desirable taste and aroma without poisonous effect and are used extensively in cooking; toxic mushrooms produce toxin, mind altering substances, antibiotics and antiviral substances, therefore, ingestion of toxic mushrooms may cause harmful effects that vary from mild symptoms such as gastric upset to severe life-threatening organ-failure which may result in death. Medicinal mushrooms on the other hand have extracts that are possibly used for treatment of diseases.

On the other hand, Chang (2007) had earlier reported that mushrooms can be grouped into four main categories, these include ―(1) those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus*; (2) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (3) those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., *Amanita phalloides*; and (4) 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‘.

Mushrooms can also be classified according to the substrates they grow on (Dzomeku, 2009). These include cellulolytic mushrooms, lignocellulolytic and termitomyces. The cellulolytic mushrooms grow mainly on cellulose such as straws; examples include *Vovariella volvacea, Agaricus bisporus etc*. The lignocellulolytics grow well on both straws and decaying wood such as sawdust; examples include *Pleurotus ostreatus*. The termitomyces grow mainly on anthills and their life cycles are completed by the help of ants or termites; examples include the termitomyces family.

2.3. The Technology of Mushroom Cultivation

Mushroom cultivation is a space-confined technology and requires relatively small capital. Over 200 species of mushrooms have long been used as functional foods around the world (Kalac, 2013), but only about 35 species have been commercially cultivated (Xu et al., 2011). They are a rich source of nutrients, particularly proteins, minerals as well as vitamins B, C and D (Panjikkaran and Mathew, 2013).

Research 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*).Cultivation of mushrooms is completely different from growing green plants.

Mushrooms do not contain chlorophyll and therefore depend on other plant material (the "substrate") for their food. Commonly cultured mushrooms are saprophyte fungi that feed on dead plant material. Mushrooms cultivation offers benefit 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" (Beteez 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). Shah *et al*. (2004) reported that mushrooms contain about 85 – 95% water, 3% protein, 4% carbohydrate, 0.1% fats, 1% minerals and vitamins. For different species of mushrooms, the preferred growing medium varies. Some species can grow on a wide range of materials and others cannot. Humidity, light, temperature, and carbon dioxide-to-oxygen ratio are conditions, which typically determine when a mushroom will fruit (Chang and Buswell, 2008). Mushroom survival and multiplication are related to a number of factors, which may act individually or have interactive effects among them. Chemical composition, water activity, ratio of carbon to nitrogen, minerals, surfactant, pH, moisture, sources of nitrogen, particle size, and amount of inoculum, antimicrobial agents and the presence of interactions between microorganisms are considered as chemical, physical and biological factors that are linked to mushroom production (Eira, 2003). The main environmental factors encompass temperature, humidity, luminosity and air composition of the surrounding substrate, such as concentration of oxygen and carbon dioxide (AMGA, 2004).

2.4. Mushroom Production and Consumption in Ethiopia

In Ethiopia, mushroom cultivation is a very recent practice and technology. Previously mushroom consumption was confined to rural inhabitants and picked from farmlands, forests and around waste dumpsites when environmental conditions particularly humidity favor their sporocarp formation.

Mushrooms are now cultivated and marketed in urban centers (Kumela, 2012). Dawit (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. The local demand for mushrooms is steadily growing to about 36 tons per year (button 50%, oyster 40% and Shiitake 10%) at present (Kumela, 2012). The same author indicated that 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. According to (Dawit 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.

2.5. Oyster Mushroom Cultivation

Pleurotus species is one of most extensively studied white-rot fungi for its exceptional ligninolytic properties (Bellettini, *et al*., 2017). This genus cleavages cellulose, hemicellulose and lignin from wood, whereas brown rot fungi only cleavage cellulose and hemicellulose (Machado *et al*., 2015). The process for the cultivation of oyster mushroom is simple because its cultivation does not lend itself to complex scientific procedures. Mediocre farmers can cultivate the fungi with less supervision. According to Rangel (*et al.,* 2006) the cultivation of the fungi relies on the interaction of a particular set of physical, chemical and biological factors. The important areas to note in the cultivation of oyster mushroom in order to balance the three factors include substrate selection, composting, pasteurization spawn running, fruiting and harvesting (Rangel *et al*., 2006).

2.5.1. Mushroom spawn production and spawn running

Mushrooms are to spawns as plants are to seeds; however, spawns are only pure mushroom mycelia (the vegetative part of the fungus) which are growing on a sterilized grain medium (Maheshwari, 2013). By nature fungi such as mushrooms produce spores as a means of reproduction, but these spores are too tiny to hold and to work with, therefore the technology of spawn (the vegetative method of mushroom propagation) production makes cultivation of mushrooms easier (Mensah, 2015). Additionally, the spores are likely to yield a new strain and performance would be unpredictable (Mensah, 2015). Mushroom spawns serve as growing material for mushroom production and they are prepared in a sterilized medium with a substrate for mushrooms to grow around.

Mostly grains are used to produce spawns due to their faster ramification of the substrate and their ease of planting (Stanley and Awi-Waadu, 2010). The first stage in the production of the spawn is the culturing of the mushroom mycelia on nitrified agar media before the starter culture is used to make the grain spawn. The grain spawn is then used to make the final fruiting substrate (Ogden and Prowse, 2004). After the mycelia is added to the grain, the grain and the mycelia are shaken 3 times at 4days intervals over a 14 day period for active mycelia growth (Mensah, 2015). Stanley and Awi-Waadu (2010) performed experiments on using different grains to produce mushroom spawns and then concluded that the best grain to use for mushroom spawn was white maize grain. The maize grain ensured faster mycelia growth than the red sorghum that was normally used. The factors that affect spawn preparation include CO_2, oxygen, light, pH, temperature and humidity (Nwanze *et al*., 2005). After the spawns are made or are bought, they are then mixed with the pasteurized compost for the mycelia to colonize the compost. The time needed for the mycelia to colonize the compost depends on the rate and distribution of the spawns, the moisture content, quality and nature of the compost and temperature; as such, a completed spawn run usually requires 14 to 21 days for the mostly used substrates (Mensah, 2015).

Spawn run is a term used to describe the situation whereby the mycelia colonize the substrate and use the available nutrients (Mensah, 2015). The mycelia take about 21 – 22 days to fully colonize sawdust substrate (Randive, 2012).

During spawn running, the required temperature is 20 - 25^{o}C with a humidity of 65 -70% and water content of substrate of 65% (Mensah, 2015). Many factors such as mushroom cultivar used, compost factors, sanitation etc. can determine the proper spawn growth (Royse, 2014). Mensah (2015) reported that inoculated bags must be kept in the dark until the mycelia fully invades the substrate (The reproductive phase is reached when the mycelia fully colonize the substrate and perhaps when some nutrients run out. It is only the reproductive structure that comes out of the substrate and forms the fruiting body.

2.5.2. Substrate selection

A substrate is any material that serves as a medium of growth for a living thing in which enzymes can act upon and break it to release nutrients for the growing organism. Rouse (2010) defined a substrate as any solid substance or medium to which another substance is applied and to which that second substance adheres. Mushroom is to a substrate as a plant is to a soil (Kwon and Kim 2004). A good substrate should be rich in nutrients, have good aeration and water holding capacity. Diego *et al.* (2011) grouped mushroom substrates into three; thus bulky (e.g., sawdust, straw bagasse etc.), concentrates (e.g., meals, brans, urea, etc.) and conditioners (e.g., Gypsum and calcium carbonates). If the mushroom is to be marketed in the fresh condition, then it must be a species that is acceptable on the basis of its taste appeal to the people in the area where it is cultivated. This can be determined for previously cultivated species by examination of import records, if available, or by testing for market acceptability with fresh mushrooms imported for that purpose. Once it has been determined that the species is acceptable to the local consumer, there remain other significant considerations before a decision to cultivate a particular species should be made. Both the temperatures necessary for vegetative growth and that required for fruiting must be considered in selection of an acceptable mushroom, and it should be pointed out that strain or dikaryotic stocks of species may differ in their temperature ranges and optimal values so that even within a single species selections can be made.

2.5.3. Pasteurization

Pasteurization is a partial sterilization of substrates at a temperature that destroys harmful microorganisms without major changes in the chemistry of the substrate. It is a term used to apply to the process of heating mushroom substrates, in order to reduce weeds, diseases and pests (Kurtzman, 2010). Pasteurization is used to reduce competitors in a substrate, thereby giving the mycelia an advantage over harmful organisms, allowing it to take over the substrates and eventually producing mushrooms (Mensah, 2015). Pasteurization removes competitive fungi thereby permitting faster and better uniform spawning as well as ensuring resistance to future infections (Ficior *et al*., 2006). Pasteurization occurs between 71^{O}C and 82^{O}C and anything more than that risks the proliferation of healthful microorganisms (Mensah, 2015). The compost must be pasteurized for at least 6 hours at 60^{O}C to kill possible pathogenic fungi and harmful bacteria (Kang *et al.*, 2004). As it is imperative that a soil must be prepared for the growth of plants, so it is for the growth of mushroom in order to destroy harmful microorganisms that might compete with the healthful microorganisms during the mushroom growth (Kurtzman, 2010).

