COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOTECHNOLOGY

EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATIONS ON GROWTH RATE AND YIELD PERFORMANCE OF OYSTER MUSHROOM (*Pleurotus ostreatus*), IN WOLDIA, NORTH WOLLO, ETHIOPIA

MSc. THESIS

By

Sualih Gobeze

Advisor: Abebe Girma (Associate Professor)

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APPROVAL SHEET

This is to certify that the thesis “effect of different substrates and their combinations on growth rate and yield performance of oyster mushroom (*pleurotus ostreatus*), in woldia, north wollo, Ethiopia” submitted in partial fulfillment of the requirements for the degree of Master's with General Biotechnology, and has been carried out by Sualih Gobeze under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to biotechnology department.

Abebe Girma(**Associate Professor**) _____ _____

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DECLARATION

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Name: Sualih Gobeze Hailu

Place: Woldia University

Date of submission: May, 2025

Signature: _____

BIOGRAPICAL SKETCH

The author, Sualih Gobeze, was born on 25 may 1983, at Gubalafto Woreda, North Wollo Zone to his father Gobeze Hailu and his mother Melgom Abera. He attended Elementary education (grade one to eight) at Melkakole Elementary School from 1996 -2002 and secondary education (grade nine to twelve) at Woldia Secondary School from 2003-2006. After completing his elementary and secondary education, he joined to Arba Minch University and studied biotechnology for four years and he has been graduated in the year 2010. One year after graduation, he was employed by Woldia Univesity on sep 2012 as a senior technical assistance in biotechnology department. He was promoted and worked as graduate assistant (GAII) from 2013 -2014. The University offers him free scholarship for Master of Science (MSc.) study in General Biotechnology and attended his MSc. program.

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LIST OF ABBREVATIONS

FWM	Fresh Weight Of Mushroom
DWS	Dry Weight Of Substrate
SPP.	Species
P. species	Pleurotus Species
NFB	Number Of Fruiting Body
MFB	Maturation Of Fruiting Body
BY	Biological Yield
BE	Biological Efficiency
TBY	Total Biological Yield

ABSTRACT

This research examined the effect of various substrate compositions on the growth rate and yield performance of oyster mushroom (*Pleurotus ostreatus*) *under controlled conditions at* ***Woldia University, Ethiopia****. Given the national need for sustainable, low-input food sources amid rising food insecurity, the study addresses a critical gap in knowledge regarding optimal substrate utilization using locally available materials such as teff straw, grass straw, and sawdust. A completely randomized design with seven treatments and three replications was employed. Significant differences ($p < 0.05$) were found among treatments for key variables including colonization period, pinhead formation, stipe length, cap diameter, number of fruiting bodies, total fresh yield, and biological efficiency. The highest total yield (530.87 g) and biological efficiency (53.09%) were recorded in the substrate mixture of 33.3% teff straw, 33.3% grass straw, and 33.3% sawdust. This treatment also produced the most consistent regrowth across three flushes. In contrast, the 50% sawdust + 50% teff straw treatment yielded the lowest biological efficiency (49.03%). Substrate composition significantly influenced cap diameter (ranging from 2.87 to 7.73 cm) and number of fruiting bodies (9–26 per flush), indicating that blended substrates optimize physical structure and nutrient availability. These findings underscore the importance of strategic substrate formulation to enhance mushroom productivity and support food security initiatives through cost-effective, environmentally friendly cultivation systems.*

Keywords: biological efficiency, fruiting body, mycelium colonization, oyster mushroom, substrates,

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1. INTRODUCTION

1.1. Background and Justification

Oyster mushroom (*Pleurotus* spp.) is a widely cultivated edible fungus known for its adaptability, rapid growth, and nutritional value. Belonging to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, and family Pleurotaceae, this mushroom is native to both temperate and tropical regions and thrives naturally on dead or decaying wood, especially in moist forest environments (Sher *et al*., 2011). There are about 38 species under the *Pleurotus* genus, with approximately 25 being cultivated commercially across different parts of the world (J. Raman et al., 2021). Prominent cultivated species include *Pleurotus ostreatus*, *P. florida*, *P. sajorcaju*, and *P. eryngii*. These mushrooms can grow on a wide variety of lignocellulosic substrates such as teff straw, grass straw, sawdust, and corn cobs, making them suitable for cultivation in diverse environmental conditions(Tesfaw *et al.*, 2015). They require moderate temperatures, good ventilation, and high humidity for optimal growth, and their ability to flourish in varying climates makes them an ideal crop for both small-scale and commercial production.

Mushroom cultivation particularly that of oyster mushrooms, has witnessed significant growth worldwide due to its low input requirement and high yield potential. Among edible mushrooms, *Pleurotus* species are the second most cultivated after *Agaricus* spp., contributing substantially to global mushroom production(Sánchez, 2017). China alone accounts for approximately 87% of global oyster mushroom production, showcasing the crop's economic importance(Barh *et a*l., 2019). These mushrooms have a relatively short cultivation cycle, are less prone to diseases and pests, and efficiently convert agricultural waste into protein-rich biomass. Depending on the substrate used, yield and productivity can vary significantly, highlighting the need to identify the most suitable materials for enhanced production performance (J. Raman et al., 2021).

Oyster mushrooms play a crucial role in food security, nutrition, and environmental sustainability. They are an excellent source of high-quality protein, essential amino acids, vitamins, minerals, and dietary fiber, making them an important addition to the human diet, especially in developing countries where malnutrition is prevalent(Torres-Martínez *et al.*, 2022). Beyond their nutritional benefits, oyster mushrooms contribute to environmental management by

recycling agricultural, food, and industrial wastes into valuable food products, thereby reducing environmental pollution (A. Doroški et al., 2022).Their cultivation also promotes socioeconomic development by creating employment opportunities and supporting income generation, particularly in rural areas (Nongthombam *et al*., 2021). With over 3,283 species classified as edible or conditionally edible globally, mushrooms are increasingly being recognized for their functional and therapeutic properties (Li & Xu, 2022).

Oyster mushrooms (*Pleurotus* spp.) are increasingly recognized for their exceptional nutritional and medicinal value, making them a vital component of both functional foods and alternative medicine. Numerous studies affirm their rich nutrient profile and health-promoting properties, positioning them as a dual-purpose organism serving as both nourishment and therapeutic agent (Maray et al., 2018). Nutritionally, *Pleurotus ostreatus* is an excellent source of essential vitamins including vitamin E (7.23 mg/g), vitamin A (0.363 mg/g), and vitamin C (0.363 mg/g), while other species like *P. citrinopileatus* are rich in B-complex vitamins such as B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), and B9 (folic acid) (Olukemi et al., 2021). In terms of fatty acid composition, oyster mushrooms contain a variety of beneficial unsaturated fatty acids. Notably, *P. ostreatus* is high in linoleic acid (19.1%), which is essential for human health, while *P. pulmonarius* and *P. sajor-caju* also contribute palmitic acid (18.4%) and linoleic acid (13.5%) respectively. Other fatty acids such as stearic, oleic, pentadecanoic, and ethyl linoleate are also present, contributing to cardiovascular benefits (Kaur et al., 2014; Rathod et al., 2021).

The macronutrient profile of *P. ostreatus* further underscores its dietary importance. Per 100 grams of edible portion, it contains approximately 3.31 g of protein, 6.09 g of carbohydrates, 2.3 g of dietary fiber, and 1.01 g of ash. It is also a valuable source of essential minerals such as calcium (3 mg), iron (1.33 mg), magnesium (18 mg), phosphorus (120 mg), potassium (420 mg), and selenium (2.6 µg), along with several trace elements including copper, manganese, and zinc (Rathod et al., 2021). Though its protein content is lower than that of meat, it surpasses that of milk and provides all essential amino acids, making it a suitable supplement in plant-based and low-resource diets.

In addition to its nutritional benefits, oyster mushrooms exhibit powerful medicinal properties. Bioactive compounds such as polysaccharides, particularly 1,6-branched 1,3-β-glucans, have demonstrated immunomodulatory and antitumor effects. These compounds activate immune cells and stimulate cytokine production, leading to inhibited tumor growth. For instance, hot water extracts of *P. ostreatus* have been shown to suppress the proliferation of human breast cancer cells (MCF-7) and prostate cancer cells (PC-3), indicating its potential as a complementary cancer therapy (Martin et al., 2010; Roupas et al., 2012). Furthermore, mushrooms from the *Pleurotus* genus are rich in antimicrobial substances, including phenolics, protein-polysaccharide complexes, free fatty acids, and secondary metabolites. These compounds display significant antibacterial, antifungal, and antiviral activities, supporting their application in combating infectious diseases (Rathod et al., 2021). Additionally, compounds such as pleurotin, found in *P. griseus*, exhibit potent antibiotic properties, expanding the therapeutic versatility of these mushrooms (Chang et al., 2017).

In summary, *Pleurotus* mushrooms are not only valuable food sources due to their dense nutritional profile, but also possess considerable pharmacological potential through their bioactive constituents, which offer antitumor and antimicrobial effects. Their integration into diets and therapeutic regimens holds promise for addressing malnutrition and managing chronic and infectious diseases.

