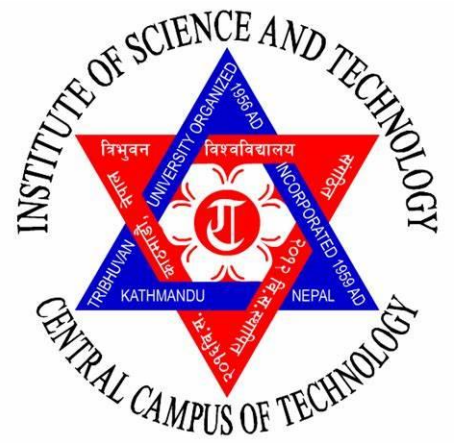
EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATION ON THE YEILD OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS)*



A

Dissertation

Submitted to the **Department of Microbiology**,

Central Campus of Technology,

Tribhuvan University Dharan, T.U,Nepal

In Partial Fulfillment of the Requirements for the Award of

Degree of Master of Science in Microbiology

(Agriculture)

By:

PRAKASH BAJAGAIN

Exam Roll No.: MB1464

Batch No.: 075/076

T.U. Regd. No.: 5-2-3-455-2011

©Tribhuvan University

2023

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE & TECHNOLOGY
Central Campus of Technology

Phone No. : 025-520228
025-526530
Post Box No. 4

DHARAN-14, HATTISAR
SUNSARI, NEPAL

Department of .....

Ref. .....

Date : .....

Date: 08/15/2023

RECOMMENDATION

This is to certify that Mr. Prakash Bajagain has carried out the dissertation work entitled **"EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATION ON THE YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus)*"**, under our supervision. The entire work is based on the collection of primary data by the student. This outcome has not been submitted for any other academic degree elsewhere. Therefore, we advise using this dissertation to partially fulfill the requirements for Tribhuvan University's Master of Microbiology degree.

.....

Mr.Dhiren Subba Limbu
(Supervisor)
Program Co-Ordinator
Msc.Microbiology Program
Central Campus of Technology

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE & TECHNOLOGY
Central Campus of Technology

Phone No. : 025-520228
025-526530
Post Box No. 4

DHARAN-14, HATTISAR
SUNSARI, NEPAL

Department of .....

Ref. .....

Date : .....

Department of Microbiology
Central Campus of Technology
Hattisar, Dharan

CERTIFICATE OF APPROVAL

The dissertation paper submitted by Mr. Prakash Bajagain entitled **"EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATION ON THE YEILD OF OYSTER MUSHROOM (*Pleurotus ostreatus)"*** has been accepted as a partial fulfillment of the requirements for the Masters in Microbiology (Agriculture).

.....

Assoc.Prof.Dr.Dil Kumar Limbu
Campus Chief
Central Campus of Technology
Tribhuvan University

.....

Mr. Dhiren Subba Limbu
Program Co-Ordinator
Msc.Microbiology Program
Central Campus of Technology

Date: 2080-04-30

Department of Microbiology
Central Campus of Technology
Hattisar, Dharan

BOARD OF EXAMINERS

Recommended by

.....

Supervisor

Mr. Dhiren Subba Limbu

Department of Microbiology,

Central Campus of Technology, Dharan

Approved by

.....

Program Co-ordinator

Mr. Dhiren Subba Limbu

Department of Microbiology,

Central Campus of Technology, Dharan

Examined by

.....

External Examiner

Asst. Prof. Janak Raj Dhungana

Department of Microbiology,

Tri-Chandra Multiple Campus, Kathmandu

.....

Internal Examiner

Mr. Arjun Ghimire

Department of Microbiology,

Central Campus of Technology, Dharan

Date: 08./.15./.2023

ACKNOWLEDGEMENT

Central Campus of Technology
Hattisar, Dharan

Without the help and support of numerous people and organizations, this dissertation report could not have been made possible. I consider myself incredibly fortunate to have received this while working on my thesis. I am grateful to **Mr. Dhiren Subba Limbu**, the department head of microbiology and **Msc. Microbiology Program Coordinator** at the Central Campus of Technology, and **Dr. Dil Kumar Limbu**, an associate professor for providing me instruction I needed to complete my dissertation.

For their excellent cooperation and assistance, I also want to thank each and every instructors **Mr. Sagar Gautam, Mr. Purna P. Chapagai, Mr. Basistha Acharya, Mr. Gopal Parajuli** and **Mr. Ramesh Kumar Yadav** and staff, including **Ain Bdr. Karki,** librarian **Mr. Omnath Khatiwada**, and library staff at the **Central Campus of Technology**.

My sincere gratitude also include out to Belbari-9, **Ms. Pratima Khanal**, the accountant, **Mrs. Devyani Basnet**, chairman, and the rest of the staff at **Bahumukhi Krishi Farm Pvt. Ltd.** For approval for this research to conduct at their own facility.

My sincere gratitude goes out to Sundar Dulari Municipality, Morang, **Mr. Suraj Nepal**, Director, and all the staffs of **Nepal Brothers mushroom and Agro research Pvt. Ltd for** providing ***Pleurotus ostreatus*** Spawn to perform this research work.

Finally, I want to express my gratitude to God, My beloved wife and my Parents for helping me get to where I am today through their unwavering support, blessings, and encouragement.

Prakash Bajagain

Date: 2080/04/30

ABSTRACT

Of all the cultivated mushrooms that can grow all year round, *Pleurotus* has the species that is most developed commercially. They can use a variety of agricultural waste products to transform lignocellulose biomass into delicious, nutritious food. Using agricultural and agricultural waste as a substrate for cultivation of edible mushrooms is an effective and economical technology for turning these waste products into a food that is high in protein and a commercially valuable cash crop. Paddy straw, Wheat husk, and Maize cob were used as substrates in this study for the cultivation of *Pleurotus ostreatus* mushrooms. The data was analyzed on various aspects such as time required for colonization, time required for pinhead appearance, number of fruiting, and fresh weight of different treatments compared by One-way ANOVA using IBM SPSS Statistics version 29.0.1.0(171) and post hoc multiple comparison was done by Tukeys hsd at 5% level of significance to determine significance differences between the means of mushroom yields. Paddy straw (control) had the quickest colonization time (16.67days) compared to maize cob (20.33 days), but wheat husk (control) had the slowest (31.33 days). The shortest time for primordial formation was in Paddy straw (21.33 days), while the longest in Maize cob (29.67 days) and wheat husk (36.67 days) respectively. Paddy straw had the highest mean fruiting number (619.33), followed by wheat husk (196.67) and maize cob (66.33), respectively. Paddy straw (2519.5gm) was highest in three flushes than Wheat husk (775gm) and Maize cob (775gm) in two flushes. This study also proved that substrates such as wheat husk, maize cob, and paddy straw can be used to grow mushrooms.

Keywords: Biological efficiency, Oyster mushroom, Wheat husk, Substrate, Yield

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATION

ANOVA	:	Analysis of Variance
BE	:	Biological Efficiency
BKFPL	:	Bahumukhi Krishi Farm Pvt. Ltd
C/N	:	Carbon and Nitrogen
Ca	:	Calcium
CRD	:	Completely Randomized Design
DNA	:	De-oxyribo Nucleic Acid
MC	:	Maize Cob
MT	:	Metric Ton
NARC	:	Nepal Agricultural Research Council
NBMARPL	:	Nepal Brothers mushroom and Agro research Pvt. Ltd.
P S	:	Paddy Straw
SPSS	:	Statistical Package for the Social Sciences.
WH	:	Wheat Husk

CHAPTER-I

INTRODUCTION

1.1 Background

The name "Pleurotus" is derived from the Greek term "Pleuro," which means "formed laterally or lateral location of the stalk or stem." The oyster mushroom, a member of the genus *Pleurotus* and family Pleurotaceae, is among the most popular edible mushrooms. *Pleurotus* is widely grown because of its beneficial organoleptic and therapeutic properties, straightforward and affordable production process, and increased biological effectiveness(Alkoaik et al., 2015).The presence of a pileus, or spatula-shaped cap, has been used to describe the exterior appearance of oyster mushrooms. This area is fleshy. The oyster mushroom's fruiting body has a stalk that can be short, long, lateral, or central. Stipe is the name of this stalk. The oyster mushroom has long ridges and furrows underneath the pileus, which are referred to as gills or lamellae, which is an intriguing and appealing feature. The oyster mushroom's gills contain the spores that help with reproduction. Smooth and cylindrical in shape, these spores can germinate on any kind of mycological media within an incubation period of 48 to 96 hours(Rathod et al., 2021).

The term "oyster mushroom" refers to the 40 species of the genus *Pleurotus* (Deepalakshmi & Mirunalini, 2008). Higher elevations have the best growing season from March/April to September/October, while lower elevations have the best growing season from September/October to March/April. It can also be grown in the summer by providing the additional humidity required for growth (Dubey et al., 2019).Approximately 25 species are currently grown for commercial purposes in various parts of the world, *Pleurotus spp* includes, *Pleurotus florida, Pleurotus ostreatus, Pleurotus columbinus, Pleurotus salignus, Pleurotus spodoleucus, Pleurotus pulmonarius*, and subspecies *Pleurotus sapidus, Pleurotus populin, and Pleurotus sajor-caju*(Nadir et al., 2016).