2.5.4. Factors that affect oyster mushroom cultivation

Mushroom survival and multiplication are related to a number of factors, which may act individually or have interactive effects among them. Chemical composition, water activity, ratio of carbon to nitrogen, minerals, surfactant, pH, moisture, sources of nitrogen, particle size, and amount of inoculum, antimicrobial agents and the presence of interactions between microorganisms are considered as chemical, physical and biological factors that are linked to mushroom production (Eira, 2003). Oyster mushroom needs different environmental condition at each growing stage. During incubation, appropriate relative humidity of 65–70% and water content of 65% substrate is required (Kang, 2004).The main environmental factors encompass temperature, humidity, luminosity and air composition of the surrounding substrate, such as concentration of oxygen and carbon dioxide (AMGA, 2004).

According to Bellettini, *et al*., (2017), the growth and development of *Pleurotus* mushroom depends on both intrinsic (Composition of substrates, C:N ratio, Levels of spawning, PH,

Moisture and Particle size, aswellas Extrinsic factors: Humidity, light, temperature, and carbon dioxide-to-oxygen ratio are conditions, which typically determine when a mushroom will fruit (Chang and Buswell, 2008).

2.5.4.1. Composition of substrates

Substrates used in mushrooms cultivation have effect on chemical, functional and sensorial characteristics of mushrooms (Oyetayo and Ariyo, 2013). *Pleurotus* spp. is a saprophyte, and it extracts its nutrients from the substrate (grasses, wood and agricultural residues) through its mycelium, obtaining substances necessary for its development, such as carbon, nitrogen, vitamins and minerals (Urben, 2004). The low contamination might have occurred due to a high substrate quality. Therefore, it is important to keep the dry substrate in dry conditions. When contaminants, such as green molds, as Trichoderma or Aspergillus, are scarce in the substrate, they do not offer a competence for the mycelium of *Pleurotus* which quickly colonizes it.

The use of different types of substrate by fungus will depend on its capacity to secrete enzymes such as oxidative (ligninase, laccase, manganese peroxidase) and hydrolytic (cellulase, xylanase and tannase) enzymes which are involved in utilizing lignocellulosic substrates (Luz *et al*. 2012). According to Bellettini, *et al*., 2017), in fungus growth and development, both quality and quantity aspects (biological productivity and efficiency), are closely linked to nutrient type and growth conditions. For that, it is important to know the chemical composition of substrates before making its use in mushroom cultivation (Patil *et al*., 2010).

2.5.4.2. Ratio of carbon to nitrogen (C/N)

Mushrooms need to strike a balance in the substrate as the carbon and nitrogen ratio. The total carbon value in the C/N ratio represents the carbon contents, including recalcitrant cellulose and hemicelluloses (Ryu *et al*., 2015). According to Bellettini *et al*. (2015), the C/N ratio (28–30% carbon and 1% nitrogen) is an important condition for mushroom production (spawn running). However, recently, Schuttmann *et al* (2014) studied the effect of different natural substrates on versatile peroxidases activity in *Pleurotus sabidus.*

They showed that the highest versatile peroxidases activity was measured when fungus was cultured on biogas plant material residues, in which the C/N ratio was 10:1. Yang *et al* (2013a) reported the slower rate of spawn running on the cottonseed hulls substrate may be due to its high C/N ratio, because nitrogen deficiency is known to inhibit mycelial growth, whereas slow growth on perilla stalks substrate may be caused by an excess of nitrogen, which is known to delay the formation of the fruiting body). Li *et al* (2015) related that higher C/N value was beneficial to high levels of crude protein, amino acids, 50-nucleotides and equivalent umami concentration, while lower C/N value was beneficial to carbohydrate, polysaccharides and trehalose production.

2.5.4.3. pH

Each mushroom has its optimal pH range for development, and it is variable; for example, pH between 4.0 and 7.0 for the mycelium and 3.5 to 5.0 for formation of basidio carp (Urben, 2004). The optimum pH for mycelial growth and subsequent fruiting body development is obtained at between 6.5 and 7.0 (Kalmis *et al*., 2008). With fungal colonization, the substrate pH is reduced to values close to 4.0 for the reduction of organic acids, primarily oxalic acid in step preceding the cutting of the package fruiting crop of solid-state fermentation.

2.5.4.4. Moisture

Water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow (Oei and Nieuwenhuijzen, 2005). High moisture content in the substrate will result in difficult breathing for the mycelium, inhibiting perspiration, rendering the development of fruiting body impossible, even with elevated inoculum amounts or number of holes in mushroom cultivation packages, resulting in the development of non-desired organisms such as bacteria and nematodes (Urben, 2004).

Low moisture content will result in the death of the fruiting body. The optimum moisture content for growth and substrate utilization depends upon the organism and the substrate used for cultivation. Increasing moisture level is believed to reduce the porosity of the substrate, thus limiting oxygen transfer.

For this reason, the use of high moisture content limited the growth within the whole substrate, resulting in surface growth (Patel *et al*., 2009). According to Chang and Miles (2004), the appropriate moisture in the substrate should encompass a range between 50% and 75% in the substrate; enabling the satisfactory growth of *Pleurotus* species. Moisture above 70% makes the development of diseases and competing molds possible.

2.5.4.5. Particle size

A desirable property in solid-state fermentation is that its small particles (substrates can be cut into 5–6 cm) provide a larger surface area used by the microorganism (average bulk density of 428 lb/yard3) (Yildiz *et al.,* 2002). However, very small particles result in a compressed substrate, interfering with the aeration system and in oxygen used by microorganisms (Bellettini *et al*., 2015). Zhang *et al*. (2002) found that when straw was ground into too small particles, the mushroom yield decreased. On the other hand, particles with large size cause an increase in space between particles, thus improving the oxygen transfer related processes, however, limiting the surface area of the particles, which cause mass transfer processes (nutrients and moisture) required for the microorganism (Pandey *et al*., 2000).

2.5.4. 6. Levels of spawning

Increasing spawning rate shortens mycelial colonization time, primordia formation, the time to the first mushroom crop (Yang *et al*., 2013a) and narrows the gap of opportunity for competitor invasion (Stamets, 2000). Alananbeh *et al*. (2014) studied three levels of spawning (5%, 7.5%, and 10%) and related that the yield, biological efficiency, and total fruiting bodies increased as the percentage of cultivating P. *ostreatus* increased. A lower inoculum level may not be sufficient to initiate growth, whereas a higher level may cause competitive inhibition (Sabu *et al*., 2005). An increase in inoculum size enhanced the utilization of solid substrate, thereby improving laccase activity. However, with further increase in inoculum above the limits, laccase production is decreased due to a fast depletion of nutrients, resulting in a decreased metabolic activity (Patel *et al*., 2009)

2.5.4.7. Temperature and relative humidity

Pleurotus ostreatus can be widely cultivated, and it can adapt to different temperatures. It exists on every continent except Antarctica and grows throughout the year (Qu *et al*., 2016). Li *et al*. (2015) related that the substrate containing inoculum was subsequently kept in a darkened spawn-running room at 23 ^{o}C. According to Ahmed *et al*. (2013), for the cultivation of *Pleurotus* high-king, *Pleurotus ostreatus* and *Pleurotus geesteranus*, temperature of culture house was maintained at between 22 and 25 ^{o}C. Neelam *et al*. (2013) indicated that the optimal temperature for mycelium growth in oyster mushroom *Pleurotus florida* was 25–30 ^{o}C. Fruiting body development is often induced after drastically altering environmental circumstances (Pandey *et al*., 2008). Dahmardeh (2013) related that temperature was controlled by electric heaters at 25 ^{O}C for spawn running and at 17–20 ^{o}C for fruiting body formation. Lower temperatures and dry condition reduced stalk height and cap size of mushroom (Sher *et al*., 2010). On the other hand, high temperatures in growing environment can reduce mushroom development in different ideal growth tracks, allowing the development of competitive microorganisms better adapted to high temperatures (Urben, 2004). According to Li *et al*. (2015), the appropriate humidity during the darkened spawn-running and mycelia stimulation should encompass a range between 60–75% and 85–97%, respectively, in the environment, enabling a satisfactory growth of *Pleurotus* species. High humidity is favorable for pining and fruiting (Pandey *et al*., 2008).

2.5.4.8. Oxygen and carbon dioxide concentration

Since growth of the fungus produces carbon dioxide as it decomposes the substrate, introduction of 'outside' air reduces carbon dioxide build up and increases oxygen levels. Fungal mycelium is extremely tolerant of carbon dioxide, thriving at 20% CO_2 levels. Oxygen is required for formation of fruit bodies.