Despite its potential, mushroom cultivation faces several challenges, particularly concerning substrate availability, standardization, and productivity optimization. The efficiency of oyster mushroom cultivation largely depends on the type and quality of substrate used, as different substrates affect growth parameters such as mycelial colonization time, pinhead formation, and total yield (Tesfaw *et al.,* 2015). Inadequate research on locally available and economically viable substrates has limited the potential for maximizing yields in some regions. Moreover, the lack of awareness, technical know-how, and support infrastructure continues to hinder the expansion of mushroom farming in many developing countries (Oseni *et al*., 2012).Therefore, this research aims to investigate the effects of various substrates namely teff straw, grass straw, sawdust, and their combinations on the growth and yield performance of oyster mushrooms. The goal is to compare, select, and standardize the most appropriate substrate(s) for optimizing oyster mushroom production under local conditions.

1.2. Statement of the problem

Ethiopia is currently grappling with the challenges of rapid population growth, urbanization, shrinking arable land, and increasing food insecurity. Traditional agricultural practices, which many rural communities heavily rely on, are proving insufficient to sustainably meet the nutritional and economic needs of the expanding population. In this context, there is a critical need for innovative and resource-efficient agricultural solutions that can thrive under limited land and input conditions.

Mushroom cultivation, particularly that of oyster mushrooms (*Pleurotus ostreatus*), offers a promising alternative due to its low input requirements, short cultivation cycle, and ability to grow on various organic wastes. However, despite its potential, optimal substrates for mushroom cultivation under Ethiopian conditions remain under-researched. Locally available agricultural residues such as teff straw, grass straw, sawdust, and crop stalks are abundant, yet their comparative suitability for oyster mushroom cultivation is not well understood.

The substrate type significantly influences the biological efficiency and yield of oyster mushrooms, with organic wastes like maize stalk and faba bean stalk demonstrating promising results. Similarly, a study conducted at Aksum University revealed that cotton seed hull and barley straw significantly outperformed other substrates in yield and colonization rate (Tekeste *et al.*, 2020). Furthermore, a recent study published by E. Mesele et al. (2024) in *PubMed* emphasized that combinations of substrates enhanced both growth rate and productivity, pointing to the importance of optimizing substrate mixes for better performance. A study from Wondo Genet College also found that while cottonseed showed the highest biological efficiency, sawdust was the least effective (Kenenı, 2023).

Despite these findings, the data remain fragmented, and no comprehensive study has yet systematically compared the performance of key locally available substrates under similar growing conditions. As a result, farmers lack clear, evidence-based guidelines on which substrates to use for optimal mushroom production. This knowledge gap hinders the adoption and scalability of mushroom farming as a viable livelihood and nutrition strategy in Ethiopia and this investigations fills the geographical gap since it has not been well done in case of amhara region.

Therefore, it is imperative to investigate the effects of different locally available substrates both individually and in combination on the growth, yield, and biological efficiency of *Pleurotus ostreatus* to establish sustainable and low-cost mushroom cultivation practices tailored to the Ethiopian context.

1.3. Objective

1.3.1. General objective

- The general objective of this study was to evaluate the effect of different substrates and their combinations on the growth rate and yield performance of oyster mushrooms.

1.3.2. Specific objectives

1. To assess the impact of individual substrates (e.g., teff straw, grass straw and sawdust) on mushroom growth.
2. To determine the effects of substrate combinations on yield and growth rate.
3. To identify the most effective substrate or combination for optimal yield in the local climate.

1.4. Significance of the Study

This research plays a pivotal role in advancing the cultivation practices of oyster mushrooms by focusing on the optimization of growing techniques and the identification of the most effective substrates. By systematically evaluating various substrate materials, the study aims to determine which options yield the highest mushroom production, best quality, and most efficient resource use. These findings will directly benefit mushroom growers by offering practical, science-based strategies to enhance productivity, reduce production costs, and ultimately increase their profitability.

Oyster mushroom (*Pleurotus ostreatus*) holds substantial significance due to its remarkable enzymatic activity, nutritional value, and environmental benefits through waste bioconversion. It produces an array of extracellular enzymes such as laccase, cellulase, and lignin peroxidase

which enable the degradation of complex lignocellulosic materials. These enzymes not only facilitate mushroom growth on organic waste but are also valuable in biotechnological applications like bioremediation, paper processing, and biofuel production. Nutritionally, oyster mushrooms are rich in high-quality protein, comprising 20–35% of their dry weight, and contain all essential amino acids, making them a vital dietary supplement, especially in regions where meat is scarce or costly. Their ease of digestion and low fat content further enhance their role in combating protein-energy malnutrition. Moreover, oyster mushrooms play a critical role in sustainable agriculture by converting agricultural and industrial residues such as teff straw, grass straw, and sawdust into edible biomass and nutrient-rich spent mushroom substrate. This process not only reduces environmental waste but also generates valuable byproducts usable as organic fertilizer or livestock feed. Altogether, the cultivation of *P. ostreatus* offers a low-cost, eco-friendly approach to improving food security, nutrition, and waste management.

2. LITERATURE REVIEW

2.1 Over View of Oyster Mushroom

Oyster mushrooms, scientifically classified under the genus *Pleurotus*, are among the most suitable fungi for cultivation due to their ability to grow on a wide range of agro-industrial wastes without the need for composting (A. Doroški et al., 2022; Mondal et al., 2010) . Commonly referred to as "wood fungi" and locally known as Dhingri in India, they were first described scientifically by Nikolaus Joseph Freiherr von Jacquin in 1775 under the name *Agaricus ostreatus*. Later, Paul Kummer reclassified them into the *Pleurotus* genus in 1871(Keneni & Kebede, 2016). *Pleurotus* species are saprophytic fungi that grow naturally on decaying wood and other plant materials rich in cellulose and lignin (da Fonseca *et al*., 2015; Tikdari & Bolandnazar, 2012). Their morphological and physiological adaptability makes them ideal for both tropical and temperate climates (Shukla & Jaitly, 2011). These mushrooms have evolved efficient enzyme systems that allow them to thrive on unprocessed lignocellulosic materials, making them highly sustainable for cultivation across diverse geographies (Grimm & Wösten, 2018).

Oyster mushrooms have a global distribution and can be found in both wild and cultivated environments across Asia, Europe, Africa, and the Americas (J. Kumla et al., 2013; Rahi & Malik, 2016). Their natural habitats include decaying logs, fallen branches, and rich humus soils, particularly in areas with high humidity and moderate temperatures (Keneni & Kebede, 2016). In African countries, they are widely harvested in forests during rainy seasons, while in Asian countries like China and India; they are extensively cultivated for both subsistence and commercial purposes (Li & Xu, 2022; Sileshi *et al.*, 2023). The adaptability of *Pleurotus ostreatus* to various agro-climatic conditions, coupled with its low input requirements, has facilitated its widespread cultivation even in resource-limited settings(Grimm & Wösten, 2018; Seid, 2023) . Moreover, oyster mushrooms are known for their resilience against pests and diseases, allowing for successful outdoor and indoor cultivation in both developed and developing regions (Ragupathi *et al.*, 2018; Uddin *et al.*, 2011) .

Oyster mushroom cultivation is a growing sector within global agriculture, with *Pleurotus ostreatus* ranking as the second most commercially important mushroom species worldwide after *Agaricus bisporus* (Grimm & Wösten, 2018; Li & Xu, 2022). Mushrooms are grown mostly on waste materials like sawdust, composting materials, straw and garbage's. In China, mushroom farming has emerged as the fifth largest agricultural sector, valued at USD 24 billion, with a sustained annual growth rate of 10% over the last three decades (Li & Xu, 2022; Sileshi *et al*., 2023). The productivity of oyster mushrooms is significantly influenced by substrate type, temperature, humidity, and pH levels, making environmental optimization crucial for commercial yields (Silva *et al*., 2018). Substrates such as teff straw, sawdust, and grass have proven effective in enhancing biological efficiency, especially when used in mixed forms (Bellettini *et al*., 2019). Adams *et al*. (2022) studied the growth of King Oyster mushroom (Pleurotus eryngii) on sawdust and rice straw in Bangladesh and found that sawdust showed the highest biological efficiency (73.5%). Although the sawdust substrate produced better yield and efficiency, rice straw produced the mushrooms with larger fruiting bodies. Sawdust substrate, due to its lignocellulosic content, is also very efficient in mushroom cultivation provided that it is composted and can be supplemented with nitrogen sources, such as wheat bran or vermicompost (Adams *et al*., 2022; Viriato *et al*., 2022). Mushroom cultivation is common in Guyana but there is limited knowledge about its nutritional benefits. This study also investigated that whether sawdust, grass clippings and rice straw are suitable substrates for the growth of P. ostreatus. Adams et al. (2022) estimated that P. ostreatus is easy to grow, requires few environmental controls, has the ability to grow on a wide variety of substrates, produce high yields, and their fruiting bodies are not as susceptible to pests (Adams *et al*., 2022). Recent studies indicate that the optimum temperature for *Pleurotus* mycelial growth lies between 25–30°C, with a preference for 75–90% relative humidity and a substrate pH of 6.5–7.0 for best results. Spawn can be prepared from different carriers of grains such as wheat, sorghum, barley and rice. It is reported that as compare to grains of wheat and barley, grains of sorghum are better mycelium carrier. Oyster mushrooms hold immense significance due to their nutritional, economic, and environmental benefits. They are rich in proteins, vitamins, minerals, and dietary fibers, making them a valuable food source, especially in addressing malnutrition and food insecurity in vulnerable communities (Keneni & Kebede, 2016; Sileshi *et al*., 2023). Beyond nutrition, oyster mushrooms exhibit potent medicinal properties, including antioxidant, antimicrobial, and