The second most popular type of mushroom in the world, after *Agaricus*

bisporus, is *Pleurotus* (Sánchez, 2010). Due to its potent ligninolytic abilities, *Pleurotus spp*. is a well-researched white-rot fungus. Compared to other types of mushrooms, *Pleurotus species* have a shorter growing season. *Pleurotus*, which can grow on various varieties of agricultural and forestry waste than any other species, is the best wood decomposer. Nearly every kind of hardwood, wood byproducts (sawdust, paper, pulp sludge), cereal straws, corn cobs, sugar cane bagasse, coffee residues (coffee grounds, hulls, stalks, and leaves), banana fronds, cottonseed hulls, agave waste, soy pulp, and a variety of other materials that are both too numerous to list and difficult to imagine are all sources of food for them(E. & D., 2015). The chemical composition, carbon (C) to nitrogen (N) ratio, nitrogen (N) sources, surfactant, minerals, pH, water activity, moisture, particle size, and amount of inoculum, antimicrobial agents, and presence of microorganism interactions are all chemical, physical, and biological aspects of mushroom production. Oyster mushroom yield and quality are significantly influenced by light, humidity, temperature, and the air composition of the surrounding substrate, such as the levels of carbon dioxide and oxygen(Muswati et al., 2021).

*Pleurotus species e*volutionary relationships are still unclear, and many taxonomic questions are still up for debate. When grown under various environmental and dietary conditions, size varies between species and even within the same species. The fruit bodies are typically a few to several centimeters wide; the smallest size ranges from two to three centimeters to fifteen to twenty centimeters. The fruiting bodies of oyster mushrooms can be white, cream, grey, yellow, pink, or light brown in color and have distinctive shell, fan, or spatula shapes depending on the species (Money, 2016a).*Pleurotus* mushrooms contain large amounts of dietary fiber, proteins, carbohydrates, essential amino acids, water-soluble vitamins, and minerals(Raman et al., 2021a). *Pleurotus*, one of the most diverse genera of cultivated mushrooms, displays the typical life cycle of the Basidiomycetes fungus(Adebayo & Oloke, 2017). *Pleurotus species* are well-known commercial and necessary mushrooms that are widely grown around the world because of their exceptional ligninolytic properties(Bellettini et al., 2019).

Pleurotus ostreatus, also known as the tree oyster or pearl oyster mushroom, is

a common edible fungus in the oyster mushroom family. Similar to oyster mushroom colonies, this species produces grouped fruiting bodies of various sizes. The fruiting body measures 4 to 15 cm and is pink, gray-to-dark brown in color. Their cap is 3–15 cm in diameter, broadly convex, flat, or depressed flat, kidney- to fan-shaped in outline, or nearly spherical when growing on tree trunks, young, fresh, and slightly greasy; bald; pale–to–dark brown; fade–to–buff; occasionally–slowly–fading–and–becoming–two-toned; and the edge is slightly curled when young(Mitsou et al., 2020).In particular, hornbeam (Carpinus sp.), beech (Fagus sp.), willow (Salix sp.), poplar (Populus sp.), birch (Betula sp.), and common walnut (Juglans regia) trees are home to *Pleurotus ostreatus*, which can be found in both dead and living tree branches(Piska et al., 2017). Gills are typically close and short, and they can be white or grayish in color. As they age, they turn yellowish and occasionally have brownish edges. Gills can run down the trunk (or pseudo stem). Their flesh is also thick, white, and unchanging when cut. These species are crucial to the global movement toward the "zero-waste economy" because they have the capacity to transform or biodegrade waste for biomass production using a variety of enzyme properties like endoglucanase and laccase(Melanouri et al., 2021).

Agro-waste materials, which are primarily composed of lignin, cellulose, and hemicellulose, serve as a significant source of carbon and energy for the cultivation of *Pleurotus* mushroom. Out of all the cultivated mushrooms that are suitable for year-round growth, *Pleurotus* has the species that is most commercially developed. They can use a wide range of agricultural waste materials and convert the biomass of lignocellulose into high-quality food with flavor and nutritional value. An effective and financially sound technology for turning these materials into valuable protein-rich food and a commercially valuable cash crop is the cultivation of edible mushrooms on agricultural and agro-industrial residues as a substrate(Hossain, 2017).

1.2 Statement of the Problem

Around 998 million tons of agro-waste, including paddy, wheat, and cereal straws, are produced globally each year. By cultivating *Pleurotus* mushrooms, which use these agricultural wastes as substrates for growth, we can fill a

nutritional gap while also recycling agricultural waste(Raman et al., 2021b).

Government agencies and other private organizations have conducted limited research on mushroom farming, and there are currently no specific policies in place for its promotion. Horticulturists have yet to recognize it as a commodity, and due to its fungal nature, it has been assigned to the Plant Pathology division of NARC, considering fungi's role in causing crop diseases. Nepal is primarily an agricultural country with abundant raw materials for substrate preparation and inexpensive labor. Agricultural waste, including corn stover, paddy and wheat straws/brans, sugarcane bagasse, sawdust, and coffee husks, has been used for mushroom cultivation. However, most farmers prefer rice straw for oyster mushroom cultivation due to a lack of knowledge about effective parameters and techniques for growing *Pleurotus ostreatus* mushrooms using other agricultural byproducts and their different combinations.

During the harvesting season, agricultural waste such as maize cobs, wheat husks, paddy straw, and corn stalks are plentiful and often burned by farmers for disposal. This burning practice contributes to environmental pollution. Educating farmers about the benefits of mushroom cultivation can help reduce waste burning and convert agricultural byproducts into value-added mushrooms. Nepal's diverse agro-climatic regions provide suitable climates for mushroom cultivation, making it a high-yielding and fast-maturing crop. If ground corn cobs, wheat husks, paddy straw, and their different combinations can support oyster mushroom development, it could provide mushroom growers with a cost-effective source of substrate. Therefore, farmers should be educated on the use of other raw materials available in their area. *Pleurotus species* are widely farmed, employing a range of agricultural products and low-tech, low-cost production methods (Raman et al., 2021b). Mushrooms act as nature's recyclers, transforming agricultural and industrial waste into high-quality protein-rich vegetables within a certain period.

While numerous studies have been conducted on the cultivation of *Pleurotus ostreatus* mushrooms using various substrates, the specific combination of 25% maize cobs with 75% paddy straw, 75% wheat husks with 25% maize cobs, and 50% paddy straw with 40% wheat straw, supplemented with 10%

rice bran, has not been well-documented. Cultivating mushrooms on these byproducts could be one solution to convert inedible waste into acceptable edible biomass. Research on these substrates and their combinations is necessary to identify the factors influencing the production of *Pleurotus ostreatus* mushrooms. Therefore, the purpose of the current study was to investigate the effects of different substrates and their combinations on the yield of *Pleurotus ostreatus* mushrooms.

1.3 Rationale of the study

The cultivation of oyster mushrooms is gaining popularity on a global scale due to their ability to consume various lignocelluloses and adapt to a wide range of temperatures. Cellulosic wastes contribute to the ecological burden and pose health risks and disposal issues. Research into recycling waste as an alternative energy source can reduce health risks and benefit the environment. Mushroom farming serves as a recycling method that produces mushroom biomass rich in protein from agricultural waste.

Oyster mushrooms play a crucial role in the forest ecosystem, aiding in the stabilization and restoration of forest communities. *Pleurotus species* are known for their abundance of amino acids, making them valuable as a flavoring ingredient. Mushroom farming, especially in less developed countries, is a labor-intensive activity that can increase income and support livelihoods (Raman et al., 2021b). Oyster mushroom cultivation requires minimal substrate preparation, and composting is not necessary, unlike in button and straw mushroom cultivation. Lignocellulolytic fungi, particularly *Pleurotus species*, have drawn attention from researchers as potential biomass degraders for large-scale applications, given their ability to produce valuable compounds and extracellular lignocellulolytic enzymes. Oyster mushrooms and their enzymes serve as effective biodegradation and bioconversion agents for lignocelluloses and other resistant contaminants. Oyster mushroom lignocellulose biotechnology has the potential to produce fruit bodies, extracellular enzymes, animal feed, and other value-added goods (E. & D., 2015).

The market today offers a variety of mushroom products, including pickled

mushrooms, spices, beverages, extracts, dried and canned mushrooms, vitamins made from mushrooms, cosmetics, and more. Additionally, inventive products in other industries are emerging, such as mycelium-based platforms, mycelium-based construction materials, mycelium-based drugs, and mycelium-based leather, among others. Mushroom cultivation is easy, generates little waste, and does not release carbon dioxide. Fungi provide producers with a good source of income and additional benefits through processing (Alkoaik et al., 2015).

The growth of the mushroom-based sector also opens the door to the possibility of exporting mushroom products, providing job opportunities for the unemployed. Mushrooms are commercially farmed all over the world (Ferdousi et al., 2020).Oyster mushroom cultivation is advantageous for farmers of other cash crops as they can use inexpensive substrate seasonally and do not need to buy raw materials for substrate. Sawdust, banana leaves, sugarcane bagasse, compost made from wheat and rice straw, and other materials can all be used to grow mushrooms (Dubey et al., 2019).