A significant decrease in ambient CO_2 level and increase in oxygen is critical for the initiation and development of primordia. Thus sufficient air circulation within a mushroom fruiting site is vital. Excessive influx of outside air, however, greatly affects both temperature and humidity of the environment (Stamets, 2000).

2.5.4.9. Light

Photoperiod is not necessary to induce the primordium formation but it is needed for fruiting body production. There are species that develop in the dark and other ones in partial light. Some mushrooms such as *Pleurotus* species or L. *edodes* require light for primordia formation (Nakano *et al*., 2010). In general, the photoperiod of mycelia stimulation to promote mushroom fruit bodies formation should be sufficient to read a sheet of paper (200– 640 lux 8–12 h a day _1) at a temperature compatible with the mushroom (Ahmed *et al*., 2013). Environments that have a lot of light can cause paleness, deformations, elongated stipe (Urben, 2004). In the complete absence of light, oyster mushrooms will form no cap but stipes (mushroom stalks) forming a coral-like structure (Oei and Nieuwenhuijzen, 2005).

2.6. Benefits of Mushroom

2.6.1 Nutritional Value of Mushroom

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 human body (Belewu, 2005; Oei, 2003). Quality of mushroom protein is superior to that of vegetable protein and proteins have high digestibility (Bhupinder and Ibitwar, 2007). According to Onokpise *et al*., 2008, musroom 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. Mushrooms are rich in essential amino acids that cannot be synthesized by our body as well as the most commonly occurring non-essential amino acids.

They are low in calories, carbohydrates and calcium. Mushrooms also contain a high proportion of unsaturated fat. 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, 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. 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).

2.6.2. Medicinal Values of Mushrooms

The second major attribute of mushrooms is their medicinal property which has long been recognized in China, Korea, and Japan. Many mushrooms possess significant medicinal attributes like hypocholesterolemia, hypoglycemic and hypotensive properties. Mushrooms also exhibit strong anti-oxidant and hepato-protective properties. Some of *Pleurotus* species have been shown to possess a number of medicinal properties, such as antitumor, immune modulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antiplatelet-aggregating, antihyperglycemic, antimicrobial and antiviral activities (Rai and Arumugonathon, 2003). According to Chang and Buswell (2003), the term "mushroom nutraceutical" is used for a new class of new compounds extractable from either the mycelium, or the fruiting body of the mushroom. Mushroom nutraceuticals possess both nutritional and medicinal properties (Rai and Arumugonathon, 2003). Due to present day, high-pressured work demands resulting in great stress to the human body and causing a weakening of the human immune system and pose many new diseases. These have developed because of lower natural body resistance (Wasser, 2002a). There is some evidence that the beneficial treatment of these diseases can be obtained by consumption of mushrooms as a functional food, or with extracted biologically active compounds as a dietary supplement, in order to enhance immune response of the human body, thereby increasing resistance to disease and, in some cases, causing regression of a diseased state.

2.6.3. Reduction of Environmental Pollution

Reduction of environmental pollution is done through use and management of vast quantities of organic wastes as growth media (substrate) for mushroom cultivation. Organic solid wastes are a kind of biomass, which are generated annually through the activities of the agricultural, forest and food processing industries. They consist mainly of three components: cellulose, hemicellulose and lignin. The general term for these organic wastes is lignocellulose.

It is a common knowledge that lignocellulosic wastes are available in abundance both in the rural and urban areas (Chang and Buswell, 2003). When carelessly disposed of in the surrounding environment by dumping or burning, these wastes are bound to lead to environmental pollution and consequently health hazards. It should be recognized that the wastes are resources out of place and their proper management and utilization would lead to further economic growth as well (Wasser, 2002b). Huge quantities of lignocellulosic and other organic waste residues are generated annually through the activities of agricultural, forest and food processing industries (Imtiaj and Rahman, 2008).

The same author indicated that, the world produced 952 million tons of bagasse; 6,476,000 tons of coffee pulps; 6,152, 000 tons of coffee wastes; 9,386 thousand of cottonseed hulls; 14,073,000 tons of waste paper; and 325 thousand tons of sisal wastes. Million tons of sawdust, wood chips, and water hyacinth are also available worldwide (Chang and Buswell, 2003). The same author indicated that, all these lignocellulosic waste residues can be used as substrate for growing mushrooms; otherwise, they would cause health hazards. Mushroom enzymes can break down lignin, cellulose and hemicellulose present in these organic materials into simpler molecules, which the mushrooms then use for their growth and metabolism (Wasser, 2002b).

3. MATERIALS AND METHODS

3.1. Experimental Site

The study was conducted at Haramaya University from February 2018 to July 2018. The experimental site was at the Mushroom Research, Production and Training Laboratory of Haramaya University located within the campus at 42°3ˡE longitude, 9°26ˡN latitude and at altitude of 1980 m.a.s.l. (AUA, 1996).The experimental room average minimum and maximum temperatures were 12.96°C and 25.72°C respectively. Relative humidity was in the range of 57.67-91.93% have been managed by an instrument known as data logger (Hygrometer)

3.2. Sources of Experimental Materials

3.2.1. Source of Spawn

Pure cultures of three edible oyster mushroom species (*Pleurotus ostreatus, Pleurotus florida*, and *Pleurotus sajor–caju*) were obtained from Mushroom Research, Production and Training Laboratory of Haramaya University (MRPTLH). The pure cultures were initially multiplied on sterilized potato dextrose agar (PDA) medium to get enough stock culture of the pure species needed to prepare the required quantity of spawn (Sara, 2007). Stock culture was grown by transferring the cultures into flasks containing grain (maize) as described under section 3.2.3.1

3.2.2. Source of Growth Substrates

Leaves of *Eichhornia crassipes (*Water hyacinth) were collected from Lake Tana, while sugarcane bagasse and Leaves of *Argemone ochroleuca* were collected from Metahara Sugar Factory, and Haramaya University and its surroundings, respectively.

3.2.3. Spawn Preparation

3.2.3.1. Preparation and Sterilization of Spawn Substrate

Mother spawn was prepared according to the procedure described by Singh and Chaube (1995). Nine kg of the maize seeds were boiled in 15 liters of water for 15 minutes and then allowed to remain soaked in the hot water for another 25 minutes.

The water was drained off and the grains were put in a sieve to dry overnight. Next day, 120 g calcium sulfate and 30 g calcium carbonate were mixed with each 9 kg of the boiled grains. The calcium sulfate and calcium carbonate were used to maintain the pH close to neutrality (6.8-7.5) and reduce grain adhesion (Smith and Love, 1995). The supplemented grains were filled in half or one liter serializable bottles (225 or 450 g/ bottle). Bottles were plugged with non-absorbent cotton and sterilized in an autoclave at 121^{o}C for half hour. After cooling, the bottles were inoculated with pure stock culture by taking a piece of agar with mycelium. The culture and grains were thoroughly mixed to uniformly distribute the mycelium and were incubated at 25^{o}C. After all the grains were fully covered with mycelium, the bottles were used as mother spawn.

3.2.3.2. Inoculation of Spawn Substrate

Inoculation of spawn substrate in flasks was done by transferring a small amount of agar with mycelium from stock culture to substrate flask under aseptic condition over a flame. The mycelium was thoroughly mixed with grains and the flask was then incubated at 25^{o}C in an incubator. After 7 days of incubation, the content of flasks was shaken to mix the mycelium uniformly over the grains. The flask was then incubated until all grains become fully covered with mycelium in about 15 days. Contamination free flasks were used for further multiplication of spawn (Sara, 2007).

3.2.3.3. Multiplication of Spawn from Mother Culture

Based on the amount of spawn required for the experiment, the spawn substrate was prepared and sterilized under controlled condition.

The substrate flask were inoculated under aseptic condition in laminar flow cabinet by transferring a small quantity of mycelium covered grains from master spawn flask to freshly sterilized spawn substrate flasks. Inoculated substrate was incubated and mycelium was allowed to grow. One flask of master spawn culture was used to make 30-40 flasks of spawn (Kidane, 2006).

3.2.4. Preparation of Substrates for Inoculation

The selected leaf samples were chopped to 2–4 cm size pieces, and then seven different substrates and substrate combination preparations were made for each of the three mushroom species (*Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida*) by mixing leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca* (Pale Mexican prickly poppy) and sugarcane bagasse in varying proportions as shown in Table 1. The substrates were spreaded and allowed to dry for about one week at a regular turning interval of 3 days. Then it sterilized for 2 hours at a temperature of 121°C in a dry fire-heated drum to avoid contamination. The sterilized substrates was kept in a clean room and allowed to cool down over night (for 12 hours) (Atikpo *et al*., 2008). After cooling, the three substrates (sugarcane bagasse and dried leaves of Water hyacinth and *Argemone ochroleuca*) were soaked in 80 liters of water and 110ml of 2% formalin for about 24 hours for further sterilization (Diriba *et al*., 2013).