cholesterol-lowering effects, classifying them as functional foods or nutraceuticals (M. L. Gargano et al., 2017; Rahi & Malik, 2016). Their cultivation offers a sustainable avenue for income generation, particularly for smallholder farmers and rural households, due to low capital investment and the use of agricultural residues as substrates (Dhanapal & Rajoo, 2023; Seid, 2023). In addition, their role in environmental conservation through biodegradation and waste recycling enhances their ecological importance (da Fonseca *et al.*, 2015; Mortimer & Karunarathna, 2014)). The growing interest in mushroom-based biotechnologies, including biofuel production and biodegradable packaging, further underscores their multifaceted value. The superior number of effective fruiting bodies and biological yield found in mixtures indicates that substrate blending enhances nutritional diversity and physical properties (like aeration and moisture balance), maximizing mushroom productivity. This is consistent with the findings of who advocated for mixed substrates to improve biological efficiency (Bellettini *et al*., 2019; Li & Xu, 2022). Substrates like; teff straw, barley straw, sesame stalk, saw dust and cotton seed hull were used as potential substrates for Pleurotus ostreatus mushrooms cultivation (Tekeste *et al*., 2020) .

2.2 Origin, Taxonomy and Category of Oyster Mushroom

2.2.1. Origin of oyster mushroom

Among many species, oyster mushroom is the most suitable for cultivation by utilizing various agro wastes without composting. Oyster mushroom is generally known as the wood fungus and in India commonly known as Dhingri. Oyster mushroom is scientifically known as *pleurotus sp*. Oyster mushroom was first described scientifically in 1975 by a Dutch naturist Nikolaus Joseph Freiherr von Jacquin (1721-1817) and named *Agaricus ostreatus*. Later on, these species of oyster mushrooms were included in the genus *Pleurotus* by a German mycologist Paul Kummer in 1871 (Doroški *et al.*, 2022). Oyster mushrooms are one kind of edible saprophytic fungi growing on dead organic matters of vegetative origin belonging to the genus *pleurotus* under the class *basidiomycetes* (Mondal *et al.*, 2010).

2.2.2. Taxonomy of oyster mushroom

This species has been taxonomically classified as kingdom *fungi*, phylum-*basidiomycota*, class-*agaricomycetes*, order-*agaricales*, family-*pleurotaceae* and genus-*pleurotus*. There are about

thirty eight species described under genus *pleurotus* from different part of the world, out of which 25 species are under cultivation (Raman *et al.,* 2021). They are having outstanding flavor and taste with fan shaped *pileus* which is rich source of both macro and micro nutrients.

2.2.3. Morphology

The external appearance of oyster mushroom has been characterized by the presence of a spatula shaped cap called *pileus*. This is fleshy part. An oyster mushroom's fruiting body has a stem that can be short, long, lateral or central and thick (Shukla *et al.,* 2011). This stalk is called stipe. An interesting and attractive feature of oyster mushroom is presence of long ridges and furrows underneath the *pileus* which is called *gills* or *lamellae*. The oyster mushroom's gills contain the spores that help in reproduction. These spores are characterized as smooth and cylindrical which can germinate on any type of mycological media within incubation period of 48 to 96 hrs. The mycelium colour of *pleurotus ostreatus,* is purely white (Rathod, Mukundraj G, et al., 2021). Mostly, the colors of mushrooms are white, brown, or yellow. Sometimes, it can be very colorful. The spore print of the mushroom is white to lilac-grey, and best viewed on dark background.

2.2.4. Category of oyster mushroom

All mushrooms can be grouped into a few major categories. These categories overlap as well because some mushrooms frequently fall under more than one heading (Rahi *et al.,* 2016). For instance, oyster mushrooms can be found in the wild in addition to being grown all over the world (cultivated).

2.2.4.1. Cultivated mushrooms

Commercially grown mushrooms are referred to as "cultivated mushrooms." Mushroom producers use a wide range of diverse techniques and settings to take care of their crops as they cultivate mushrooms in large quantities for consumers. Any variety of mushrooms that customers can purchase at the market is considered to be cultivated (Niazi *et al.,* 2021).These include shiitake, oyster mushrooms, *enoki*, *portabello*, etc.

2.2.4.2. Wild mushrooms

Those mushrooms are obtained by mushroom *shikarionce* from their natural growing spot in undomesticated areas. Some mushroom varieties, like those that enlarge on the living root structures of trees, only originate in nature. Many times, the qualities of wild mushrooms that can only be found in nature make it difficult or practically impossible to cultivate them on a large agricultural scale. It is difficult to recognize the species of mushroom before harvesting (Kumla *et al.,* 2013). You must be mushroom hunting alongside an expert who can confirm and recognize mushroom types. Most of the wild mushrooms are poisonous but exactly look like edible mushrooms.

2.2.4.3. Medicinal mushrooms

Shiitake, lion's mane, and porcini are a few well-known edible mushrooms with health advantages. Other medicinal mushrooms cannot be consumed because they are either too bitter or too woody (Gargano *et al.,* 2017). To benefit from the health advantages of these kinds, are prepared into teas and used as supplements or capsules.

2.2.4.4. Poisonous mushrooms

Several wild varieties of mushrooms are identified as poisonous species; it is essential to make sure those foraged specimens are positively recognized without a doubt before ingesting them. The toxins contained in various species are very different in chemical composition, thus the effects of poisoning differ considerably according to the species involved (Dhanapal *et al*., 2023). Poisonous mushrooms can cause a person to become extremely unwell or even permanently harm their organ systems.

2.2.4.5. Useful mushrooms

While some types of mushrooms are not consumed by people, they nonetheless serve a vital purpose, such as breaking down oil and other environmental pollutants or being added to compost (Mortimer *et al.,* 2014). Every year, new ideas utilizing mushrooms are made and launched by scientists, including the use of mushrooms in biofuels, packaging, cleaning goods, and other applications.

2.2.4.6. Psychoactive mushrooms

Mushrooms that can induce hallucinations and have a psychotropic impact are known as psychoactive mushrooms. The hallucinogenic component psilocybin is present in most of them. Most nations forbid the cultivation of certain kinds of mushrooms, and doing so can be harmful to people's health (Rahi *et al.,* 2016) .

2.3. Growth and development of oyster mushroom

Mushrooms in nature grow on roots of trees and in the soil as mycelium like as the case of *pleurotus species*. *Pleurotus species* grown on the parts of plants which was generally rich in nutrients and vitamins. *Pleurotus species* have extensive enzyme systems capable of utilizing complex organic compounds which were derived from agricultural wastes and industrial by-products. This would lead to avoid processing of substrates for cultivation of *pleurotus species* (da Fonseca *et al.,* 2015). Oyster mushrooms are mainly saprophytes that get their nutritional requirements from host substrates or from the agricultural wastes that are rich in lignin, cellulose and hemicelluloses (Tikdari *et al.,* 2012). Oyster mushroom can be grown on various substrates including wheat straw, maize stalks or cobs, vegetable plant residues, bagasse, eucalyptus sawdust, eucalyptus bark, coffee husks, paddy straw, all cereal straws, paper, wood shavings, sawdust, nutshells etc . In this research sawdust, grass and teff straw in pure and mixed form will be used as a substrate to grow *pleurotus ostreatus* mushroom (Kinge *et al*., 2016).

2.4. Life Cycle and Feeding of Oyster Mushroom

2.4.1. Life cycle

The oyster mushrooms are *basidiomycetes* that bear their spores externally on *basidia*. The *basidia* grow to release the reproductive *basidiospores*. The oyster bears so many spores. The spores on landing in a favorable environment germinate into haploid mycelia. When the mycelia meet other haploid mycelia, they mate and then undergo *plasmogamy* (Wang *et al.*, 2018). This result in the fusion of their cell membrane to create a dikaryotic cell, one with two genetically different haploid nuclei (Dorr *et al.,* 2021).The oyster mushroom has four mating types to ensure the chance of successful mating. The new dikaryotic cell multiplies and divides to live as a

multi-cellular dikaryotic organism. This is the dominant stage for growing and gathering of nutrients. The dikaryotic mycelia then mature to a mushroom.

The nuclei in the basidia finally undergo *karyogamy*, fusion of the nuclei, and at last form a diploid nucleus that quickly undergoes meiosis. Each diploid nucleus yields four haploid nuclei of different mating types that develop into a *basidiospore* to repeat the cycle (Pelkmans *et al.*, 2016).The oyster really begins its life with a spore, from the spore the oyster grows in a thread-like, branching formation known as a hypha. The hyphae continue to elongate and branch repeatedly to form a network of vegetative hyphae which is known as mycelia. The spores and the initial hyphae are haploid (contains only one copy of each chromosome (Izza *et al.,* 2023). A haploid mycelia meeting another haploid mycelia of the same species join together and exchange nuclear materials. The process is called diploidization .This is normally done before meiosis and spore production – *Karyogamy*. The two nuclei duplicate themselves, fuse, mix-and- match their genes, divide, and divide again to produce four new sexual nuclei which become the nuclei of the four spore produced on a typical *basidium* (Wang *et al.*, 2018).