Oyster mushrooms have gained popularity due to their ease of cultivation and low cost. In a natural setting, the only constraints to mushroom production are the season and available space. Unlike white button mushrooms, oyster fruit bodies can be dried and stored. Dried oyster mushrooms can be rehydrated in hot water for 5 to 10 minutes and used directly or ground and added to a variety of dishes. Fresh mushrooms have a shelf life of 24 to 48 hours, even at room temperature.

1.4 Objectives of the study

1.4.1 General objective

The study's main objective was to examine how different substrates and their mixtures affected the yield of oyster mushrooms *(Pleurotus. ostreatus)*

1.4.2 Specific objectives

- To identify *Pleurotus ostreatus* Colonization Days, Primordia formation Day, Fruiting Number and Yield.
- To determine biological efficiency of mushroom.

CHAPTER-II

LITERATURE REVIEW

2.1 Conceptual review

Fungi are classified into two types due to alterations in environmental factors such as temperature, oxygen, or nutrition: mold and yeast. Yeast is a unicellular creature with a spherical shape. It creates colonies that are smooth and round. Mold, on the other hand, is multicellular and has a filamentous structure known as hyphae. It creates brightly colored colonies with a woolly appearance(Patel et al., 2022).Throughout their lives, filamentous fungus have complex morphologies. A single reproductive spore usually germinates to start the life of a filamentous fungus. The germ tube transforms into a hyphal, which is a tubular filament. The fundamental units of fungi, known as hyphae, are capable of absorbing and transmitting nutrients from their surroundings. The hyphae continue to grow and branch, eventually forming mycelium, a cross-linked structure (Watkinson, 2016). During the Precambrian period, fungi evolved from a different group of unicellular eukaryotes. According to molecular clock studies, the emergence of the fungal kingdom occurred between 760 million and 1.06 billion years ago (Money, 2016b).The majority of fungal species are classified as belonging to the phyla Ascomycota and Basidiomycota (Hawksworth & Lücking, 2017). Because of basic biochemical changes in cell wall composition, several of these species have migrated out of the Kingdom Fungi.

The cell wall of the majority of true fungi is mostly made of chitin and other polysaccharides(Abdelghany, 2016). As evidenced by these and other characteristics, the Eumycota (real fungi or Eumycetes), which constitute the fungi, are a single group of related animals with a common ancestor (a monophyletic group) (Md Zulfekar, 2013). The Spitzenkörper, a dense assembly of secretory vesicles found in hyphal tips, and the Woronin body of Ascomycota, which acts as an intracellular plugging mechanism that prevents cytoplasmic leakage, are two examples (Money, 2016a). Fungi can be found in soil, air, and water, as well as decaying organic plant or animal waste. Organic carbon and energy are required by all fungi, as well as a combination of

nitrogen, phosphorus, and sulfur, cations potassium, calcium, and magnesium, and trace amounts of several other elements. The majority of culturable saprotrophic fungi will grow in a specific synthetic medium(Watkinson, 2016).

Due to their holomorphic nature, most fungi have both sexual and asexual reproduction (Gould, 2009). An anamorph is a fungal stage that generates asexual spores. The sexual reproductive stage is known as teleomorph. Sexual reproduction helps fungus to adapt to changing and demanding conditions while also encouraging genetic variety. Four steps are involved in sexual reproduction: 1) Plasmogamy, the nuclear fusion that results in the production of diploid nuclei; 2) Karyogamy, the development of haploid spores; 3) Meiosis, the development of haploid spores; and 4) Spore Germination, the development of multicellular mycelium(Parker et al., 2017).Sex organs, sex cells, or sex nuclei are not joined during asexual reproduction. Since the "offspring" are genetically identical to the parent fungus, asexual reproduction enables fungi to move throughout their environment and effectively maintain genotypes in the population. Asexual fungi can reproduce in one of three ways: 1) Mycelial fragmentation; 2) Mycelial fission (as in Schizosaccharomyces spp.); and 3) Budding (for example, in yeast strains of Saccharomyces spp).

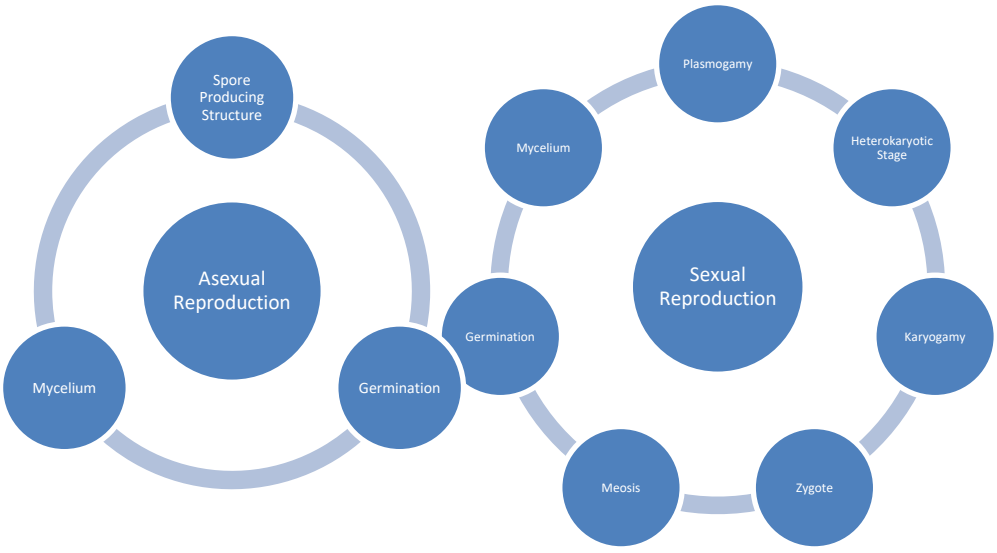


Figure 1: Reproduction of fungi

2.1.1 History of mushroom cultivation

The first mushroom farms were established in French caves. The Chinese made another attempt at it in 600 AD. It only generated 60,000 tons in 1978. Following that, China and Japan started to grow mushrooms artificially. The production of mushrooms increased significantly over time, rising from 0.30 million tons in 1961 to 18.58 million tons in 2016, thanks to the development of new, simple growing methods. The cultivation of various types of mushrooms is practiced in about 100 different countries, with button, shiitake, oyster, wood ear, and paddy straw mushrooms accounting for nearly all of the world's production. Mushrooms are currently significantly increasing in importance almost everywhere in the world(Thakur, 2020).*Pleurotus ostreatus* is widely cultivated, but their demand on the world market is lower than that of shiitake and button (*Agaricus bisporus*) mushrooms(Raman et al., 2021b). Many industrialized nations in Europe and America have access to a wide variety of mushrooms. Agriculture has evolved into a high-tech industry with extensive automation and mechanization (Zied & Pardo-Giménez, 2017).

2.1.2 Nepal's history of mushroom farming

The cultivation of mushrooms is a new industry in Nepal. Mushroom cultivation research was started in 1974, under the supervision of the Nepal Agricultural Research Council (NARC). The first mushroom farming by farmers took place in 1977 with the cultivation of white button mushrooms. Plant pathology at the NARC began spreading spawn. In 1984, oyster mushrooms were made available to growers. This cultivation was initially begun in Bhaktapur and the Kathmandu area by a small group of farmers. After oyster mushrooms were successfully produced, there were 50 farmers. In Kathmandu alone, there were about 5000–6000 mushroom farmers(Poudel & Bajracharya, 2011). Mushrooms that can be eaten have come to represent the production of protein from waste. About 200 species have so far been successfully cultivated in a lab, and 7000 species were thought to have varying degrees of edibility. The potential for commercial production existed for sixty species. Although there were many different kinds of mushrooms in Nepal naturally, only 1150 of them have been formally identified as of yet (Acharya & Tiwari, 2021). Out of these, 147 were thought to be edible, 100 are deemed

potentially harmful, and 73 were thought to serve a medicinal purpose.(Kant Raut, 2019).

2.1.3 Mushroom

A particular kind of fungus called a mushroom spends most of its life cycle as a microbe and only develops the reproductive fruit body known as a "mushroom" before becoming visible to the naked eye(Dhar & Shrivastava, 2012). Mushrooms are heterotrophic macro-fungi that generate spores and are part of the kingdom Fungi(Kumar et al., 2015).They mostly belong to the phyla Basidiomycota and Ascomycota. Mushrooms are the first known fungus and are found in abundance in nature. The diversity of mushroom species is closely related to habitat. They are discovered on terrestrial soil, rotting wood, lignified wood, dead leaves, manure, and other organic materials (coprophilous). Macro-fungi are classified into three groups based on their nutrition: saprophytes, parasites, and symbiotic. All species of mushrooms have a wide range of physical characteristics(Ganguly et al., 2021). Toadstools are poisonous or inedible equivalents that are occasionally mistaken for mushrooms. Around 14000 of the estimated 1.5 million species of fungi in the world produce fruiting bodies big enough to be called mushrooms or toadstools(Clarke & Crews, 2014).