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 water stopped dripping, the substrate was ready for inoculating. The substrate was then filled into 21x3 a sterile polyethylene bags arranged for three species spawning. Thus, 21 plastic bags for each species or 63 transparent plastic bags for all 3 species were filled with substrates weighing 2 kg of dry weight. Sixty three (63) transparent plastic bags were arranged corresponding to the three sets of seven substrate preparations in triplicates.

Among these, seven substrate preparations in triplicates were used (7x3) for *Pleurotus ostreatus,* seven substrate preparations in triplicates (7x3) for *Pleurotus sajor-caju,* and seven substrate preparations in triplicates (7x3) for *Pleurotus florida*. The bags were labeled or arranged according to substrate type and mushroom species they contained.

Table 1. Arrangements of Growth Substrates for each Selected Mushroom Species.

Mushroom species	Growth substrates preparation						
	S1	S2	S3	S4	S5	S6	S7
Pleurotus ostreatus	50%LEC +50%LAO	50%LEC +50%SCB	50%LAO 50%SCB	100% SCB	100% LEC	100% LAO	33%SCB +33%LEC +33%LAO
Pleurotus sajor-caju	50%LEC +50%LAO	50%LEC +50%SCB	50%LAO +50%SCB	100% SCB	100% LEC	100% LAO	33%SCB+ 33%LEC+ 33%LAO
Pleurotus Florida	50%LEC +50%LAO	50%LEC +50%SCB	50%LAO +50%SCB	100% SCB	100% LEC	100% LAO	33%SCB+ 33%LEC+ 33%LAO

SCB=Sugar bagasse, LEC=Leaves of *Eichhornia* crassipes, LAO=Leaves of *Argemone ochroleuca*

3.2.5. Spawning and Spawn Running

After the substrate preparation was over, the wet substrate was spreaded on a clean alcohol swabbed polyethylene sheet. Then after, 200 g (which was equal to 10% of the weight of the substrate mixed) of the edible mushroom (*Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida*) spawn was added and thoroughly mixed with the substrate kept in the polyethylene bags using sterile spoons under the laminar flow hood.

Then, rubber bands tied the open ends of the bags and nine small holes were made using sterile needle to allow air exchange of bags (Dawit, 2008).

3.2.6. Incubation, Control of the Environment and Cropping

All inoculated bags were incubated for 18 days at 28-31°C on shelves 15cm apart in a completely randomized design in a clean and disinfected dark room according to (Dawit, 1998). Fresh air exchange between the dark room and the outside environment was allowed by opening windows at night and closing during the daytime to enhance the quick colonization of the substrate. After fully colonization, the bags was transferred to the cropping room, whose environment was kept illuminated by sunlight through the improvised windows and a temperature and humidity of 29°C and 75-85%, respectively, was maintained by sprinkling the bag with water twice a day. The temperature and humidity range was adjusted by a room heater and by spraying water to the walls and floors of the cropping room and additionally an instrument known as Data logger was used. Formation of a complete mushroom occurs one week after the colonized substrates was transferred to the cropping room (Oei, 2005). After 32 days of incubation, fully matured mushroom species on each substrate was collected and analyzed for their proximate composition (moisture, crude protein, crude fat, crude fiber, ash and carbohydrate content) and biological efficiency.

3.3. Experimental Design

The experiment was designed in a Completely Randomized Design (CRD) with three replications involving a 3 X 3 factorial arrangement with seven preparations of growth substrates (Sugarcane Bagasse, Leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca* (Pale Mexican prickly poppy) and their four different mixtures) and three selected *Pleurotus* species namely: *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida*.

3.4. Data Collection

3.4.1. Phenological Observation

Phenological observation: data on days to fully colonization of a substrate by mycelium (DMF), days to appearance of pin heads formation (DPF) and days to formation of fruiting bodies (DFF) from the day of spawning in the selected substrates was recorded.

3.4.2. Total Yield

Weight of fresh fruiting bodies harvested from each substrate block was measured using sensitive balance.

Data on weight of mushroom from each substrate block at first, second and third flush harvesting stages were recorded separately and their total weight was considered as total yield which were also used for calculating biological efficiency (Dawit, 1998).

3.4.3. Determining Biological Efficiency of Cultivated Mushroom

The biological efficiency (BE) of the mushroom species was calculated using the formula recommended by Chang and Miles, (1989) as follows:

$$\% \, \mathbf{BE} = \frac{W_2}{W_1} = \frac{\boldsymbol{weight\ of\ fresh\ musroom\ harvested\ per\ bag}}{\boldsymbol{weight\ of\ dry\ substrate\ \ before\ inoculation\ per\ bag}} * \mathbf{100}$$

3.4.4. Determination of proximate composition (Analysis)

Proximate analysis (percentage of moisture content, percentage of crude protein, percentage of crude fiber, percentage of crude fat, percentage of ash content and carbohydrate content), of the three edible oyster mushrooms (*Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida*) was performed following the methods described by AOAC (1995).

3.4.4.1. 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 (%MOC) was generally calculated using the following formula:

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\boldsymbol{Wet\ Weight - Oven\ Dry\ Weight}}{\boldsymbol{Wet\ Weight}} \times \mathbf{100}$$

3.4.4.2. 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 priory 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 (Na2SO4 was mixed with CuSO4 in the ratio of (10:1) of 1g has also been added into digestion flask followed by the addition of 10ml of concentrated H2SO4. 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% of 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% boric acid 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).

Percentage nitrogen was calculated using the following formula.

$$\% \textbf{ of Nitrogen } = \frac{\mathbf{V_{HCl} \text{ in litre} * N_{Hcl}(ca.0.1) * 14 \frac{g}{mol}}}{\textbf{Sample weight in g on dry matter basis (db)}} * \mathbf{100}$$

Then Crude protein was determined as follows,

% CP = 6.25 x % N

3.4.4. 3. Determination of Crude 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 0C 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:

(%) Crude Fat Content (CFC) = ((W_2-W_1))/ (Sample mass in g on dry matter bases (db)) x100

Where: W_2 = Weight or mass of flask and fat (oil) extract, W_1 = the mass of extraction or dried flask

3.4.4.4. Determination of Crude 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% H2SO4 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 550^0C in a muffled furnace (AOAC, 1990).

The fiber content in percentage was calculated using the formula shown below:

(%) Fiber Content= W_3-W_2/ W_1 X 100

Where, W_3= Weight of crucible with dry residue before ashing, W_2 = Weight of crucible with ash after ignition and W_1= Weight of sample used in g

3.4.4.5. 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 550^0C 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).

Total ash content was determined the following method:

(%) (Ash) AC= W_2 (g)-W_1 (g) / W (g) X 100

Where: W_1 = Weight of empty crucible, W_2 = Weight of crucible + ash, and W = Weight of sample.

3.4.4.6. Determination of Total Carbohydrate

The available carbohydrate content was determined using the following equation (Raghuramulu *et al*., 2003):

% Carbohydrate = 100 − (moisture content +crude fat + crude protein + total ash + crude fiber) /100

3.5. Data Analysis

The data collected during the study was subjected to analysis of variance (ANOVA) for factorial combination of CRD following the procedure described by Gomez and Gomez (1984) and the Fisher-Hayter procedure least significance difference (modified LSD) at 5% level of significant was used for the treatment mean separation. Correlation between the treatments was also computed using SAS computer.

4. RESULTS AND DISCUSSION

4.1. Phenological Observation, Effect of Organic Substrates on Growth and Yield of the Selected *Pleurotus* species

4.1.1. Days for Mycelium to Fully Colonize of Substrate

Days for *Pleurotus* mycelium to fully colonize substrate (DMF) was largely affected by the type of substrate and substrate combination ($p<0.05$). From the three organic substrates and their combination, the fastest fully colonization of substrate (17.33 days) by mycelium of *Pleurotus florida* was recorded for the bag containing100% SCB (Table 2). Sugarcane bagasse supported the fast mycelial growth during cultivation of *Pleurotus* species. This was because of the presence of more amorphous lignocellulosic material in sugar cane bagasse, which is easy to be attacked by the fungus mycelia (Singh, 2005). The second best substrate in fully mycelium colonization was recorded for combination of the three substrates: sugarcane bagasse, Leaves of *Eichhornia crassipes* and *Argemone ochroleuca* leaves (22.67 days). This could be the nature of the three substrates in combination with their nutritional and chemical and composition, and also narrow C/N ratio. The slowest fully colonization of substrate (28.33days) was recorded for the bag containing dried *Argemone ochroleuca* leaves. This might be due to its high C/N ratio or it may be because of absence of sufficient fungal nutrient. Yang and his coworkers (2013) reported that, low nitrogen level can stimulate ligninolytic enzyme production, whereas a high nitrogen level represses it. The results of the present study is in harmony with the findings of Shah *et al*. (2004) except for *Pleurotus* species grown on dried *Argemone ochroleuca* leaves. Shah and his coworkers reported that the spawn running took 16-25 days after inoculation. But the current results are in accordance with those reported by Stanley, (2010) that the duration of mycelia invasion differs depending on the type of substrate used.