2.4.2. Mode of feeding system

Oyster mushrooms are unable to manufacture their own food; they feed by excreting digestive enzymes through the tips of their hyphae. The hyphae branch to form thick mass mycelia to increase their surface area through which feeding can be maximized. The oyster mushrooms feed by secreting a range of enzymes such as peroxidases, lactases, cellulases, hemicellulases and xylanases (Patel *et al.,* 2012). This makes the oyster mushroom well adapted on lignin and cellulose containing substrates such as sawdust, rice straw etc. These mushrooms, as a result of their saprophytic and the lignocellulotic ability, are able to grow on lignocellulosic substrates with little composting. This is because oyster prefers the lignin that makes up the secondary cell walls of hard woods from angiosperm trees (González *et al.,* 2021). The hypha of the fungi then secretes enzymes to digest these microorganisms, and then the mushroom absorbs the nutrients within them. That is how oyster mushrooms solve their nitrogen problem and defend themselves from a potential predator.

2.5. Mushroom Cultivation in Ethiopia

In Ethiopia, mushroom cultivation on different substrates was started in the early 1990's at Addis Ababa University. But, the domestication and consumption habit remain low (Dejene *et al*., 2023). However, the cultivation and consumption of commercial mushroom types increasing from time to time and spreading to other places in Ethiopia (Megersa & Tolessa, 2024). The cultivated mushrooms dominated by oyster mushrooms *(pleurotus ostreatus, pleurotus sajor-caju, pleurotus florida, p. citrinopileatus, p. eryngii, p. sapidus,lentnus edodes, agarcus bisporus)*. In the production of mushroom *pleurotus ostreatus*, the cultivation of this species does not require a specific substrate (Emiru & Zenebech, 2016). Since the economy of Ethiopia largely depends on agriculture, huge amount different types of biomasses are produced that are suitable for the cultivation of mushroom. Mushroom is a well-known product found/grow in humus rich forest and agricultural fields. In small land size, production of mushroom with vertical cultivation practice may produce high amounts of mushrooms (Dissasa, 2022). Thus, mushroom can be cultivated on agricultural crop residues, industrial biological waste and on the forest residues of sawdust, branches (Bulti *et al*., 2021). Studies have been conducted in Ethiopia to determine the diversity of wild Mushrooms occurring in different habitats such as natural and plantation forests, grazing land, farming area, termite mounds, and swampy areas (Megersa & Tolessa, 2024).

Some of the ethnomycological studies revealed wild mushrooms that are consumed by some ethnic groups, while others are used for various purposes. The list of areas investigated for mushrooms include Oromia region (Dagaga and Gambo forests), Amhara and Sidama regions Mesele *et al.* (2024), and Benshangul Gumuz Region (Asossa Zone, Menge District) (Bulti *et al.*, 2021).

2.6. Agro climatic conditions required for growth of oyster mushroom

The general requirements for growing mushrooms are stated below, however, bear in mind that each variety has specific needs. Although oyster mushrooms can be grown all year long, for commercial purposes they need optimized particular environmental factors. Optimizing the growth conditions, which include temperature, humidity, and light levels, is one of the most critical elements for generating optimum yields (Silva *et al.,* 2018).

2.6.1. Effect of temperature

The optimum temperature for maximum mycelial growth is 25- 30ºC (Ragupathi *et al*., 2018). Variations in any of the factors directly affect the primordial initiation, yield and biological efficiency. This denotes that high or low temperature slow down the growth of mycelium (Uddin *et al.,* 2011). Among the eight temperature regimes viz., 15, 18, 20, 22, 25, 28, 30 and 32◦C for *pleurotus ostreatus* mycelial development and growth it is found significantly differ under different test temperatures. The temperate of 22◦C was found to be optimum for the mycelia development of *p. ostreatus* (Hu *et al.,* 2022).

2.6.2. Relative humidity (Moisture)

The key condition for healthy mushroom growth is moisture. Mushrooms should be kept out of direct sunlight to promote wetness and relative humidity should be 75-90 per cents. The most favorable months for higher production and best biological efficiency were during the winter (December to February). The cultivation practices during the summer months can be done by delivering redundant moisture needed for its growth and development (Deora *et al*., 2021) .

2.6.3. Light

Although mushrooms don’t need darkness to thrive, mushrooms don't need light either. However, darkness promotes the moist conditions that mushrooms require; individuals frequently grow mushrooms in low light or complete darkness (Zawadzka *et al*., 2022).

2.6.4. PH

The pH is a crucial factor to consider when preparing the substrate, as different raw materials can influence this aspect. Incorporating corncobs and sugarcane bagasse into substrate formulations led to a decrease in both the C: N ratio and pH value in comparison to substrates composed solely of sawdust. For the fruiting phase, the optimal pH is between 4.0 and 7.0. However, as colonization occurs, the pH decreases, so the initial adjustment should be made to a pH between 6.5 and 7.0 through the use of a corrective, such as Ca_2CO_3 (Bellettini *et al.,* 2019). When pH levels ranged from 5.0 to 6.4, the duration from pinhead emergence to the first harvest varied

significantly (Sultana *et al.*, 2024).Therefore, the pH value influences the proper growth and development of mushrooms.

2.6.5 Effect of pore size

After substrates were filled to plastic bags, different size holes were made to evaluate effect of aeration, contamination and moisture loss. Pine holes, 16.18mm^2 and 28.16mm^2 holes were made using circular cutter with different diameter. Here pine holes were made after substrates are filled in the bags (Tembe, 2018).

2.7 The Values of Mushroom

2.7.1 Nutritional values

Many researchers have assessed nutritional value of dietary mushrooms and found to be satisfactory to keep us healthy. *pleurotus species* have been recognized as edible mushroom with dual functions to humans both as food and medicine (Maray *et al*., 2018).

2.7.1.1. Vitamins composition

Edible mushrooms have been reported to be a good source for several vitamins. Vitamin B3 (nicotinic acid), B5 (pantothenic acid), B2 (riboflavin), B1 (thiamine), B6 (pyridoxine), B7 (biotin) and B9 (folic acid) are found abundantly in *pleurotus citrinopileatus*. Maximum amount of vitamins were reported from *pleurotus ostreatus* as vitamin E (7.23 mg/g), vitamin A (0.363 mg/g) and vitamin C (0.363 mg/g) (Olukemi *et al*., 2021) .

2.7.1.2. Fatty acid composition

Fatty acids are structurally characterized as straight-chain-mono-unsaturated and polyunsaturated with branched chain building blocks of dietary fats and oils. The essential fatty acids viz. linoleic and linolenic acids are two long chain fatty acids that are fundamental to human diets. Total fat or lipid content production varied from one species of *pleurotus* to the other. Linoleic acid (19.1%) is found in *pleurotus ostreatus*. palmitic acid (18.4%) in *pleurotus pulmonarius*, linoleic acid (13.5%) in pleurotus sajor-caju. Several other fatty acids produced by these organisms are pentadecanoic acid, stearic acid, oleic acid, methyl hexadecanoate, ethyl hexadecanoate, methyl

8,11-octadecadienoate and ethyl linoleate (Kaur *et al*., 2014); (Rathod, Mukundraj G, et al., 2021).

2.7.1.3. Protein, carbohydrate, vitamin and mineral composition

Crude protein content of oyster mushroom is lower than animal meats but higher than milk which is an animal product. nutrient content in oyster mushroom as protein (3.31g), ash (1.01 g), carbohydrates (6.09 g), dietary fibre (2.3 g), calcium (3 mg), copper (0.24 mg), iron (1.33 mg), magnesium (18 mg), manganese (0.11 mg), phosphorus (120 mg), potassium (420 mg), selenium (2.6 µg), sodium (18 mg), zink (0.77 mg), thiamin (0.125 mg), riboflavin (0.35 mg), niacin (4.96 mg), and pantothenic acid (1.29 mg) per 100 g edible portion (Rathod, Mukundraj G, et al., 2021).

2.7.2. Medicinal values

The health-promoting properties of mushrooms have long been recognized in some countries, especially in China. Application of modern analytical techniques has identified various mushroom-derived compounds, polysaccharides and tri-terpenoids for example, which exhibit a wide range of medicinal properties including immuno-enhancing, anti-tumor, antiviral and hypocholesterolemic activities (Chang *et al.*, 2017). Mushrooms produce many bioactive proteins and peptides, primarily including lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial/antifungal proteins, ribonucleases and laccases. pleurotin is an aromatic compound found in *pleurotus griseus* which exhibited antibiotic properties (Chang *et al.*, 2017).

2.7.2.1. Antitumor activity

Mushrooms produced bioactive compound1,6-branched1,3β-glucans which have been reported to inhibit tumor growth by activating immune compatible cells and cytokine production (Roupas *et al*., 2012). Water soluble extract from *pleurotus ostreatus* showed significant effects against prostate cancer PC-3 cells. Hot water extracts of *pleurotus ostreatus* also suppressed proliferation of MCF-7 human breast cancer cells (Martin *et al.*, 2010).