In Nepal, there are 108 families, 357 genera, 1291 species of wild mushrooms, including 34 endemic species (Ascomycota 165 species and Basidiomycota 1126 species). There are 159 edible mushrooms among them, 74 medicinal mushrooms, and 100 lethal mushrooms(Devkota & Aryal, 2020).Edible mushrooms are a diverse and interesting group of fungi, accounting for about 20% of all mushroom taxa recorded in the sources around the world, or 3283 mushroom species(Zhang et al., 2021).Temperature, humidity, light, and the substrate over which mushrooms grow are all environmental elements that influence their growth.

Contrary to green plants, mushrooms are heterotrophs. They must obtain nutrients from outside sources because they lack chlorophyll and are unable to produce them through photosynthesis. In contrast, mushrooms have the capacity to produce a wide variety of enzymes that allow them to initially

digest the complex substrate on which they grow before absorbing the soluble material for nourishment. Usually, soil or a synthetic substrate are used for mushroom cultivation above ground. The fruiting structures of specialized fungi known as mushrooms have the extraordinary capacity to decompose organic matter and recycle nutrients back into the soil. These amazing fungi can convert lingo-cellulosic agricultural wastes into valuable biomass rich in protein and other nutrients. Byproducts from using mushroom fungus to transform wastes include manure, animal feed, improved soil, and bio remediation, all of which reduce air pollution(Thakur, 2020).It has been used as food and medicine by numerous civilizations since antiquity because of its exquisite flavor, nutritional benefits, and numerous medicinal properties (Martínez-Ibarra et al., 2019).

Mushrooms reproduce by spores. Spores germinate into hyphae under the right circumstances (collectively, mycelia). Mycelia are filamentous and, in most cases, imperceptible to the human eye. Mushroom mycelium, which normally blocks the ground, has a proclivity to grow out from a central point to build a large, invisible circular colony. When the time comes for sporulation, the sporophores are formed along the colony's edge, forming a ring. Because of an old idea that mushrooms growing in a circle reflect the route of dancing fairies, this ring is known as a fairy ring. Primary mycelia are formed by germinating hyphae, whereas secondary mycelia are formed through plasmogamy (hyphal fusion). In addition to colonizing the substrate, they also store its nutrients (soil for plants). When the temperature, humidity, and other factors are right, the mycelial colony grows pins and eventually forms fruit bodies. The bodies of young fruits resemble pins.The fruit bodies that are produced by pins become a cap and a stalk(Alexopoulos et al., 1996).

Recently the most prevalent species of *Pleurotus* genera was classified on Lentinus genera which include: *P. ostreatus* (oyster mushroom), *P. djamor* (pink oyster), *P. citrinopileatus* (golden oyster), *P. eryngii* (king oyster), *P. tuber-regium* (king tuber oyster), *P. pulmonarius* (phoenix oyster), *P. nebrodensis* (white ferula mushroom), *P. cystidiosus* (abalone mushroom), *P. cornucopiae* (branched oyster mushroom) and *P. sajor-caju* (grey abalone oyster)(Corrêa et al., 2016). There is a great deal of diversity among the

Pleurotus species and strains, including *Pleurotus ostreatus*, *Pleurotus citrinopileatus var cornucopiae*, *Pleurotus eryngii*, *Pleurotus pulmonarius*, and *Pleurotus djamor* (Barh et al., 2019).

In fiscal years 2013/14, 2014/15, and 2015/16, fresh mushroom production totaled 1900 MT, 2700 MT, and 9300 MT, respectively, while seed production was 333505, 425000, and 1488000 bottles, respectively. This data shows that both fresh and seed mushroom production have been rising quickly in recent years (MoAD, 2015/16).

In the fiscal years 2016–17, 2017–18, and 2018–19, fresh mushroom production totaled 10850 MT, 10500 MT, and 11255 MT, respectively (MoAD, 2019). There is currently not enough domestic production to meet local demand. In order to meet demand, Nepal imports a lot of mushrooms and mushroom-related products from other nations every year. In recent years, Nepal's consumption of mushrooms has dramatically increased. The home market is quickly growing due to changes in eating habits and the recognition of its nutritional and therapeutic benefits(Kant Raut, 2019).

2.2 Empirical Review

Ashraf et al.,(2013)compared three species of *Pleurotus*, namely *P. sajor-caju* (V1), *P. ostreatus* (V2), and *P. djmor* (V3), grown on three different substrates: cotton waste (T1), wheat straw (T2), and paddy straw (T3). The study aimed to examine the effects of various agricultural wastes on the growth and yield of mushroom production. Cotton waste was discovered to be the most productive treatment for mushroom cultivation among all those examined.

Yang et al.,(2013)cultivated Oyster mushrooms *(Pleurotus ostreatus)* on rice straw, wheat straw, cotton seed hull basal substrate, and wheat straw or rice straw supplemented with different proportions (15%, 30%, and 45%) of cotton seed hull. They aimed to find a cost-effective substrate and investigate the impact of autoclaved sterilized and non-sterilized substrates on growth and development. The findings showed that compared to oyster mushrooms grown on cotton seed hull basal substrate, those grown on rice straw and wheat basal substrate exhibited faster mycelial growth rates, relatively low surface

mycelial densities, shorter total colonization times, fewer days from bag opening to primordia formation, lower yields, and lower biological efficiencies. Both sterilized and non-sterile substrates were consistent with these results.

The addition of cotton seed hull to the substrate of wheat and rice straw slowed the formation of fruit bodies, primordial development, and spawn running. However, adding more cotton seed hull improved the mushrooms' weight, cap diameter, stipe length, yield, biological effectiveness, and whiteness and uniformity of the mycelium. The non-sterilized substrate grew mycelium more quickly, colonized faster, and took less time from bag opening to primordium formation than the sterilized substrate. The non-sterilized substrate did not yield noticeably more mushrooms or have a higher biological efficiency than the sterilized substrate, despite having some undesirable traits like a smaller mushroom cap diameter and a relatively long stipe length. Therefore, more study is needed to determine how to improve the quality of mushrooms grown on non-sterile substrate.

Hoa et al., (2015)compared the effects of different agricultural wastes on the development, production, and nutritional makeup of the oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). They tested seven substrate combinations, including sawdust (SD), corncob (CC), and sugarcane bagasse (SB), individually and in mixtures of 80:20 and 50:50. The total colonization period, fruiting body characteristics, yield, biological efficiency (BE), nutritional composition, and mineral concentrations of both oyster mushrooms PO and PC were significantly different when grown on various substrate formulas.

According to the findings, increasing CC and SB decreased the C/N ratio and raised the mineral content (Ca, P, and Mg) of the substrate formulations. The mineral (Ca, K, Mg, Mn, and Zn) and protein content of both mushrooms' fruiting bodies increased when CC and SB were added to substrate formulas. The best substrate formulations for growing oyster mushrooms PO and PC had 100% CC and 100% SB, and they exhibited the highest values of cap diameter, stipe thickness, mushroom weight, yield, BE, protein, fiber, ash, mineral content (Ca, K, and Mg), and short stipe length.

Girmay et al., (2016)studied the effectiveness of four substrates (cotton seed, paper waste, wheat straw, and sawdust) in the growth of oyster mushrooms. The results of the study showed that oyster mushrooms developed at different rates on cotton seed, paper scraps, sawdust, and wheat straw. Sawdust produced the least amount of oyster mushrooms compared to cotton seed, which also had the highest biological efficiency and the largest biological and economic yield. According to the study, paper scraps are the next best substrate for growing oyster mushrooms after cotton seed.

Hossain, (2017b)carried out an experiment to investigate how *Pleurotus florida* oyster mushroom performs on various substrate types. Five substrates were used in a complete randomized design (CRD) with four replications, and they were contrasted with the control. The mycelium running rate was highest (0.78 cm/day) in the banana leaves and rice straws (1:1) and lowest (0.50 cm/day) in the control. In banana leaves and rice straw, mycelium running time completion was lowest (1:3 and 3:1, respectively), but it was highest in the control. Primordia initiation and harvesting took very little time in the event of control.

The largest pileus thickness (measured from rice straw) was found to be 0.668 cm, and the control had the most total primordia (52.25) and effective primordia (37.25). Individual weight was found to be lowest in the control group and highest in the rice straw. The highest biological yield (164.4 g) and economic yield (151.1 g) were both significantly higher than the control and were produced by rice straw.

Sitaula et al., (2018)performed an entirely randomized experiment on the growth and yield of oyster mushrooms *(Pleurotus ostreatus)* (CRD). Four substrates, paddy straw (100 percent), maize cob + paddy straw (1:1), sugarcane bagasses + paddy straw (1:1), and sawdust + paddy straw (1:1), were used in the treatment (1:1). The experiment tracked variables such as the colonization period, the fruit initiation period, the length, diameter, and weight of the pileus of the first and second flushes of mushrooms, as well as the biological efficiency (BE) of various substrates.