Pleurotus ostreatus, *Pleurotus florida* and *Pleurotus sajor-caju* had no significant effect on mycelium colonization of the substrate ($p<0.05$). This could be due to the nature of the *Pleurotus* species lignocellulosic enzymes during the invasion time. Sugar cane bagasse and dried *Argemone ochroleuca* leaves recorded significant differences ($p<0.05$) with regard to days to fully colonization of substrate by mycelium (Table 2).

Interaction of substrates with species of the mushroom was non-significant for days to fully colonization of substrate by mycelium.

Table.2. Days for Mycelium to fully colonize a substrate (DMF).

	Pleurotus species		
Treatments	*Pleurotus ostreatus*	*Pleurotus sajor- caju*	*Pleurotus florida*
100% SCB(**control**)	17.67^{Dd}	17.67^{Dd}	17.33^{Dd}
100% LEC	23.67^{Bb}	24.67^{Bb}	23.00^{Db}
100% LAO	27.63^{Aa}	27.33^{Aa}	28.33^{Aa}
50% SCB+50% LAO	24.00^{Bb}	24.00^{Bb}	26.00^{Bb}
50% SCB+50% LEC	23.00^{Bb}	23.00^{Bb}	24.67^{Cb}
50% LAO+50% LEC	25.33^{Bb}	24.67^{Bb}	25.00^{Bb}
33%SCB+33%LAO+33%LEC	22.67^{Cc}	23.67^{Cc}	23.67^{Cc}
CV=5.86			
LSD=2.29			

CV=Coefficient of Variation, LSD= Least Significant Difference, LAO= leaves of *Argemone ochroleuca,* LEC= *Eichhornia crassipes,* SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.1.2. Days to Pine Head Formation

The earliest number of days (20.67) to pin-head formation after *Pleurotus* mycelium fully colonizes substrate was recorded for *Pleurotus ostreatus* cultivated on sugarcane bagasse alone (Table 3). These three *Pleurotus* mushroom cultivated on sugarcane bagasse recorded the shortest time for pine head formation, 20.67, 21.33 and 22.67 to *Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida* respectively. The second best earlier pin-head formation was recorded on combination of the three substrates: sugarcane bagasse, Leaves of Water hyacinth and *Argemone ochroleuca* (24.33 days). The slowest pin-heading was

observed for *Pleurotus sajor-caju* with dried *Argemone ochroleuca* leaves (30.00 days). This might be the chemical nature (phenolic contents) of the substrate which becomes difficult to be degraded. The presence of excess nitrogen also delay growth period of *Pleurotus* mushroom.

Pleurotus ostreatus reached pin-head formation earlier than *Pleurotus florida* and *Pleurotus sajor-caju*, and this could be due to nature of the fungus (*Pleurotus ostreatus*) which is also known as wood mushroom that it may be more competent in degrading the woody substrates as compared to the other species. But statically the difference observed between the species with regard to the time taken from spawning to pin head formation was non- significant ($p<0.05$). Interaction of substrates and substrate combination with species of the mushroom was non-significant for the days Pin head formation.

Table.3. Days to pin head formation (DPF).

	Pleurotus species		
Treatments	*Pleurotus Ostreatus*	*Pleurotus sajor- caju*	*Pleurotus florida*
100% SCB(**control**)	20.67^{Dd}	21.33^{Dd}	22.67^{Dd}
100%LEC	25.02^{Ca}	26.33^{Ba}	26.33^{Ca}
100%LAO	29.33^{Aa}	30.00^{Aa}	29.67^{Aa}
50%SCB+50%LAO	26.67^{Bb}	27.00^{Bb}	27.67^{Bb}
50%SCB+50%LEC	25.00^{Cb}	25.00^{Cb}	26.67^{Cb}
50%LAO+50%LEC	27.00^{Bb}	27.33^{Bb}	27.67^{Bb}
33%SCB+33%LAO+33%LEC	24.33^{Cc}	25.33^{Cc}	25.67^{Cc}
CV=3.68			
LSD=1.58			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC=*Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.1.3. Days to Fruiting Body Formation

Pleurotus mushroom had no significant effect on days to fruiting body formation of the substrate ($p<0.05$) (Table 4). The fastest fruiting body formation after spawning (23.67days) was observed for *Pleurotus ostreatus* grown on sugarcane bagasse alone followed by combination of the three substrates: sugarcane bagasse, dried leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca* (25.67 days). This might be the nature of the plant. The slowest fruiting body formation (34.33 days) was recorded *Pleurotus sajor-caju* on the bag containing dried leaves of *Argemone ochroleuca.* This could be the high lignin content which is difficult be degraded by fungal enzyme, high phenolic compounds and high antifungal characteristics.

Table.4. Days to fruiting body formation (DFF).

	Pleurotus species		
Treatments	*Pleurotus Ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB(**control**)	23.67^{Dd}	25.00^{Dd}	24.00^{Dd}
100%LEC	28.33^{Bc}	27.67^{Cc}	27.33^{Cc}
100%LAO	33.00^{Aa}	34.33^{Aa}	32.33^{Aa}
50%SCB+50%AO	28.67^{Bb}	29.33^{Bb}	30.67^{Bb}
50%SCB+50%LEC	26.33^{Cc}	28.67^{Bb}	30.00^{Bb}
50%LAO+50%LEC	28.00^{Bb}	29.00^{Bb}	28.33^{Cb}
33%SCB+33%LAO+33%LEC	25.67^{Cc}	27.67^{Cc}	29.67^{Bb}
CV=3.25 LSD=1.52			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC=*Eichhornia crassipes*, SCB=Sugar cane bagasse. CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

In the fruit body development phase, the occurrence of a lower C/N ratio in the cultivation substrate is more favorable. Besides affecting the formation of fruit bodies, nitrogen excess may have affected the degradation of lignin, which may prevent the mycelium from developing. The results of this study are in harmony with Robert (1996) who reported that long period of fruiting body formation is related to high nitrogen content of a different lignocellulosic substrate (enzymatic activity). However the interaction between substrates and mushroom species were significant in terms of taken to fruit body formation.

4.1.4. Effects of Substrate and Substrate Combination on Total Yield of Selected *Pleurotus* Species

In terms of yield performance, *Pleurotus ostreatus* grown on sugarcane bagasse demonstrated better performance compared to all the treatments used with a total yield of 1697.00g in Table 5. The second better yield of mushroom (1485.00g and 1443.67g) were recorded for *Pleurotus florida* and *Pleurotus ostreatus* cultivated on sugarcane bagasse alone and mixture of bagasse, leaves of *Eichhornia crassipes* and *Argemone ochroleuca* respectively.

The lowest yield of mushroom (556.7g) was recorded for *Pleurotus sajor- caju* cultivated on dried leaves of *Argemone ochroleuc*a. Vijayakhader, (2002) reported the inhibitory effect of phenolic compounds leading to lower fruit body formation and as a result low value had formed. Lowest yield of mushroom also could be due to the decreasing nutrient content of the substrate consumed by mushroom during growth from one flush stage to the next.

Table 5. Effect of substrate types on total yield (g /2kg) of *Pleurotus* species.

	Pleurotus species		
Treatments	*Pleurotus ostreatus*	*Pleurotus sajor- caju*	*Pleurotus florida*
100%SCB (**control**)	1697.00^{Aa}	1330.33^{Ac}	1485.00^{Ab}
100%LEC	1178.3^{Db}	973.7^{Dd}	1217.00^{Bb}
100%LAO	714.7^{Ff}	556.7^{Fg}	616.7^{Ff}
50%SCB+50%AO	834.3^{Ee}	762.3^{Ee}	796.00^{Ee}
50%SCB+50%LEC	1316.3^{Cc}	1113.00^{Bb}	1380.7^{Ac}
50%LAO+50%LEC	769.00^{Ee}	715.00^{Ee}	741.00^{Ee}
33%SCB+33%LAO+33%LEC	1443.7^{Bb}	1310.00^{Ac}	1391.00^{Ab}
CV=6.85			
LSD=120.11			

CV= Coefficient of Variation, LSD= Least Significant Difference, LAO= leaves of *Argemone* ochroleuca, LEC=*Eichhornia crassipes*, SCB=Sugar cane bagasse. CV= Coefficient of Variation, LSD= Least Significant Difference, LAO= leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes,* SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.1.5. Effect of substrate and substrate combination on biological efficiency

Biological efficiency which is used as a measure of substrate conversion into mushroom (Oseni *et al*., 2012) was calculated on the basis of percentage fresh mushroom production per unit dry weight of substrate. The three species of *Pleurotus* mushroom were also evaluated for their biological efficiency (BE) and the results are provided below in table 6. The substrates types significantly ($p<0.05$) affected the biological efficiencies (BE) the *Pleurotus* mushroom.