2.7.2.2. Antimicrobial activity

Bioactive substances of several *Pleurotus sp*. have showed antibacterial, antifungal, antiviral, and antimicrobial activities. Many studies have demonstrated the antimicrobial efficacy of extracts obtained from oyster mushrooms. Secondary metabolites, protein-polysaccharide compounds, some phenols and phytochemicals, free fatty acids and their derivatives may have the role in producing antimicrobial effects (Rathod, Mukundraj G, *et al*., 2021).

2.7.2.3. Antioxidant activity

Antioxidant compounds help to prevent from oxidative damage of cells and tissues. Stress on the body due to aging, obesity, and detrimental lifestyle choices is another serious health issue, which often takes the form of oxidative damage to tissues. The requirements for natural sources of antioxidant foods have been identified in edible mushrooms (Kozarski *et al.*, 2015) .

2.7.2.4. Immunomodulators

Are also known as biological response modifiers, immunoaugmentors, or immunorestoratives. In any healthy organism, the immune system produces a wide range of immunomodulators to maintain homeostasis within the body. Lectins, terpenoids, proteins, and polysaccharides are some classes of immunomodulators, classified on the basis of their chemical nature; whereas in clinical practice they are classified into immunosuppressants, immunostimulants, and immunoadjuvants. Top ten mushrooms with immunomodulatory activity are *agaricus subrufescens, cordyceps sinensis, ganoderma lucidum, grifola frondosa, hericium erinaceus, inonotus obliquus, lentinula edodes, pleurotus ostreatus, poria cocos and trametes versicolor* (El Enshasy *et al.*, 2013*)*. So, cultivating and eating edible mushroom has several nutritional and medicinal importances.

Therefore; mushrooms can be able to grow in different parts of Ethiopia and this research will be conducted in order to cultivate oyster (*pleurotus ostreatus*) mushroom on sawdust, grass straw and teff straw to show and aware of the society around the study area.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at the Biotechnology Laboratory of Woldia University, located in Woldia town, the administrative capital of the North Wollo Zone in the Amhara Regional State, Ethiopia. Woldia is situated at a latitude of 11°49′ N and a longitude of 39°36′ E, at an elevation of approximately 2,112 meters above sea level with the total population of more than 83,000 residents. The area falls within the Weyna Dega (midland) agro ecological zone and is characterized by moderate temperatures and seasonal rainfall patterns. The climate in Woldia is typically subtropical highland, with average annual temperatures ranging from 10°C to 28°C and annual rainfall between 700 mm and 1,200 mm, mainly occurring from June to September. The predominant soil types in the area are verti soils and cambisols, which support diverse agricultural production under both rains, fed and irrigated conditions. Agriculture is the primary livelihood activity in Woldia and its surrounding areas, employing the majority of the population. The region is known for growing a variety of crops, including cereals (e.g., teff, sorghum, and maize), pulses (e.g., lentils and chickpeas), and horticultural crops, particularly tomatoes, onions, and peppers. Livestock farming is also common, with cattle, goats, sheep, and poultry being the main animals. This research was conducted from January to April 2017 EC.

3.2. Experimental Material

The materials those were used in this study includes mushroom mycelium, sorghum grain, wheat bran, substrates, hood, plastic bag, autoclave, Petri dish, aluminum foil, rubber band, tissue paper, sprayer, bunsen burner, hot plate, ph meter, spatula, incubator, string balance, metric ruler, measuring cylinder, electronic balance, hammer mill , thermometer, humidity tester, glove, glass bottles, metal barrel, cotton etc.

3.3. Sample Collection

The pure culture of oyster mushroom (*Pleurotus ostreatus)* mycelium was collected from Mycology Laboratory, Department of biotechnology in Hollata agricultural research center. The mouth of the slant culture was folded by aluminum foil and Para film in order to protect it from contamination during transportation. Then, the culture was transported to Woldia University,

biotechnology laboratory at -4 by using portable ice box. After reaching to the laboratory it was kept at -25^{0}C in a deep freezer until it was sub cultured and refreshed (Mondal *et al*., 2010).

Impurity and pest free sorghum grains was obtained from the market in woldia town for spawn preparation and Wheat bran was also taken from a mill house as a nutrient supplies.

Agricultural residues and industrial wastages like; teff straw, grass straw and sawdust were collected from local farmers in enough amount around woldia university.

3.4. Treatments and experimental design

The treatments consisted of seven treatments (teff straw, teff straw + grass straw, grass straw, grass straw + sawdust, sawdust, sawdust+teff straw, teff straw+grass straw+sawdust) arranged in Complete Randomized Design (CRD) design with three replications. The total experiment set up was 7*3 = 21 trials.

Table 3. 1: treatment composition ("<Fathima_Afrah_Mohideen_BMS_Journal.pdf>,")

Treatments	**Substrate code**	**Compositions**
Trt 1	TS	100% teff straw
Trt 2	TS+GS	50% teff straw + 50% grass straw
Trt 3	GS	100% grass straw
Trt 4	GS+SD	50% grass straw + 50% sawdust
Trt 5	SD	100% sawdust
Trt 6	SD+TS	50% sawdust + 50% teff straw
Trt 7	TS+GS+SD	33.3% teff straw + 33.3% grass straw + 33.3% sawdust

Trt = treatment, TS = teff straw, GS = grass straw only, SD = sawdust, TS+GS = teff straw + grass straw, SD+TS = sawdust + teff straw, GS+SD = grass straw+ sawdust, TS+GS+SD = teff straw + grass straw + sawdust.

3.5. Experimental Procedure and Preparation

3.5.1 Mycelium culture and PDA Preparation

To obtain pure culture, PDA culture or tissue culture planting method was used. The PDA media was prepared by using of 250 g peeled and sliced potato, 20 g dextrose, 30 g agar and 250 mg aspergine in one liter of water. About 5 ml of PDA mixture was poured in each test tube followed by plugging. The media was sterilized in an autoclave for 15 minutes at 121°C with 1.5 kg /cm2 pressure. The sterilized PDA containing test tube was kept in a slanting position. The mushroom was thoroughly pre-washed in distilled water. A scalpel was then dipped in alcohol and flamed until it was red hot. Then it was cooled for 10 seconds. The cut was made length wise from the cap to down wards. Small piece of the internal tissue of the broken mushroom was cut and removed with a flamed needle. The needle with the tissue attached was then immediately inserted in to test tube slant and the tissue laid on the agar surface. The mouth of the test tube was flamed before the needle was inserted. The mouth of the test-tube was plugged with cotton plug. After 3 to 4 days, the tissue was covered with a white mycelium that was spread on the agar surface (Mondal *et al*., 2010).

3.5.2. Spawn preparation

Spawn is the vigorous mycelia growth of a single fungus on a chosen substrate (Musakhail *et al.*, 2011). About 3.5 kg of sorghum grains was washed and dead floating was removed. Then, it was soaked overnight in 8L of water and was rinsed three times in distilled water. The excess water was drained off. About 12% wheat bran, 2% gypsum ($CaSo_4$. $2H_20$) and 1% ($CaCO_3$) was added and mixed (Alemu & Fekadu, 2014). Moisture was maintained at the level of 55 %, and distributed equally in to 500 ml volume glass bottle at the rate 100g of grains per bottle.

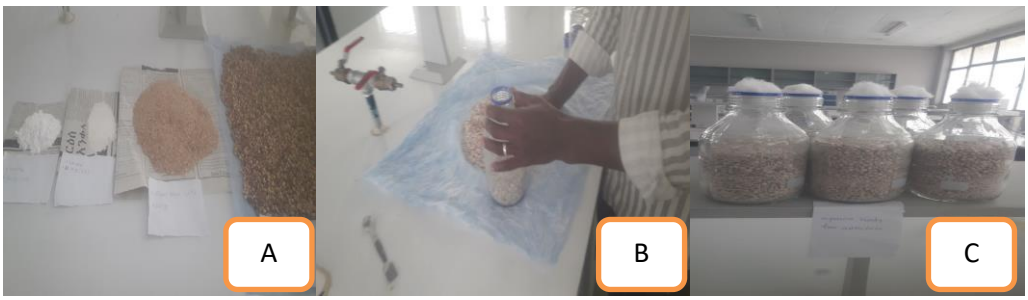

Figure 3.1: Spawn preparation

A: sorghum and other components, B: pouring the spawn into bottles, C: spawns ready for autoclave

3.5.3 Sterilization and incubation of spawn

Once the mouth of the bottles was plugged with cotton the spawn was autoclaved at 121°C and 15 Pascal for 2 hrs. After sterilization allowed for cooling, each bottle was inoculated with eleven days old mycelium culture aseptically in laminar airflow cabinet by using a cork borer and the borer was flame sterilized between inoculations of each spawn bottles. Finally the inoculated spawn bottles were incubated at 27^0C in the dark in an incubator until the substrate was fully colonized; at ten days interval mycelia invasion and contamination was recorded

Figure 3.2: Sterilization and incubation of spawn

A: incubated spawn B: colonized spawn

3.4.3. Substrate preparation

The following substrates teff straw and grass straw “except sawdust which is already in proper size” was chopped into 2-3 cm pieces using an axe. These was then separately soaked in sufficient amount of water over night and the excess water drained off by squeezing (Kinge *et al*., 2016). Then, it was mixed with 10% wheat bran, 3% gypsum and 2 % $CaCO_3$. Substrates was transferred and distributed equally into sterilizable polyethylene bags of 36x60 cm size at the rate of 1 kg substrate per polyethylene bag was filled.