Paddy straw had the quickest colonization and fruit initiation times among the

substrates examined, at 18.25 and 21.75 days, respectively. Paddy straw had the largest pileus and stalk diameters (0.80 cm and 7.90 cm, respectively), while sugarcane bagasses and paddy straw (1:1) had the shortest stalks (6.10 cm). Similarly, paddy straw was found to have the highest biological efficiency (96.29688%), followed in that order by maize cob plus paddy straw (1:1), sugarcane bagasses plus paddy straw (1:1), and sawdust plus paddy straw (1:1).

Adeniran, (2018)determined the effectiveness of different substrates in the growth of oyster mushrooms. Florida species of *Pleurotus* (oyster mushrooms) were grown during the summer with an average daily temperature of 26-30°C and RH ranging from 80-100% on five different substrates, including banana leaves, rice straw, wheat straw, a combination of rice and wheat straw, and sawdust. Banana leaves produced the highest total yield per five kilograms of substrate, or 4.76 kg, compared to the other four treatments, making them the most effective. Sawdust produced the lowest yield.

2.2.1 Nutritional value of mushroom

Mushrooms are an important source of food. They are consumed not only because of their natural flavor and taste, but also because of their high nutritional value. The nutrient content varies according to species and growth requirements. The fresh mushroom has a moisture content of 85-90 percent, a protein content of 3 percent, a carbohydrate content of 4 percent, a fat content of 0.3-0.4 percent, a mineral(such as iron, potassium, phosphorus, calcium and copper) content ranging from 8 to 12 percent, a good source of trypsin enzyme, and a vitamin content of 1%, and certain medical properties such as decreasing blood cholesterol levels, cancer prevention, and promoting hair development (M. Kumar and Tripathi 2017; Regmi and Puri 2022). *Pleurotus citrinopileatus* contains a high concentration of vitamins B3 (nicotinic acid), B5 (pantothenic acid), B2 (riboflavin), B1 (thiamine), B6 (pyridoxine), B7 (biotin), and B9 (pantothenic acid) (folic acid). Vitamin E (7.23 mg/g), vitamin A (0.363 mg/g), and vitamin C (0.363 mg/g) were the most abundant vitamins in *Pleurotus ostreatus*. (Adebayo & Oloke, 2017). There are approximately eight essential amino acids that the human body cannot produce and must therefore be consumed in the diet on a daily basis. Free amino acid

composition varies greatly, but in general, it is high in theronine and valine and low in sulphur-containing amino acids (ethionine and cysteine)(Raman et al., 2021a). Mushroom protein appears to be nutritionally equivalent to both meat and vegetable proteins. Although they are not technically a vegetable, they are frequently used in recipes and served as such. Mushrooms are high in protein and low in calories, making them an excellent choice for people with heart disease. Mushroom proteins contain all of the essential amino acids and are especially high in lysine and leucine, which are both lacking in most cereal foods(Singh, 2017). Commercially grown mushrooms and wild varieties have similar nutritional profiles. Mushrooms rank below most animal meat in terms of crude protein content, but far above other foods such as milk, which is an animal product.

Mushrooms have about twice the protein content of asparagus and cabbage, and four and twelve times the protein content of orange and apple, respectively. Higher fungi, particularly mushrooms, stand out as a distinct class of protein sources. Edible mushrooms have a high protein content that varies greatly. Mushrooms are regarded as a complete, healthy meal that can be consumed by people of all ages, from infants to the elderly. Numerous factors, such as the mushroom's species, developmental stage, and environmental circumstances, influence its nutritional content (Jegadeesh et al., 2014).

2.2.2 Medicinal values of mushrooms

The numerous medicinal properties of mushrooms, which have long been valued for their culinary and nutritional value, are finally being recognized. As a result, they are now used as dietary supplements, nutraceuticals, and Mycotherapy products in addition to dietary foods (functional foods) (Elkhateeb, 2020; Wasser, 2014).Mushrooms have a wide range of medicinal properties. Approximately 6% of edible mushrooms have medicinal properties and are used in herbal remedies, tinctures, health tonics, teas, soups, and liqueurs(Ferdousi et al., 2020). Since ancient times, Asian countries have used them to promote and maintain good health as well as treat ailments, though this strategy is much more modern in the West. They have powerful biological activities such as anticancer, antioxidant, antimicrobial, antiviral, antiaging,

hepatic protective, hypoglycemic, and hypo-cholesterolemic due to the presence of a variety of phenolic compounds, polysaccharides, terpenoids, and other compounds.

CHAPTER –III

MATERIALS AND METHODS

3.1 Materials and equipment

The agricultural wastage such as paddy straw, wheat husk, maize cob were collected from Bahumukhi Krishi Farm Field and other equipment's, chemicals used in performing this research work. All the materials used are listed in **APPENDIX-A.**

3.1.1 Site of experiment

The research activities of the present study were carried out in Bahumukhi Krishi Farm Pvt. Ltd., Morang. This Experiment was conducted from 03-May, 2023 in Morang, Nepal are listed in **APPENDIX- D.**

3.1.2 Mushroom spawns

The oyster mushroom *(Pleurotus oestratus)* was used in the experiment, and spawn for the experiment were collected from Nepal Brothers mushroom and Agro research Pvt. Ltd., Sundarharaicha Municipality, Ward No-04,Morang are mentioned in **APPENDIX-E**

3.1.3 Preparation of substrate

To prepare the substrate, the dry weight of each component before mixing was used. In this study, Paddy Straw, Maize Cob, and Wheat husk were used as substrates for the cultivation of oyster mushrooms. After sun drying, the Maize cob was hammered into small pieces, and the remaining substrates were chopped into 1-2 inch pieces with a sickle. The substrate were 50% of Paddy straw with 40% Wheat husk supplemented with 10% rice bran, 100% Wheat husk, 100% of Maize cob, 100% of Paddy straw and a 3:1 (w:w)mix ratio of Wheat husk and maize cob, 3:1 (w:w)mix ratio of Paddy straw and maize cob respectively were filled with each ball of wet substrate weighing 6 kg on Polybag where individual bag had their three replications of each.

3.1.4 Watering of substrate material

The substrates were soaked in water for 24 hours thoroughly moisten them before being placed on the steep floor to remove excess moisture and achieve

a moisture level of 70%. Excess water is manually squeezed out with the hand. The "Palm method" was implemented.

It was used to determine whether the substrate mixture contains enough moisture. First, a fistful of substrate is squeezed tightly. The substrate mixture has the proper water content if only the palm was wet and no drop of water was released. The substrate is more susceptible to infection if the moisture content is too high. If the moisture content is too low, the spawn may grow poorly and the harvest quality may suffer.

3.1.5 Sterilization

The substrates were individually steam sterilized in a metallic drum for two to three hours before being allowed to cool to room temperature (Regmi & Puri, 2022). The most important factors influencing oyster mushroom growth and production are substrate preparation and selection. As a result, a contamination-free substrate rich in lingo-cellulosic and cellulosic substance must be prepared(Sharma et al., 2017).

3.1.6 Disinfection of room

The fruiting room was sprayed with 70 % ethyl alcohol for disinfection before starting the experiment. The room was cleaned and well ventilated. To avoid contamination, all actions were carried out aseptically.

3.1.7 Bagging and spawning

Concurrently, there was bagging and spawning. Using *P. ostreatus* spawn, spawning was done in layers. Before being placed in polypropylene bags (16 x24 cm), the various substrate compositions were thoroughly mixed on a sanitized cement floor. Four spawn layers were formed alternately with substrates, leaving a 5 cm gap between each layer, and spawning was done with 20 g of spawn at the edge of each layer (Dhakal et al., 2020).

A very thin layer of substrate was applied to the final layer of spawn. The bag was tightly packed, with no empty spaces, and the bag opening was securely fastened with rubber band. The bags were pierced with a needle for gas exhaust. Packets for all treatments were created in this manner.

3.1.8 Hanging

When the fruiting bags have fully ramified , hang them in the growing house using rope in an alternative orientation to promote optimal oyster mushroom fruit setting(Jr, 2023). Complete randomness was used to assign treatments to experimental units. In order for the mycelium to fully penetrate the substrate, the bags were left in a sterile, dark environment.

3.1.9 Bag removal

Several primordia were formed after mycelial growth was completed. When white mycelial growth had reached both the lower and upper sides of the inoculation zone, the mouths of polypropylene bags were opened, the bags were cut vertically from an upper point downward, and the bags were gently removed to allow the mushrooms to grow out.

3.1.10 Relative Humidity

A hygrometer is used to measure the relative humidity (R.H.) of the mushroom. The fruiting bags were misted with water to keep them damp. The floors were also wet to help raise the humidity to at least 85.0 percent. Until the spawn run was finished, moisture was continuously supplied while the room temperature was kept between 18 and 21°C (Muswati et al., 2021).

Water was applied to the substrates three times per day, twice during the first flush and once during the second flush and so on.

3.1.11 Fruiting and harvesting

Fruiting bodies were harvested after the majority of them had fully grown. For the collection of the fruiting bodies, a little twist on the bottom half of the stipe was applied. If they are sliced, the cut surface on the substrate that remains is a perfect entrance place for *Trichoderma* (green mold).

After each harvest, the substrate was incubated under the same conditions for the second and third flushes. Mushrooms from different treatments were separated for fresh weight measurement (Sanjel et al., 2021).