The highest values of biological efficiencies (71.67%, 70.00% and 69.67) were obtained for *Pleurotus ostreatus, Pleurotus sajor- caju* and *Pleurotus florida* grown on SCB alone respectively (Table 6). These higher values of BE could be because of narrow C: N ratio of substrates, high cellulose content and low lignin content of the substrate. The second comparable value of biological efficiency were obtained for *Pleurotus ostreatus, Pleurotus sajor- caju* and *Pleurotus florida* grown on 33.33% of SCB, LEC and LAO (68.67%,66.33% and 64.00%) respectively. This finding is supported by Owaid *et al*. (2015), which stated that productivity and biological efficiency were increased in some mixtures when compared with wheat straw alone.

Table.6. Biological efficiency of selected *Pleurotus* species.

	Pleurotus species		
Treatments	*Pleurotus ostreatus*	*Pleurotus sajor- caju*	*Pleurotus florida*
100%SCB (**control**)	71.67^{Aa}	70.00^{Aa}	69.67^{Aa}
100%LEC	60.67^{Dd}	58.00^{De}	56.33^{De}
100%LAO	43.00^{Gg}	40.67^{Gh}	39.33^{Ih}
50%SCB+50%AO	58.00^{Ee}	55.67^{Ef}	53.00^{Eg}
50%SCB+50%LEC	63.33^{Cc}	61.00^{Cd}	58.67^{Ce}
50%LAO+50%LEC	46.33^{Ff}	44.33^{Ff}	42.00^{Fg}
33%SCB+33%LAO+33%LEC	68.67^{Bb}	66.33^{Bc}	64.00^{Bd}
CV=2.15			
LSD=2.01			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC=*Eichhornia crassipes*, SCB=Sugar cane bagasse.CV=Coefficient of Variation, LSD= Least Significant Difference, LAO= leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes,* SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

On the other hand, relatively lower BE (39.33%, 40.67% and 43.00%) were obtained from the *Pleurotus florida* and *Pleurotus sajor- caju* and *Pleurotus ostreatus* grown on leaves of *Argemone ochroleuca* alone respectively. The current result shows BE value ranged from 39.33% to71.67% that was close to the finding of Moonmoon *et al* (2010) who observed that the BE ranged from 45.21% to 125.70% in case of oyster mushroom on saw dust and rice straw.

4.2. Effect of Growth Substrate and Substrate Combinations on Proximate Compositions of Selected *Pleurotus* Species.

4.2.1. Moisture Content

Interaction effect of growing substrates and types of species was significant ($p<0.01$) in relation to moisture content of the mushrooms (Table 7). The maximum moisture contents of 88.93 % and 88.53% were obtained from *Pleurotus ostreatus* grown on 100% LEC and 100% SCB respectively, whereas, the least moisture content (80.40%) was recorded for *Pleurotus ostreatus* grown on the 100% LAO, this indicates that leaf of *Argemone ochroleuca* has poor water holding capacity which in turn affects the percentage of moisture content in *Pleurotus ostreatus.* So, the difference in moisture content could be due to the difference in water holding capacity of the growing substrate and also the type of *Pleurotus* mushroom. The type of substrates and substrate combinations used for growth and type of *Pleurotus* mushroom could affect the percentage of moisture content in *Pleurotus* mushroom. The present results are in agreement with those of Hamid *et al.* (1996) who reported that moisture contents of most *Pleurotus* species lie between 80-90%.

Table 7. Percentage moisture contents of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB (**control**)	88.53^{Bb}	86.40^{Bc}	85.40^{Bd}
100%LEC	88.93^{Aa}	87.80^{Ab}	86.80^{Ac}
100%LAO	80.40^{Ff}	82.40^{Gg}	81.40^{Gh}
50%SCB+50%AO	85.53^{Ee}	83.40^{Fg}	83.80^{Fh}
50%SCB+50%LEC	87.50^{Cc}	85.20^{Ce}	85.80^{Cd}
50%LAO+50%LEC	86.67^{Dd}	85.70^{De}	84.80^{Df}
33%SCB+33%LAO+33%LEC	85.53^{Ee}	84.20^{Ef}	84.60^{Eg}
CV=0.23			
LSD=0.32			

CV=Coefficient of Variation, LSD= Least Significant Difference, LAO= leaves of Argemone ochroleuca, LEC= *Eichhornia crassipes,* SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.2.2. Crude Protein

Crude protein contents of selected *Pleurotus* species were significantly ($p<0.05$) affected by the type of growth substrate and substrate combinations (Table 8). The highest percentage of crude protein (35.52%, 33.86% & 31.96%) were obtained for *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida* grown on 100%SCB alone respectively, followed by *Pleurotus ostreatus* grown on 33% SCB+33% LAO +33% LEC% (31.67%). So the highest value of crude protein might be due to higher C/N value of the growing substrate. The lowest percentage of crude protein (14.38%) was obtained for *Pleurotus sajor-caju* grown on 100% LAO which could be due to low nitrogen content of this substrate. This finding showed that Crude protein content of selected *Pleurotus* mushroom grown on all substrate and substrate combination was significantly ($p<0.05$) different from each other. Crude protein content obtained in the current study was almost close to the value observed by Khan (2010) who

reported values of Crude protein in the range from 17-42. The values of crude proteins obtained from selected *Pleurotus* species grown on 100% SCB, 50% SCB+50% LEC and 33% SCB+33% LAO +33% LEC were also similar with the values determined by Chang *et al*. (2003 who reported crude protein content of *Pleurotus* species grown on various kinds of substrates were in the range of 26.9–37.2%, 26.6–35.5% and 26.6–35.6%. This similarity could be due to the ability of *Pleurotus* mushroom to degrade and use the nitrogen content of the selected growth substrates properly and effectively, and also as suggested by Singh and Kumar (2012), could be due to *Pleurotus* species have also the ability to fix nitrogen from air.

Table 8. Percentage of crude protein of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB (**control**)	35.52^{Aa}	33.86^{Ab}	31.96^{Ab}
100%LEC	25.00^{Dd}	22.02^{Ee}	24.78^{Dd}
100%LAO	18.75^{Gg}	14.38^{Gi}	16.08^{Gh}
50%SCB+50%AO	23.75^{Ee}	24.63^{De}	22.13^{Ee}
50%SCB+50%LEC	28.08^{Cc}	27.73^{Cc}	26.13^{Cc}
50%LAO+50%LEC	21.04^{Ff}	18.75^{Ff}	18.85^{Ff}
33%SCB+33%LAO+33%LEC	31.67^{Bb}	30.67^{Bb}	29.17^{Bb}
CV=6.43			
LSD=2.6			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of Argemone ochroleuca, LEC= Eichhornia crassipes, SCB=Sugar cane bagasse. CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.2.3. Crude Fat

The highest crude fat contents (1.91%) obtained for *Pleurotus florida* and *Pleurotus sajor-caju* grown on 100%SCB alone followed by *Pleurotus sajor-caju* and *Pleurotus florida* grown on 33% SCB+33% LAO +33%LEC and 50%LAO+50% LEC (1.87%) (Table 9). Whereas the lowest percentages of Crude fat (1.21% &1.28%) were obtained for *Pleurotus ostreatus* grown on 100% LEC and 100% LAO respectively. Crude fat content of *Pleurotus ostreatus* grown on 100%SCB and 50%SCB+50%LAO were not statically ($p<0.05$) significant. Crude fat content of *Pleurotus sajor-caju* and *Pleurotus florida* grown on100% SCB, 100% LEC, 50%SCB+50%LEC,50% LAO+50% LEC and 33% SCB+33% AO +33% LEC were not statically ($p<0.05$) significant. The current results obtained in this study which was ranged from 1.21%-1.91% were between the values obtained by Anthony (2007) who reported from 0.6-3% crude fat for mushrooms.