3.4.4 Sterilization and inoculation of substrates

The substrates in the polyethylene bags were sterilized by autoclave at 121°C for three hours. After sterilization bags were left for cooling. Then, substrates were inoculated with the spawn

(one glass bottle per bag) in a sterilized surface. Spawns were added layer by layer on the substrate and were mixed thoroughly to facilitate rapid and uniform mycelia growth.

The mouths of the bags were tightly tied using a rubber band (Alemu & Fekadu, 2014). Pin holes were made through the bags (1/100 cm2) for drainage and aeration. The holes were plugged with sterilized cotton. It was kept in a spawn running room at room temperature in the dark until primordia formation. After primordial formation, large holes were made in the polythene bag to allow normal development of fruiting bodies. Bags was transferred to mushroom house under normal environmental conditions with a relative humidity maintained at 85- 90%. It was moistened using tap water morning and evening until all flushes of fruiting bodies was harvested. Adequate ventilation was provided to prevent increased CO_2 concentration in the room by opening the door and windows for half an hour in the morning and evening (Keneni *et al.*, 2016).

Figure 3. 3: Substrate preparation

A: teff straw, B; grass straw

3.4.5. Fruiting

When the mycelium was fully colonized the substrate, it was produced fruiting bodies in response to a sudden change to the external physical environment, which first promoted the formation of initial fruiting bodies that later develop into fruiting bodies. The environmental changes that may trigger fruiting include changes in temperature, relative humidity, and light and water availability within the compost. These induction factors had an impact on the quality of the mushroom (Golian *et al.*, 2021). For pleurotus species decreasing the temperature from the

spawn run temperature (25 to 27°C) to between 12°C and 18°C is the most common induction method that was used to stimulate fruiting. The relative humidity was adjusted as 85-95% by spraying water to the bags and the room. The primordial formed at the top of the bags and developed within 3 to 4 days and was ready to be under proper fruiting conditions, the additional flushes was occurred without any new inductions, but the flushes were controlled by heating the blocks/bags followed by reducing the temperature.

3.4.6. Harvesting

The procedure for oyster mushroom harvesting involves grasping each mushroom stalk individually and twisting the mushroom until it was pulled out of the substrate. The mushroom harvesting periods vary between different mushroom strains and usually range from 6 to 12 weeks and they can be harvested on a number of flushes. During harvesting the number of flushes and production time were determined by substrate formulation and environmental conditions. In case of oyster mushroom the total time period starting from culturing mycelium to harvesting was about eight weeks. Nutrients were accumulated at resting stage (a period between mushroom flushes). During this stage, contamination was prevented to allow rapid mycelia growth by spraying 75% alcohol in to the walls, surface and surroundings of the growing room. Harvesting of mushrooms was carried out at proper stages depending on the mushroom species in order to avoid a decreased market value and quality of mushroom (Besufekad *et al.*, 2020).

3.7. Data Collection

The data was collected from different parameters such as;

3.7.1. Growth rate (days)

Time of mycelium colonization, pinhead formation and maturation of fruiting body was taken for each treatment.

3.7.2. Pileus diameter and Stipe length (cm)

The length and diameter of the stalk and cap of fruiting bodies respectively were measured by a ruler for each treatment in all the three flushes.

3.7.3. Number of fruiting bodies (number)

Number of effective fruiting bodies produced per polyethylene bag was counted for all flushes.

3.7.4. Yield of fresh mushroom (g)

Fresh weight of mushrooms produced from each treatment was measured by beam balance and recorded.

3.7.5. Biological efficiency (%)

The biological efficiency of oyster mushrooms for each treatment was calculated as;

BE = Weight of fresh mushroom /Dry weight of substrate × 100%

3.7. Data Analysis

Prior to analysis, all the collected data were checked for normality and subjected to analysis of variances (ANOVA) using SAS statistical software (SAS version 9.3). Based on Ahmed *et al*. (2013), the differences among treatment means were compared using the Least Significance Difference test (LSD) at 1% or 5% probability level. Moreover, simple Pearson's correlation analysis was used to reveal the magnitudes and directions of the relationships between selected substrate, yield and efficiency of the mushroom.

4. RESULTS AND DISCUSSION

4.1. Results

The study was conducted to find out the effect of different substrates on the growth, yield and biological efficiency of' oyster mushroom (*Pleurotus ostrealus*). The results were presented and discussed with the help of tables, graphs and possible interpretations as shown below.

4.1.1 Effect of different substrates on colonization, pinhead formation and harvesting time

Mycelia invasion was observed within eight days of inoculation in all the seven substrates. Complete colonization of the substrates was seen after 20 days of spawn inoculation. The colonization data showed significant variation among treatments. The longest colonization time 25 days was shown on 33.3% TS + 33.3% GS + 33.3% SD which was significantly greater than most other treatments, indicating a delayed onset followed by 100% GS 24 days. This result was statistically similar with 100% SD and 100% TS which were 21 and 21.33 days respectively as shown in table 4.2.

Pinhead formation varied significantly across treatments. Again the highest value 25 days, was shown on 33.3% TS + 33.3% GS + 33.3% SD while 100% GS recorded the lowest 19 days, indicating an earlier pinhead formation time. Treatments like 100% TS, 50% TS + 50% GS, 100% SD, and 50% SD + 50% TS grouped closely as indicated in table 4.2.

Days of maturation of fruiting bodies were highest on substrates of 33.3% TS + 33.3% GS+ 33.3% SD 26.67 days, suggesting extended maturation time, followed by 50% TS + 50% GS, 26 days which were statistically higher than all others and significantly different from 100% TS and 100% GS recorded as 24 and 23.67 days respectively. The lowest was seen on 100% SD as 23 days, implies taking shorter time to mature and harvest, as shown in table 4.2 below. Despite of taking longer day’s mycelium colonization, pinhead formation and maturity of fruiting bodies the combined substrates overall performance was higher.

Figure 4. 4: Colonization, pinhead formation and maturation of oyster mushroom

A: colonization, B: pinhead Formation, C: maturation time

Table 4.2: Effect of substrates on colonization, pinhead formation and maturation period of fruiting bodies in days.

TRTS	CZN (Days)	PHF (days)	MFB (days)
100% TS	21.33c	21.00bcd	24.00bcd
50% TS + 50% GS	22.00bc	21.00bcd	26.00ab
100% GS	24.00ab	19.00d	23.67cd
50% GS + 50% SD	23.67ab	23.00ab	25.67abc
100% SD	21.00c	21.67bc	23.00d
50% SD+ 50% TS	20.00c	21.33bc	25.00abcd
33.3% TS + 33.3% GS + 33.3% SD	25.00a	25.00a	26.67a
C.V (%)	5.24	5.45	5.04
LSD (0.05)	2.06	2.06	2.19

TRTS= treatments, trt=treatment, CZN= colonization, PHF= pinhead formation, MFB= maturation of fruiting body

4.1.2. Effect of different substrates on stipe length and cap diameter

Statistically significant variation was recorded in terms of stipe length and cap diameter of oyster mushroom. The length of the stipe was found highest for 100% SD 5.9cm which had a significant variation from 100% TS and 50% TS + 50% GS 3.57 and 4.6 respectively and statistically similar with 50% SD + 50% TS 5.07cm. On the other hand, the lowest stipe length

was observed on 33.3% TS + 33.3% GS + 33.3% SD, 3.47cm for the first harvest. Whereas the highest value of stipe length for the second harvest was recorded from 33.3% TS + 33.3% GS + 33.3% SD 4.23cm indicating better regrowth performance and the lowest value was 100% TS 2.13cm. In addition from 33.3% TS + 33.3% GS + 33.3% SD again obtained the highest stem length 5cm and 100% GS 2.9cm was taken as the lowest stalk length recorded from the third harvest.

Regarding to cap diameter the highest was recorded from 100% TS 7.73cm followed by 100% SD 7.03cm and both 50% TS + 50% GS and 50% SD + 50% TS were the lowest 4.23cm each, in which there was no significant variation between them in terms of cap diameter in the first flush.

But, in the second and third harvest the highest cap diameter were taken from 100% SD 4.97cm and 5.07cm for second and third flushes respectively and the lowest values were from 50% SD + 50% TS 2.87cm for the second and 50% TS + 50% GS 3.03cm for the third harvest. Among all the three harvesting periods the highest and lowest cap diameter were 100% TS 7.73cm and 50% SD + 50% TS 2.87cm as shown in table 4.3.

Table 4.3: Effect of different substrates on stipe length and cap diameter of P. ostreatus for the three consecutive harvesting periods.