3.1.12 Biological efficiency

Biological efficiency (BE): It was calculated by using the following formula.

$$\text{Biological efficiency (BE)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of Substrate}} \times 100\%$$

3.1.13 Data Collection

3.1.1.1 Colonization Days

Each treatment's mycelium colonization period was observed and noted.

3.1.1.2 Pin head days

The number of days to produce pin heads after each treatment was observed and recorded.

3.1.1.3 Number of mushroom harvested

The number of mushroom production in each substrate were collected and counted. After that, weight of fruit bodies was taken with the help of electronic balance in each fruiting bags.

3.1.1.4 Weight of harvested mushroom

The weight of the mushroom harvested from the each fruiting bags was recorded.

3.1.14 Statistical Analysis.

A completely randomized design (CRD), with three replications, and six treatments were used in the study. One-way ANOVA was used to compare the time required for colonization on substrates, the time required for the appearance of pinheads (primordia), the number of fruiting, and the fresh weight of different treatments. To determine significance differences between the means of mushroom yields, IBM SPSS Statistics version 29.0.1.0(171) and post hoc multiple comparison by tukeys hsd at 5% level of significance were used.

CHAPTER-IV

RESULT

Different substrates and their mix ratios were compared in terms of oyster mushroom colonization day, primordia forming day, fruiting number and production($p<0.05$).

Table 1 Comparison of colonization day, primordia formation day and fruiting number of *Pleurotus ostreatus*

	Substrate	Colonization day	Primordia formation Day	Fruiting Number
Paddy straw	Paddy straw	16.66±0.577 N=3	21.33±1.15 N=3	619.33±54.22 N=3
	50%Paddy straw+40%Wheat husk +10% bran	22.33±1.15 N=3	27.66±0.57 N=3	204±10.14 N=3
	75%Paddy straw+25%Maize cob	27.67±0.577 N=3	31.33±0.57 N=3	430±4.58 N=3
Wheat husk	Wheat	31.33±1.52 N=3	36.66±0.57 N=3	196.66±4.16 N=3
	50%Paddy+40%wheat husk +10% bran	22.33±1.15 N=3	27.66±0.57 N=3	204±10.14 N=3
	75% wheat husk +25%Maize cob	27.33±1.15 N=3	34.33±0.57 N=3	251.33±0.57 N=3
Maize cob	Maize cob	20.33±0.77 N=3	29.66±0.57 N=3	66.33±3.21 N=3
	75% wheat husk +25%Maize cob	27.33±1.15 N=3	34.33±0.57 N=3	251.33±0.57 N=3
	75%Paddy straw+25%Maize cob	27.67±0.577 N=3	31.33±0.57 N=3	430±4.58 N=3

Mean±SD,N=number of replicate

4.1.1 Effect of substrate on colonization Day of *Pleurotus ostreatus*

In the present study, Substrate had significance difference ($P<0.05$) in colonization day of *Pleurotus ostreatus* mushroom between groups and within groups.

Paddy Straw and wheat husk (control), Maize cob(Control), 50%PS+40%WH+10% Rice bran, 75% PS+ 25% MC, 25%MC+75%WH respectively have significance difference($P<0.05$) in colonization day *for Pleurotus ostreatus* mushroom.

Paddy straw substrate had fastest colonization (16.67day) in comparison to 50% of Paddy straw in combination with 40% of Wheat husk on supplement of 10% Rice bran (22.33 day) which is followed by 75% of paddy straw and 25% of maize cob (27.67 day).

Wheat husk had slower (31.33 day) to colonization with respect to 25% of maize cob in combination with 75% of wheat husk(27.33day) than the 50% of Paddy straw with 40% wheat husk and 10% rice bran Supplements(22.33day).

Maize cob had fastest colonization day (20.33day) than that of 25% of maize cob and 75% of wheat husk combination (27.33day). However, that mean colonization day of 75% Paddy straw and 25% Maize cob combination (27.66 day had least difference in colonization with that of 25% of maize cob and 75% of wheat husk combination).

The mean colonization day of paddy straw substrate was faster (16.67day) as compared to wheat husk substrate colonization (31.33day).

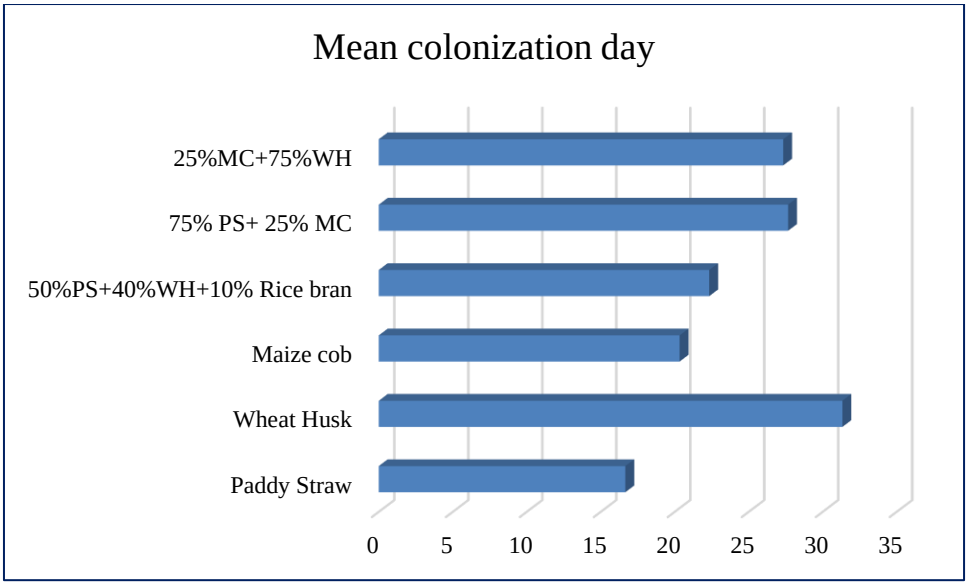


Figure 2: Effect of substrate on colonization Day of *Pleurotus ostreatus*

4.1.2 Effect of Substrate on primordia formation Day of *Pleurotus ostreatus*

In present study of primordia formation day of *Pleurotus ostreatus* mushrooms, Substrate had significance difference ($p<0.05$) in primordia formation day between groups and within groups.

There was significance difference($p<0.05$) in mean primordia formation day of paddy straw (21.33 day) substrate which was shortest primordia formation day as compared to wheat husk(36.67day), maize cob (29.67 day), 50% PS+40%WH+10% Rice bran (27.67day), 75% PS+25% MC (31.33day) substrate respectively but 25%MC+75%WH(34.33 day) substrate had shortest primordia formation day as compared to wheat husk(36.67day).

Paddy straw had fastest primordia forming day(21.33day) while that of 75% of Paddy straw in combination with 25% of maize cob had slowest primordia forming day(31.33day) which was followed by 50% of paddy straw in combination with 40% of wheat husk supplements by 10% of rice bran (27.67 day).

Wheat husk had slowest primordia forming day (36.67 day) than the combined substrate of 25% of maize cob and 75% of wheat husk (34.33day) that trend was followed by combination of 50% of Paddy straw with 40% of

wheat husk which was supplemented by 10% of rice bran(27.67 day).

Maize cob had fastest primordia forming day (29.67 day) in comparison to 25% of maize cob in combination with 75% of wheat husk (34.33day) then that trend was followed by 75% of paddy straw in combination with 25% of maize cob (31.33day).

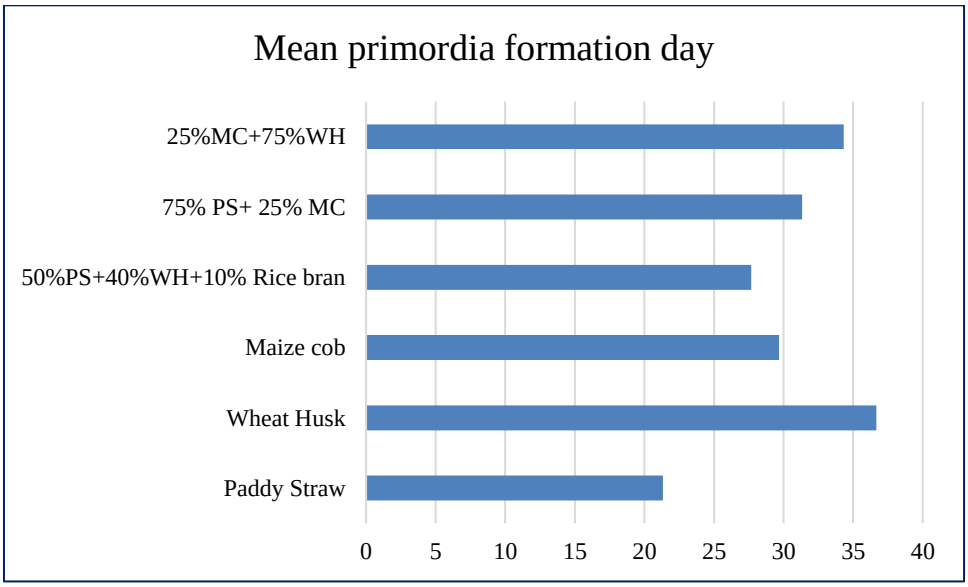


Figure 3: Effect of Substrate on primordia formation Day of *Pleurotus ostreatus*

4.1.3 Effect of substrate on Fruiting number of *Pleurotus ostreatus*

In the current investigation of the fruiting number of *Pleurotus ostreatus* mushrooms, Substrate showed a significant difference in fruiting number between groups and within groups ($p < 0.05$).