Table 9. Percentage of crude fat of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB(**control**)	1.60^{Bb}	1.91^{Aa}	1.91^{Aa}
100%LEC	1.21^{Cc}	1.81^{Aa}	1.81^{Aa}
100%LAO	1.28^{Cd}	1.65^{Cc}	1.65^{Cc}
50%SCB+50%LAO	1.63^{Bb}	1.79^{Ba}	1.79^{Ba}
50%SCB+50%LEC	1.72^{Ab}	1.84^{Aa}	1.84^{Aa}
50%LAO+50%LEC	1.36^{Cc}	1.87^{Aa}	1.87^{Aa}
33%SCB+33%LAO+33%LEC	1.75^{Ab}	1.87^{Aa}	1.87^{Aa}
CV=4.13			
LSD=0.12			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of Argemone ochroleuca, LEC= *Eichhornia crassipes*, SCB= Sugar cane bagasse. CV= Coefficient of Variation, LSD=Least Significant Difference, LAO= leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.2.4. Crude Fiber

The highest crude fiber content(12.82% &12.06%) were obtained from *Pleurotus ostreatus* grown on 100%SCB and 33% SCB+33% AO +33% LEC (Table 10). The least crude fiber content (8.22%) was recorded from *Pleurotus sajor-caju* and *Pleurotus florida* grown on 100% LAO and followed by *Pleurotus ostreatus* grown on 100% LAO (8.48%). The crude fiber content of *Pleurotus ostreatus* grown on 100%LEC, 50%SCB+50%LAO and 50% SCB+50 %LEC had no significant difference. The current results obtained in this study were close to the values obtained by Alananbeh *et al*. (2014) who reported from 5-15% Crude Fiber for *Pleurotus ostreatus* cultivated on date-palm leaves mixed with other agro-wastes.

Table 10. Percentage of crude fiber of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus Florida*
100%SCB (**control**)	12.82^{Aa}	11.02^{Ab}	11.01^{Ab}
100%LEC	9.43^{Cc}	8.89^{Cd}	8.89^{Bd}
100%LAO	8.48^{Ee}	8.22^{De}	8.22^{Ce}
50%SCB+50%LAO	9.19^{Cd}	8.97^{Cd}	8.97^{Bd}
50%SCB+50%LEC	9.47^{Cc}	9.25^{Cc}	9.25^{Bc}
50%LAO+50%LEC	8.50^{Ee}	8.57^{De}	8.57^{Ce}
33%SCB+33%LAO+33%LEC	12.06^{Ba}	10.96^{Ab}	10.96^{Ab}
CV=2.74			
LSD=0.43			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.2.5. Total Ash

The maximum total ash content (8.23%) was recorded for *Pleurotus ostreatus* grown on 100%LEC, followed by *Pleurotus sajor-caju* and *Pleurotus florida* grown on100% SCB (8.19%) (Table11). The minimum total ash content (7.02%) was obtained for the three *Pleurotus* mushroom grown on 50%LAO+50%LEC. Total ash content of selected *Pleurotus* species of the mushroom grown on 100%SCB, 100%LEC and 33% SCB+33% LAO +33% LEC were not ($p<0.05$) significantly different. Total ash content obtained from *Pleurotus ostreatus* grown on100%LAO and total ash content obtained from *Pleurotus sajor-caju* and *Pleurotus florida* grown on 50% SCB+50% LAO and 50% SCB + 50% LEC were not also significantly different.

Table 11. Percentage of total ash of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB(**control**)	8.18^{Aa}	8.19^{Aa}	8.19^{Aa}
100%LEC	8.23^{Aa}	7.95^{Aa}	7.95^{Aa}
100%LAO	7.16^{Bb}	7.11^{Cb}	7.11^{Cb}
50%SCB+50%LAO	7.36^{Bb}	7.28^{Bb}	7.28^{Bb}
50%SCB+50%LEC	7.56^{Bb}	7.61^{Bb}	7.61^{Bb}
50%LAO+50%LEC	7.02^{Cc}	7.02^{Cc}	7.02^{Cc}
33%SCB+33%LAO+33%LEC	8.06^{Aa}	7.93^{Aa}	7.93^{Aa}
CV=3.73 LSD=0.48			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

The current result of total ash obtained (7.11-8.19%) are in harmony with Oei (2003) and Dawit (1998) who reported 7.2- 8.8% total ash content for mushrooms.

But these values were lower than the total ash value of Kidane (2006) who reported higher ash content (12.32%) for *Pleurotus sajor-caju* grown on chat leaves. A number of factors such as the site of growth, type of substrate used, the developmental stage of the fungal species usually influence the nutritional composition of the mushrooms (Anthony, 2007).

4.2.6. Total Carbohydrate

In this study total carbohydrate content in the fruit bodies of three different *Pleurotus* mushrooms were recorded. There was a significant ($p<0.05$) effect of substrates on carbohydrate contents of selected *Pleurotus* mushroom (Table 12). The highest (46.65% & 39.07%) values of carbohydrate contents were obtained for *Pleurotus ostreatus* grown on 100% SCB and 33% SCB+33% LAO +33% LEC respectively, followed by *Pleurotus florida* and *Pleurotus sajor-caju* grown on 100%SCB (38.99% &38.98%) respectively. The lowest (15.18%) values of carbohydrate contents were obtained for *Pleurotus sajor-caju* and *Pleurotus florida* grown on 100%LAO. The current results are in harmony with those of Bernas *et al*. (2006) which were reported as carbohydrate contents ranged between 16-85% for the same mushroom species. In this study, the difference in carbohydrate content of *Pleurotus* mushroom grown on different substrates and substrate combinations could be due to differences carbon content of the growth substrate.

Table 12. Percentage of total carbohydrate of selected *Pleurotus* mushroom.

Treatments	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Pleurotus florida*
100%SCB(**control**)	46.65^{Aa}	38.98^{Ab}	38.99^{Ab}
100%LEC	32.77^{Cb}	30.94^{Bb}	30.93^{Bb}
100%LAO	16.07^{Ee}	15.18^{Ee}	15.18^{Ee}
50%SCB+50%AO	27.46^{Dd}	20.95^{De}	20.94^{De}
50%SCB+50%LEC	38.82^{Bb}	28.98^{Cc}	28.98^{Cc}
50%LAO+50%LEC	24.60^{Dd}	20.72^{Dd}	20.72^{Dd}
33%SCB+33%LAO+33%LEC	39.07^{Bb}	33.97^{Bc}	33.98^{Bc}
CV=8.84			
LSD=4.17			

CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. CV=Coefficient of Variation, LSD=Least Significant Difference, LAO=leaves of *Argemone ochroleuca*, LEC= *Eichhornia crassipes*, SCB=Sugar cane bagasse. Means with similar capital letters in column represent no significant difference, whereas means with similar small letters in row show no significance difference ($p<0.05$).

4.3. Correlation among Parameters

4.3.1. Correlation between Mushroom Growth and Yield Parameters

Results of from the simple linear correlation analysis (Table 13) showed that a positive and significant correlation between the days for *pleurotus* mycelium to fully colonize substrate (DMF) to pin head formation (DPF) (r = 0.97***), and to fruiting body formation (r = 0.90***). And also days to pine head formation had a positive and significance correlation with day of fruit formation (r=0.94***).

But on the other hand biological efficiency (BE) had negative correlation with DMF (r= -0.83***), DPF (r = -0.87***) and with DFF (r = -0.80***). TY had also strong negative correlation with Phenological parameters such as days for mycelium to fully colonize substrate (r = -0.82***), days to pin head formation (r = -0.87***) and also days to fruit body formation (r = -0.80***). But total yield (TY) had a high positive and significant correlation with biological efficiency (r = 0.95***). This indicated that high TY was essential for biological efficiency but low yield had negative effect on the yield parameters. A result of this study indicates that Phenological parameters (DMF, DPF and DFF) have no direct influence on the other yield parameters i.e. biological efficiency and total yield

Table 13. Correlation between mushroom growth and yield parameters.

	DMF	DPF	DFF	BE	TY
DMF	1				
DPF	0.97***	1			
DFF	0.90***	0.94***	1		
BE	-0.83***	-0.87***	-0.80***	1	
TY	-0.82***	-0.87***	-0.80 ***	0.95***	1

***, ** and ns indicate significant difference at 0.001, 0.05 and non-significant probability levels, respectively. DMF=days for mycelium to fully colonize substrate, DPF=days to pine head formation, DFF=days to fruiting body formation, BE=biological efficiency, TY=total yield

4.3.2. Correlation between Mushroom Quality Parameters

Results of the simple linear correlation analysis (Table 14) showed that percentage moisture content has non -significant correlation with percentage crude fat (r = 0.39ns) and percentage crude fiber (r =0.28ns). But it has significant correlation with percentage crude protein (r = 0.48**) and total ash content (r = 0.48**). Percentage crude fat was significantly correlated

with percentage crude protein (r = 0.78***) and percentage crude fiber (r = 0.65***), but has non- significant correlation with total ash content (r = 0.39ns).

Percentage crude protein has significant correlation with total ash content (r = 0.7***) and percentage crude fiber (r = 0.91***). Crude fiber has non-significant correlation with moisture content(r=0.28ns), but significantly correlated with crude fat (r = 0.65***), crude protein (r = 0.91***), total ash (r = 0.69***). On the other hand total carbohydrate has significant positive correlation with moisture content (r=0.63***), crude fat (r = 0.73***), crude protein (r = 0.97***), total ash (r = 0.78***), crude fiber (r = 0.89***).