	Stipe length (cm)			Cap diameter (cm)		
Treatments	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest
100% TS	3.57c	2.13d	3.00bc	7.73a	4.00ab	4.87a
50% TS + 50% GS	4.60b	3.03bcd	4.73a	4.23d	3.97b	3.03c
100% GS	4.77b	3.20abcd	2.90c	5.87c	4.30ab	4.17b
50% GS + 50% SD	4.90b	3.83ab	3.27b	4.70d	4.42ab	4.77a
100% SD	5.90a	3.63abc	2.97bc	7.03b	4.97a	5.07a
50% SD+ 50% TS	5.07b	2.60cd	4.80a	4.23d	2.87c	4.93a
33.3% TS + 33.3% GS + 33.3% SD	3.47c	4.23a	5.00a	5.97c	4.37ab	5.07a
C.V (%)	8.38	20.80	4.96	5.02	13.71	7.05
LSD (0.05)	0.67	1.17	0.33	0.49	0.99	0.56

4.1.3. Effect of different substrates on number of effective fruiting bodies of *P. ostreatus* for the three consecutive harvesting periods

With respect to number of effective fruiting body there was a significant variation in between treatments. The maximum fruiting bodies were counted from 50% SD + 50% TS 26, 100% GS 14, and 100% SD 12.67 for first, second and third harvest respectively. 100% GS 17.67, 100% TS 11 and 50% TS + 50% GS 9 were found to be the lowest values for first, second and third flushes respectively as shown in table 4.3.

Figure 4. 5: Fruiting body of oyster mushroom

Table 4.4: Effect of different substrates on number of effective fruiting bodies of P. ostreatus for the three consecutive harvesting periods.

	Number of fruiting bodies (No)		
Treatments	1st Harvest	2nd Harvest	3rd Harvest
100% TS	22.67bc	11.00c	10.00bcd
50% TS + 50% GS	20.00de	12.00	9.00d
100% GS	17.67e	14.00bc	9.67cd
50% GS + 50% SD	21.00cd	11.67a	11.67abc
100% SD	24.67ab	12.00c	12.67a
50% SD+ 50% TS	26.00a	12.00bc	12.33ab
33.3% TS + 33.3% GS + 33.3% SD	21.67cd	13.33bc	11.33abcd
CV (%)	6.74	7.32	12.28
LSD(0.05)	2.59	1.57	2.35

4.1.4. Effect of different substrates on yield of fresh mushroom and biological efficiency.

The maximum yield of fresh mushroom was obtained from 50% TS + 50% GS 290.27g which were significantly different and statistically similar with 100% SD and 50% GS + 50% SD 284g and 281.9g respectively and the lowest yield was seen from 50% SD+ 50% TS 255.3g in the first flush.

In the second flush 100% GS and 33.3% TS + 33.3% GS + 33.3% SD had the highest yields 169.37 g and 167.97 g respectively which was significantly more than all others. Whereas, the minimum yields measured for second harvest was 50% TS + 50% GS 139.73g.

The maximum yield was obtained from 100% SD 105g and 33.3% TS + 33.3% GS + 33.3% SD 104.33g in the third harvesting period and the lowest yield was from 100% TS 85.20 g indicating reduced productivity in later stages. There was a significant different at $P \leq 0.05$ between treatments as shown in table 4.5.

The highest biological efficiency was taken from 33.3% TS + 33.3% GS + 33.3% SD which was 53.09% and the least was 49.03 from 50% SD+ 50% TS substrate.

Figure 4. 6: Yield of fresh mushroom

Table 4.5: Means of yield and biological efficiency of *P. ostreatus* for all harvests

Treatments	YFM (g)				BE (%)
	1st Harvest	2nd Harvest	3rd Harvest	TYFM (g)	
100% TS	274.80c	157.33b	85.20c	517.33bc	51.73
50% TS + 50% GS	290.27a	139.73c	94.67b	524.67ab	52.47
100% GS	265.00d	169.37a	93.00bc	527.37ab	52.74
50% GS + 50% SD	281.90bc	119.40d	100.57ab	501.87de	50.19
100% SD	284.00ab	118.13d	105.00a	507.13cd	50.71
50% SD+ 50% TS	255.30e	141.00c	94.00b	490.30e	49.03
33.3% TS + 33.3% GS + 33.3% SD	258.57de	167.97a	104.33a	530.87a	53.09
CV (%)	1.59	1.86	5.03	1.41	1.02
LSD(0.05)	7.5860	4.7226	8.5112	4.18	3.87

TYFM =total yield of fresh mushroom, BE = biological efficiency

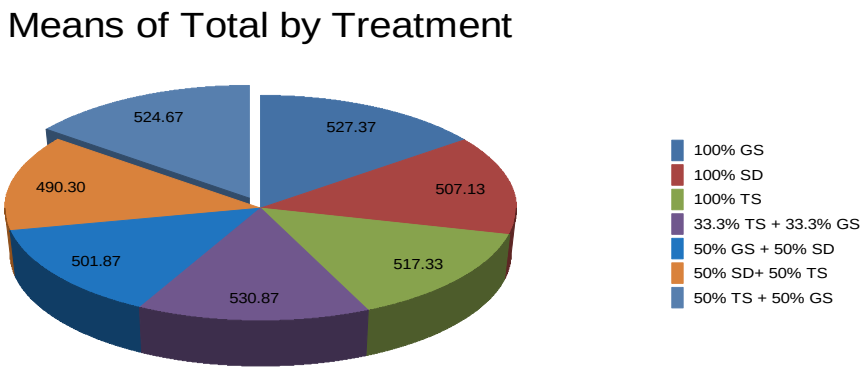

Figure 4. 7: Means of total fresh mushroom by Treatment

In recent times, mushroom production has gained attention both globally and nationally because of its nutritional, industrial and medical value as well as its ecosystem sustainability or bioremediation. Generally, it have been understood that mushrooms such as P. ostreatus has a potential to turn over various agro wastes. Thus, the substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at low cost and in huge amounts in southwestern part of Ethiopia. Utilization of these agro-wastes for the production of P. ostreatus mushrooms could be significant to alleviate food security as well as environmental rehabilitation.

Table 4.6: ANOVA results of all parameters for the first harvest

		Sum of Squares	df	Mean Square	F	Sig.
colonization	Between Groups	59.810	6	9.968	7.218	.001
	Within Groups	19.333	14	1.381		
	Total	79.143	20			
pinhead formation	Between Groups	69.810	6	11.635	8.425	.001
	Within Groups	19.333	14	1.381		
	Total	89.143	20			
maturation of fruiting body	Between Groups	32.571	6	5.429	3.455	.026
	Within Groups	22.000	14	1.571		
	Total	54.571	20			
stipe length	Between Groups	13.131	6	2.189	14.684	.000
	Within Groups	2.087	14	.149		
	Total	15.218	20			
cap diameter	Between Groups	33.932	6	5.655	69.452	.000
	Within Groups	1.140	14	.081		
	Total	35.072	20			
number of fruiting body	Between Groups	142.286	6	23.714	10.826	.000
	Within Groups	30.667	14	2.190		
	Total	172.952	20			
biological yield	Between Groups	3261.013	6	543.502	28.963	.000
	Within Groups	262.713	14	18.765		
	Total	3523.727	20			
biological efficiency	Between Groups	32.610	6	5.435	28.963	.000
	Within Groups	2.627	14	.188		
	Total	35.237	20			

4.2 Discussion

Mycelial growth was observed within eight days on all seven substrates, achieving full colonization by 20 days post-inoculation. However, colonization time varied significantly across treatments. The longest colonization (25 days) occurred on the mixed substrate (33.3% TS + 33.3% GS + 33.3% SD), followed by 100% GS (24 days), both showing delays compared to 100% TS and 100% SD, which completed colonization in about 21 days. The time to pinhead formation differed notably among treatments. The mixed substrate (33.3% TS + 33.3% GS + 33.3% SD) had the slowest pinhead emergence (25 days), while 100% GS recorded the fastest (19 days). Other treatments like 100% TS, 50% TS + 50% GS, 100% SD, and 50% SD + 50% TS formed pinheads in similar timeframes, as presented in Table 4.2.

Fruiting body maturation also varied significantly. The longest maturation time (26.67 days) was observed on the mixed substrate, followed by 50% TS + 50% GS (26 days), both significantly longer than other treatments. In contrast, 100% SD showed the shortest maturation time (23 days), indicating quicker harvest readiness. Although the mixed substrates required more time for colonization, pinhead formation, and fruiting body maturation, they ultimately demonstrated superior overall performance, suggesting a potential trade-off between growth speed and yield quality or productivity.

The shortest colonization period (20 days) was observed on 50% sawdust + 50% teff straw, comparable to the findings of (Adams *et al*., 2022) where sawdust substrate promoted faster mycelium growth due to its rich lignocellulosic content. Substrates containing sawdust and teff straw (either alone or in mixtures) consistently supported faster mycelial colonization (20-21 days), earlier pinhead formation, and shorter fruiting body maturation periods compared to grass straw-dominant treatments. This can be reasonably justified by the higher lignocellulosic content in sawdust and teff straw, which provides readily available carbon sources crucial for vigorous mycelial growth, as also indicated by (Adams *et al*., 2022; Grimm & Wösten, 2018). Regarding pinhead formation and maturation, faster development on substrates with higher percentages of sawdust and teff straw supports (Uddin *et al.*, 2011) who noted optimal mycelial development and pinhead initiation in substrates rich in cellulose at favorable pH (6.5–7.0) and temperature

(25–30°C). The study observed that substrate composition significantly influenced colonization, pinhead formation, and maturation time for *Pleurotus ostreatus*.