There was a significant difference ($p<0.05$) between paddy straw and wheat husk, maize cob, 50% paddy straw+ 40% wheat husk+ 10% rice bran, 75% paddy straw + 25% maize cob, and 25% maize cob+ 75% wheat husk in the fruiting number of *Pleurotus ostreatus* mushrooms.

Paddy straw mean fruiting number (619.33) was highest than 75% of Paddy straw in combination with 25% of maize cob(430) which was followed by 50% of paddy straw in combination with 40% of wheat husk supplements with 10% of rice bran(204).

Wheat husk had least number of fruiting (196.67)as compared to combination of 50% of paddy straw with 40% of wheat husk with supplements of 10% Rice bran(204) but 75% of wheat husk in combination with 25% of maize cob (251.61) which had highest numbers of fruits.

Maize cob had lowest number of fruits (66.33) but 25% of maize cob in combination with75% of paddy straw had highest numbers of fruits (430) and then 25% of maize cob in combination with 75% of wheat husk (251.33).

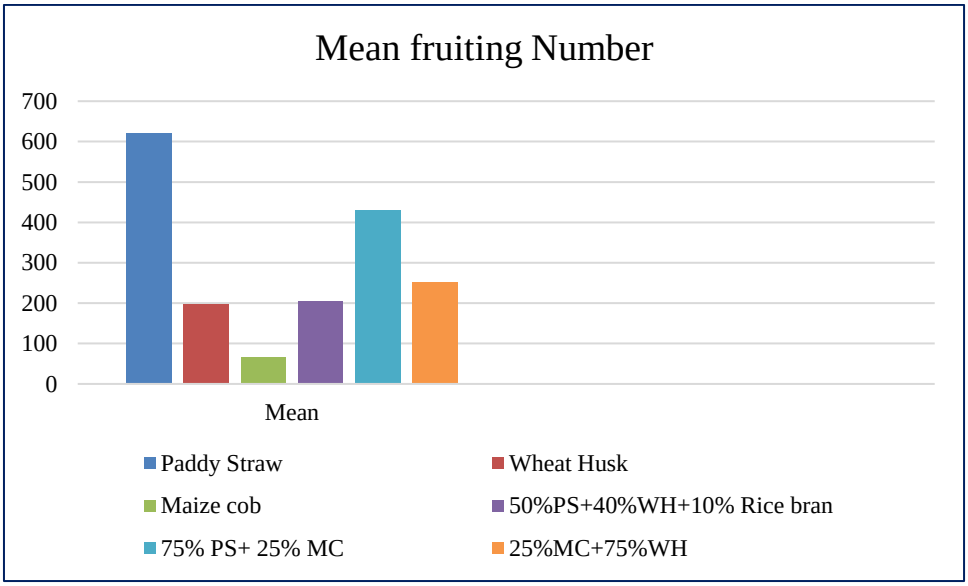


Figure 4: Effect of substrate on fruiting number of *Pleurotus ostreatus*

4.1.4 Effect of Substrate on Yield of *Pleurotus ostreatus* Production

In the current study of yield in oyster mushrooms, Substrate showed a significant difference in yield between groups and within groups ($p < 0.05$).

There was a significant difference in the yield of *Pleurotus ostreatus* mushrooms between paddy straw and wheat husk, maize cob, 50% paddy straw + 40% wheat husk + 10% rice bran, and 25% paddy straw + 25% maize cob + 75% wheat husk ($p < 0.05$).

Paddy straw had mean weight of yield(2519.5gm) was highest as compared to combination of 75% of paddy straw and 25% of maize cob(1734.33gm) but there was least yield weight in combination of 50 % of paddy straw and 40% of wheat husk which supplemented by 10% of rice bran(770.52gm).

Wheat husk had lowest yield (775gm) as compared to combination of 25% of maize cob and 75 % of wheat husk (1009.67gm) where as 50% of paddy straw and 40% of wheat husk supplemented by 10% of rice bran(770.52gm) followed by wheat husk.

Maize cob had lowest yield (254.67gm) as compared to combination substrate of 75% of Paddy straw+ 25% of maize cob (1734.33 gm) and 25% of maize cob +75% of wheat husk (1009.67 gm).

Table 2 Comparison of different flush yield of *Pleurotus ostreatus*

	Substrate	First flush yield	Second flush yield	Third flush yield	Total yield
Paddy straw	Paddy straw	1609.66±148.76 N=3	536.51±222.26 N=3	373.33±70.23 N=3	2519.50±229.36 N=3
	50%Paddy straw+40%Wheat husk +10% bran	379.93±8.78 N=3	222.16±24.16 N=3	168.42±10.36 N=3	770.52±42.85 N=3
	75%Paddy straw+25% Maize cob	782±1.73 N=3	608±2.64 N=3	344.33±4.04 N=3	1734.33±6.02 N=3
Wheat husk	Wheat husk	404.66±13.61 N=3	243±5.77 N=3	127±20.42 N=3	775.00±18.027 N=3
	50%Paddy+40 %wheat husk +10% bran	379.93±8.78 N=3	222.16±24.16 N=3	168.42±10.36 N=3	770.52±42.85 N=3
	75% wheat husk +25%Maize cob	454.66±5.03 N=3	304.33±5.13 N=3	250.66±1.15 N=3	1009.66±0.57 N=3
Maize cob	Maize cob	162.33±9.29 N=3	92.33±6.80 N=3		254.666±4.618 N=3
	75% wheat husk +25%Maize cob	454.66±5.03 N=3	304.33±5.13 N=3	250.66±1.15 N=3	1009.66±0.57 N=3
	75%Paddy straw+25% Maize cob	782±1.73 N=3	608±2.64 N=3	344.33±4.04 N=3	1734.33±6.02 N=3

Mean±SD, N=number of replicate

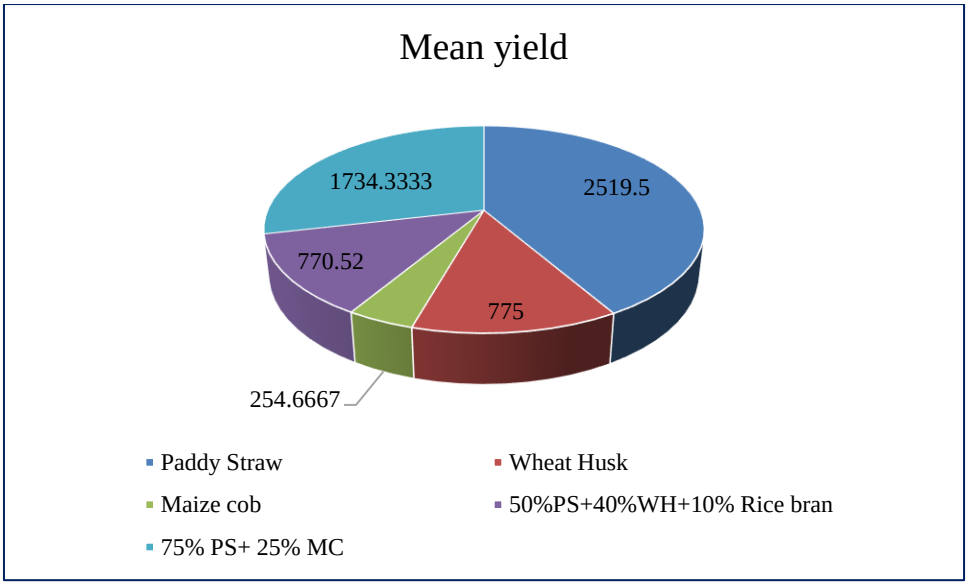


Figure 5: Effect of substrate on Yield of *Pleurotus ostreatus* mushroom

4.1.5 Biological Efficiency

The average percentage of production in 1800 gm of dry substrate was 68.51%. The highest percentage production was 139.97% (2519.5 gm from 1800 gm of dry substrate) on paddy straw substrate, followed by 96.35% in substrate 75% PS+ 25% MC(3:1). But, Maize cob and 25% MC+ 75% WH(1:3) had shown vast difference in their average biological efficiency of *Pleurotus ostreatus* mushroom production 14.14% and 74.77% respectively .However that, Wheat Husk and 50% PS+ 40% WH+10% rice bran supplement had least difference in their average biological efficiency 43.05% and 42.08% respectively.