Table 14. Correlation between mushroom quality parameters.

	MOC	CFa	CP	TA	CFi	TC
MOC	1					
CFa	0.39ns	1				
CP	0.48**	0.78***	1			
TA	0.48**	0.39ns	0.7***	1		
CFi	0.28ns	0.65***	0.91***	0.69***	1	
TC	0.63 ***	0.73***	0.97***	0.78***	0.89***	1

***, ** and ns indicate significant difference at 0.001, 0.05 and non-significant probability levels, respectively. MOC= percentage moisture content, CFa =percentage crude fat, CP =percentage crude protein, TA = Percentage total ash, = CFi = Percentage crude fiber, TC=percentage total carbohydrate.

5. SUMMARY, CONCULUSION AND RECOMMENDATIONS

5.1. Summary

Growth and yield parameters and quality of mushrooms are largely influenced by the substrate and substrate combination type on which they are grown. In view of this, the present research work was conducted at the Mushroom Research, Production and Training Center of Haramaya University. A factorial experiment involving three oyster mushroom species (*Pleurotus ostreatus, Pleurotus florida* and *Pleurotus sajor-caju*) and seven substrates and substrate combination (Leaves of *Eichhornia crassipes* and *Argemone ochroleuca*) was undertaken in a complete randomized design with three replications.

From Phenological data, the fastest and slowest fruiting body formation (23.67&34.33 days) were recorded for *Pleurotus ostreatus* and *Pleurotus sajor-caju grown* on 100% SCB and 100%LAO respectively. For yield performance, highest and lowest yield (1697.00g &556.7g) were obtained for *Pleurotus ostreatus* and *Pleurotus sajor-caju* cultivated on 100% SCB and 100% LAO respectively. The highest values of biological efficiencies (71.67%, 70.00% and 69.67) were obtained for *Pleurotus* species grown on SCB alone respectively. The second comparable value of biological efficiency were obtained for *Pleurotus ostreatus ,Pleurotus sajor- caju* and *Pleurotus florida* grown on 33%+SCB+33%LEC+33%LAO (68.67%,66.33% and 64.00%) respectively. On the other hand, relatively lower BE (39.33%, 40.67% and 43.00%) were obtained from the *Pleurotus florida ,Pleurotus sajor- caju* and *Pleurotus ostreatus* grown on leaves of *Argemone ochroleuca* alone respectively.

For quality parameters, highest percentage of crude protein (35.52% and 33.23%) were obtained for *Pleurotus ostreatus* and *Pleurotus florida* grown on100%SCB alone, followed by *Pleurotus ostreatus* grown on 33% of (SCB, LAO and LEC) 31.66%)). Lowest percentage of crude protein (14.38%) was obtained for *Pleurotus sajor-caju* grown on100%LAO. Moisture content, crude fat, crude fiber, total ash and carbohydrate content obtained from this experiment generally ranged from 80.40%-88.53%, 1.18%-1.97%, 8.2%-12.82%, 7.02%-8.28% and 15.18-46.65% respectively.

A result from simple linear correlation analysis indicates that phenological parameters (DMF, DPF and DFF have no direct influence on total yield and biological efficiency, and also have no correlation with quality parameters of mushroom.

5.2. Conclusion

In conclusion the present study revealed that organic substrate having narrow C: N ratio, high cellulose content and low lignin content of the substrate like sugarcane bagasse largely contribute to prominent growth and finally provide better yield and quality of mushroom. However substrate with poor water holding capacity, wider C: N ratio, high lignin content and insufficient growing nutrient such as dried leaves of *Argemone ochroleuca* provides unsatisfactory yield and quality of mushroom. So the present study screen out that it is better to use the test substrate (Leaves of *Eichhornia crassipes* (Water hyacinth) and *Argemone ochroleuca*) in combination with sugarcane bagasse, which shows better growth and provides comparable yield and quality parameters of the selected mushroom with that of the control substrate (sugarcane bagasse).

5.3. Recommendations

- ✓ The current finding recommends that cultivation of *Pleurotus* mushroom using the test substrate (Leaves of *Eichhornia crassipes* and *Argemone ochroleuca*) in combination with sugarcane bagasse provides better yield, better biological efficiency, and good nutritional composition.
- ✓ By taking into consideration the coasty of mushroom substrate, further study should be done on other invasive weeds (i.e. their C: N ratio and nutritional composition) so to use them as growing substrate in one hand and also to easily control and manage their distribution.
- ✓ Further detailed studies must be addressed starting from awareness creation to familiarization of production and consumption of edible mushroom.
- ✓ It is mandatory to establish spostication spawn laboratory, standardize the production techniques and accessing trainings to the rural poor and marginal farmers of Ethiopia to produce quality spawn of mushroom.

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7. APPENDICES

7.1. Appendix I. Analysis of Variance Tables

Appendix Table 7.1.Analysis of variance on days for selected *Pleurotus* species to fully colonize substrate

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	4.78	2.47	0.0970ns
Substrate	6	87.65	45.27	0.0000**
Species * Substrate	12	1.74	0.91	0.5466ns
Error	42	0.5714		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 5.86

Appendix Table 7.2. Analysis of variance on days of pinhead formation of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	1.00	1.09	0.3468ns
Substrate	6	55.44	60.22	0.0000**
Species * Substrate	12	1.46	1.59	0.1321ns
Error	42	0.92		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 3.68

Appendix Table 7.3. Analysis of variance on days of fruit body formation of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	9.96	11.63	0.0001**
Substrate	6	64.42	75.16	0.0000**
Species * Substrate	12	3.59	4.20	0.0003**
Error	42	0.85		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 3.25

Appendix Table 7. 4.Analysis of variance on Total yield of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	162748	30.63	0.0000**
Substrate	6	1056424	198.82	0.0000**
Species * Substrate	12	14589	2.75	0.0077ns
Error	42	5313		

**and ns are significant at 0.05 level and non-significant, respectively.

Coefficient of Variation: 6.85

Appendix Table 7.5. Analysis of variance on Biological efficiency of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	82.49	55.29	0.0000**
Substrate	6	1047.21	701.85	0.0000**
Species * Substrate	12	0.92	0.62	0.0170ns
Error	42	1.49		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 3.88

Appendix Table 7.6. Analysis of variance on percentage of Moisture content of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	11.06	295.32	0.0000**
Substrate	6	40.1499	1071.80	0.0000**
Species * Substrate	12	2.83ns	59.53	0.0000**
Error	42	0.0375		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 0.23.

Appendix Table 7.7. Analysis of variance on percentage of crude protein of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	51.84	20.70	0.0000**
Substrate	6	305.77	122.07	0.0000**
Species * Substrate	12	2.83	1.13	0.3615ns
Error	42	2.5		

**and ns are significant at 0.05 level and non-significant, respectively.

Coefficient of Variation: 6.43,

Appendix Table 7.8. Analysis of variance on percentage of crude fat of selected *Pleurotus* species.

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	0.67	132.72	0.0000**
Substrate	6	0.11	22.62	0.0000**
Species * Substrate	12	0.03	6.39	0.0000**
Error	42	0.005		

**and ns are significant at 0.05 level and non-significant, respectively.

Coefficient of Variation: 4.13

Appendix Table 7.9. Analysis of variance on percentage of crude fiber of selected *Pleurotus* species.

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	2.59	37.47	0.0000**
Substrate	6	15.71	227.15	0.0000**
Species * Substrate	12	0.41	5.87	0.0000**
Error	42	0.069		

**and ns are significant at 0.05 level and non-significant, respectively.

Coefficient of Variation: 2.74

Appendix Table 7.10. Analysis of variance on percentage of total ash content of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	0.032	0.41	0.6673ns
Substrate	6	1.98	24.62	0.0000**
Species * Substrate	12	0.01	0.15	0.9995ns
Error	42	0.08		

**and ns are significant at 0.05 level and non-significant, respectively.

Coefficient of Variation: 3.73

Appendix Table 7.11. Analysis of variance on percentage of total carbohydrate content of selected *Pleurotus* species

Source of variation	Degree of Freedom	Mean square	F-value	P-value
Species	2	152.65	23.78	0.0000**
Substrate	6	718.37	111.91	0.0000**
Species * Substrate	12	6.7	1.04	0.4288ns
Error	42	6.41		

**and ns are significant at 0.05 level and non-significant, respectively.
Coefficient of Variation: 8.84

Appendix Figure 7.1. Appendix Partial view of the research work from spawn preparation up to final fruiting body formation of the selected *pleurotus* mushroom

Prepared mother spawn (left) and multiplication of mother spawn (right)

Serialization of substrate, soaking of substrate and decantation of the soaked substrate (A) and inoculation of spawn substrate (B)

Mycelium colonization (left) and fruiting body formation (right)

Fruiting body formation