Moreover, the highest stipe lengths and cap diameters observed on 100% sawdust and 100% teff straw substrates suggest that the structural quality and moisture-holding capacity of these substrates create optimal physical conditions for fruit body development. Particularly, larger cap diameters, seen with teff straw, are associated with better marketability and higher consumer preference, further enhancing the economic viability of these substrates (Hu *et al*., 2022). The study observed that substrate composition significantly influenced colonization, pinhead formation, and maturation time for *Pleurotus ostreatus*. These results align with the findings of (Rathod, Mukundraj, et al., 2021; Silva et al., 2018) who reported that substrate type, temperature, humidity, and pH critically affect the biological efficiency and productivity of oyster mushrooms. This result was consistent with the reports suggest that a balanced substrate composition enhances fruiting body quality, particularly cap expansion, which is critical for market value.

The highest number of fruiting bodies recorded from the 50% sawdust + 50% teff straw substrate mirrors the findings of (Adams *et al*., 2022) where sawdust-based substrates yielded abundant and healthy fruiting bodies due to sufficient nutrient availability and substrate aeration.

The highest total yield was observed in 33.3% TS + 33.3% GS + 33.3% SD 530.87 g followed closely by 100% GS 527.37 g and 50% TS + 50% GS 524.67 g. These treatments consistently performed well across harvests, particularly in the 2nd and 3rd cuts. The lowest total biological yield was produced from 50% SD+ 50% TS 490.30 g, with modest performance in all harvests as indicated in table 4.5. LSD values (as low as 4.18 g for total biological yield) indicate statistically significant differences even between treatments with small yield gaps. The CVs were impressively low (1.41%–5.03%), indicating high experimental precision.

Biological efficiency is calculated as total yield divided by dry weight of substrate and multiply by 100% it increases with increasing total yield. When comparing yield and biological efficiency, the study found that the highest total yield and efficiency (530.87g and 53.09%) were achieved using a mixed substrate (33.3% teff straw + 33.3% grass straw + 33.3% sawdust). This

agrees with results reported by emphasizing that substrate mixtures often outperform than single substrates due to complementary nutrient profiles and better physical properties e.g., water retention and aeration as shown in table 4.5. Moreover, the high biological efficiency aligns with the global standards described by (Grimm & Wösten, 2018), who noted that biological efficiency above 50% indicates good substrate optimization and effective mushroom cultivation practices. In addition, the highest biological efficiency (53.09%) was a clear indicator of optimal nutrient availability and substrate decomposition rate, leading to enhanced mushroom conversion rates. Adams *et al*. (2022) studied the growth of King Oyster mushroom (Pleurotus eryngii) on sawdust and rice straw in Bangladesh and found that sawdust showed the highest biological efficiency (73.5%) and in this study 100% sawdust substrate gives a biological efficiency of 50.7%, even if it is above the standard it is significantly different from the above result. This is because the species difference. Since biological efficiency directly reflects the effectiveness of substrate utilization, these results substantiate that balanced substrate compositions are superior to single-material substrates for sustainable and profitable mushroom cultivation (Silva *et al.*, 2018). In general, the rate of mycelia invasion in most treatment group was highly related with completed invasion and pinning days. However, stipe length, pilus diameter and other parameters is not always indicator for higher yield obtained as also observed (Gume *et al*., 2013). Both pinhead maturation and abortion are affected by the type of substrates and environmental factors.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study assessed the impact of different substrates and their combinations on the growth parameters, yield, and biological efficiency of *Pleurotus ostreatus* cultivated under controlled laboratory and environmental conditions. The results clearly demonstrated that substrate composition significantly influences the vegetative and reproductive development of oyster mushrooms. Among the seven treatments tested, the combination of 33.3% teff straw, 33.3% grass straw, and 33.3% sawdust yielded the best overall performance. It recorded the highest total biological yield (530.87 g) and biological efficiency (53.09%), alongside consistent fruiting body development and superior morphological traits across multiple harvests. In contrast, the 50% sawdust + 50% teff straw treatment, while producing a relatively high number of fruiting bodies, underperformed in both total yield and efficiency metrics.

These findings confirm that mixed substrates enhance mushroom growth by balancing nutrient availability, moisture retention, and structural support. Additionally, they highlight the viability of using locally available agricultural residues for sustainable, low-cost mushroom cultivation, which is crucial for food security and income generation in resource-constrained settings like Ethiopia.

5.2. Recommendations

Based on the findings of this study, it is recommended that mushroom growers adopt a mixed substrate composed of 33.3% teff straw, 33.3% grass straw, and 33.3% sawdust, as it consistently demonstrated superior performance in terms of yield and biological efficiency. Substrate-specific strategies should also be considered depending on the cultivation goals; for instance, 100% sawdust or 100% grass straw can be used when rapid colonization and early pinhead formation are desired, while a 50% teff straw and 50% grass straw mix is suitable for achieving good yields in shorter growing cycles. Additionally, the use of locally available agricultural residues should be promoted to encourage low-cost, sustainable production systems that also help reduce environmental waste. Further research is needed to assess the adaptability and performance of these substrate combinations across different agro-ecological zones and

under varying climatic conditions. Moreover, conducting a comprehensive cost-benefit analysis of each substrate type will support farmers in making economically sound decisions. Lastly, capacity-building efforts, including the training of local farmers and extension workers in substrate preparation and proper cultivation techniques, are essential for scaling up oyster mushroom production in rural and semi-urban areas. Therefore, further research should be done on mixture of sawdust, teff straw and grass straw at 33.3% of each substrate at 85-90% relative humidity, 27^{o}c spawn running and 18^{o}c growing room and PH 6.5-7.5 with better aeration would be good for better production.

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7. APPENDICES

Appendix 7.1: ANOVA results of first flush

		Sum of Squares	df	Mean Square	F	Sig.
colonization	Between Groups	59.81	6	9.96	7.21	.001
pinhead formation	Between Groups	69.81	6	11.63	8.42	.001
maturation of fruting body	Between Groups	32.57	6	5.42	3.45	.026
stipe length	Between Groups	13.13	6	2.18	14.68	.000
cap diameter	Between Groups	33.93	6	5.65	69.45	.000
number of fruting body	Between Groups	142.28	6	23.71	10.82	.000
biological yield	Between Groups	3261.01	6	543.50	28.96	.000
biological efficiency	Between Groups	32.61	6	5.43	28.96	.000

Appendix 7. 2: ANOVA result of second flush

		Sum of Squares	df	Mean Square	F	Sig.
maturation of fruiting body	Between Groups	46.47	6	7.74	3.32	.030
Stipe length	Between Groups	9.51	6	1.58	3.49	.025
cap diameter	Between Groups	7.52	6	1.25	3.91	.017
number of fruiting body	Between Groups	18.95	6	3.15	3.90	.017
biological yield of mushroom	Between Groups	8080.85	6	1346.80	185.19	.000
biological efficiency	Between Groups	80.80	6	13.46	185.19	.000

Appendix 7.3:ANOVA result of third flush

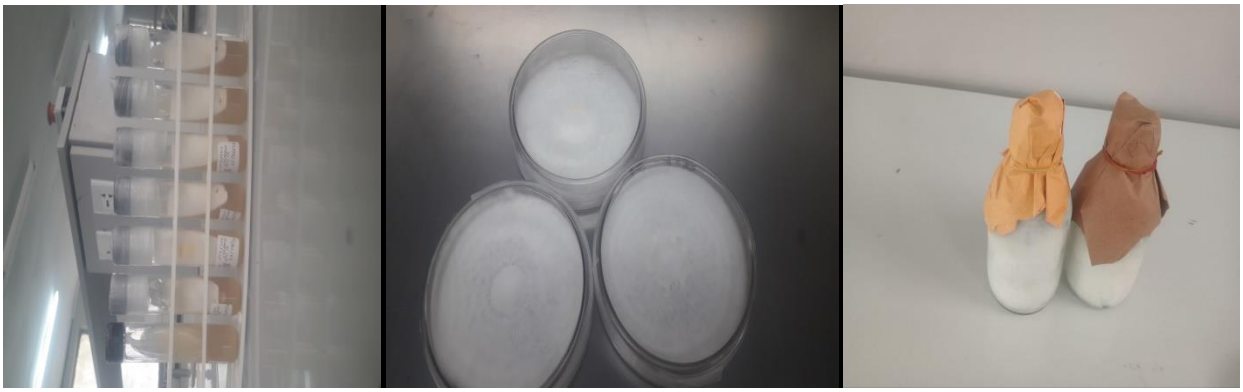
		Sum of Squares	df	Mean Square	F	Sig.
maturation of fruiting body	Between Groups	30.47	6	5.07	4.26	.012
Stipe length	Between Groups	17.21	6	2.87	80.35	.000
cap diameter	Between Groups	9.82	6	1.63	15.84	.000
number of fruiting body	Between Groups	35.61	6	5.93	3.28	.031
biological yield	Between Groups	898.41	6	149.73	6.33	.002
biological efficiency	Between Groups	8.98	6	1.49	6.33	.002

Appendix 7.1: spawn preparation




Appendix 7.2: substrate preparation and inoculation

Appendix 7. 3: from colonization to harvesting



Appendix 7. 4: long term preserved mycelium and spawn as a seed