Table 3 Biological Efficiency of *Pleurotus ostreatus*

Substrate	Average Biological Efficiency
100% Paddy Straw	139.97%
100% Wheat Husk	43.05%
100% Maize cob	14.14%
50% PS+ 40% WH(10% rice bran supplement	42.80%
75% PS+ 25% MC(3:1)	96.35%
25% MC+ 75% WH(1:3)	74.77%
Total Average B.E%	68.51%

CHAPTER-V

DISCUSSION

Nepal is an agricultural country, there are abundant raw materials available for preparing substrates, and labor costs are low. Farmers often burn maize cobs, wheat husk, paddy straw, and corn stalks to dispose of them during the harvest season. Nepal, with its diverse agro-climatic regions, offers an ideal environment for mushroom cultivation. *Pleurotus ostreatus* is among the most commercially developed species of cultivated mushrooms that can grow year-round. These mushrooms have the ability to convert lignocellulose biomass from agricultural waste into foods with high nutritional value and quality, making it effective and economical to cultivate them on agricultural and agro-industrial waste substrates (Hossain, 2017).

Using ground corn cob, wheat husk, paddy straw, and different substrate combinations may support *Pleurotus* mushroom growth, allowing mushroom growers to obtain substrate at a reasonable cost. Currently, most farmers grow *Pleurotus* mushrooms using rice straw, mainly due to their lack of awareness regarding the techniques and methods for growing *Pleurotus ostreatus* mushrooms using other agricultural byproducts and various substrate combinations. Nonetheless, *Pleurotus ostreatus* mushrooms have been successfully grown using a variety of substrates in numerous studies, as any source containing lignin and cellulose can serve as a suitable growth substrate for this fungus (Custodio, 2004).

The purpose of this study was to assess the efficacy of various agricultural wastes as substrates for mushroom growth, focusing on factors such as colonization day, primordia forming day, fruiting number, and yield. In the study, when paddy straw alone was used for *Pleurotus ostreatu*s cultivation, the Paddy straw (control) substrate demonstrated the fastest colonization from spawn inoculation (16.67 days), primordia forming day (21.33 days), the highest mean fruiting number (619.33), the mean weight of yield (2519.5 gm), and the highest biological efficiency (139.69%) which is similar to the result reported by Yang, Guo, and Wan (2013), oyster mushrooms *(Pleurotus ostreatus)* grown on rice straw, wheat straw, cotton seed hull basal substrate,

and wheat straw or rice straw supplemented with various amounts of cotton seed hull (15%, 30%, and 45%).

The results showed that oyster mushrooms grown on rice straw and wheat basal substrate exhibit faster mycelial growth rates, relatively low surface mycelial densities, shorter total colonization times, fewer days from bag opening to primordia formation, lower yields, and lower biological efficiencies than oyster mushrooms grown on cotton seed hull basal substrate. These findings were found to be true for both sterilized and non-sterile substrates. However, this contrasts with the findings of Ashraf et al.,(2013)who used both a factorial and a complete randomized design (CRD) to assess the growth of *Pleurotus Spp*. on various substrates. They utilized the least significant difference (LSD) test to compare the differences between the means of the different varieties at the same level of significance, while analysis of variance (ANOVA) techniques were used to test the overall significance of the data ($p<0.05$).

According to Sitaula et al., (2018)they also used the least significant difference (LSD) test to determine whether differences in the means of the different varieties were significant at the same level, and analysis of variance (ANOVA) techniques were used to determine the overall significance of the data ($p<0.05$), which is similar to the differences in the means of the different varieties significant at the same level and analysis of variance (ANOVA). Khadka & Parajuli, (2015)have explained why paddy straw is a suitable substrate for *Pleurotus ostreatus*, and our experiment also confirmed this. This could be because rice straw contains enough nutrients for fungus growth. The specific nutrients involved need to be specified.

Several researchers have reported that various *Pleurotus spp*. produce the highest yield on wheat and paddy straw (Baysal & Peker, 2001). The only factor influencing how quickly mushrooms emerge from the bed is the amount of cellulose in the substrate (Sivaprakasam & Kandaswamy, 1981). While non-cellulosic materials are difficult to degrade, cellulosic materials can be easily degraded by growing mushrooms. Degraded materials are used for metabolic functions. As a result of a more straightforward technique for extracting sugars from cellulosic materials, the yield of mushrooms in paddy

straw has increased (Ponmurugan et al., 2007). In a similar way, Hoa et al., (2015)compared the impact of various agricultural wastes on the nutritional composition, growth, and development of the oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). They discovered that when two oyster mushrooms, PO and PC, were grown on various substrate formulae, their total colonization period, fruiting body characteristics, yield, biological efficiency (BE), nutritional composition, and mineral concentrations were significantly different.

(Hossain, 2017)) Conducted an experiment to determine how the *Pleurotus florida* oyster mushroom responds to different types of substrate. His findings revealed that the control had the most total primordia (52.25) and effective primordia (0.668 cm), and the largest pileus thickness (measured from rice straw) was found to be 0.668 cm (37.25). He discovered that individual weight was lowest in the control group and highest in the rice straw group. Rice straw produced the highest biological yield (164.4 g) and economic yield (151.1 g), both of which were significantly higher than the control.

In the present study of other substrates, the maize cob showed the fastest mycelial growth and rapid primordial formation but resulted in fewer fruiting and the lowest fresh yield which is similar to the finding of Dhakal et al. 2020). Higher levels of water-soluble sugars, particularly hemicelluloses, which may have a longer growth phase before degrading into lignin and cellulose, may be responsible for this outcome. High mycelial densities resulted from the substrate being heavily colonized. The results of this experiment showed that the quantity and yield of mushrooms do not directly correlate with the mycelium's penetration of the substrate since growth-restricting minerals and nutrients were not added. The fruiting stage is associated with the degradation of polysaccharide compounds, and the mycelial growth rate can be increased by adding growth-limiting minerals and nutrients (Bano et al., 1963).

Similarly, another study reports on 75% PS + 25% MC substrate colonization taking 27.67 days, primordia forming duration from inoculation is 31.33 days, fruiting number 430, and an average yield of 1734 gm from per 6 kg of wet substrate. Likewise, 25% MC + 75% WH substrate has an average

colonization time of 27.33 days, primordia formation occurring on day 34.33, 251.33 pinheads, and a mean yield production of 1009.66 gm per replication (Dhakal et al., 2020). The same study also reported that colonization on various substrates took between 17 and 30 days to complete. Bhatti et al., (1987)counted the number of days it took for oyster mushrooms to completely colonize different substrates and found significant differences between them, likely due to variations in the C:N ratio and chemical composition of the substrates.

Similarly, in this study, Wheat Husk exhibited a slower colonization rate than the 50 percent PS + 40 percent WH + 10 percent, with a primordial formation time of 36.67 days, 196.67 fruiting days, and an average yield of 775 gm. The results of this study differed from those of Ganjikunta et al., (2020)which showed faster rice bran colonization (22.33 days) and primordia formation (27.67 days), but higher fruiting numbers (204) and an average yield of 770.52 gm per 6 kg of wet substrate. Ganjikunta et al.,(2020)reported that when locally available wheat straw and brans were combined in various ratios (100, 90:10, 85:15, and 80:20 inoculated WB and RB) at variable concentrations, wheat bran (WB) performed better than rice bran (RB) (10 percent, 15 percent, and 20 percent).

Zhang et al., (2014)reported that nitrogen content improved yield, as it did in wheat bran supplementation, and the inclusion of bran supplements increased mushroom yield due to the carbohydrates, amino acids, and mineral components present in the brans. However, Kimbonguila et al., (2019)found that the amount of fresh weight or yield of oyster mushrooms and the number of fruiting bodies were significantly impacted by various substrates. In all flushes, paddy straw (50%) plus wheat straw (50%) plus @ 10% pulse husk produced the most fruiting bodies and the highest fresh weight, while paddy straw + @ 10% rice bran produced the least.

CHAPTER-VI

CONCLUSION & RECOMMENDATIONS

6.1 Conclusion

According to the results obtained in the present research, it was concluded that among all substrates paddy straw showed superiority in terms of mycelial growth time, primordial formation day, fruit numbers, yield, and biological efficiency. Therefore, paddy straw is recommended for the cultivation of *Pleurotus ostreatus* mushrooms.

.

6.2 Recommendations

1. *Pleurotus* has the most commercially developed species among all cultivated mushrooms suitable for year-round growth. Therefore, farmers should receive training to carry out these practices using locally available substrates throughout the year to improve their economic status.
2. During the harvest season, farmers burn maize cobs, wheat husks, paddy straw, and corn stalks to dispose of them. Further studies should be conducted with different concentrations and mixtures of corn cobs, paddy straw, corn and wheat straw, with varying percentages of rice bran, as they have the potential to generate a substantial quantity of *Pleurotus ostreatus* mushrooms in a short period of time.

REFERENCES

Abdelghany, T. M. (2016). *Mycology* (Vol. 13, Issue July). OMICS Group eBooks 731.

Acharya, R., & Tiwari, U. (2021). Economic analysis of mushroom enterprise in Chitwan district, Nepal. *Archives of Agriculture and Environmental Science*, *6*(4), 408–415. https://doi.org/10.26832/24566632.2021.060401

Adebayo, E. A., & Oloke, J. K. (2017). Oyster mushroom (pleurotus species); A natural functional food. *Journal of Microbiology, Biotechnology and Food Sciences*, *7*(3), 254–264. https://doi.org/10.15414/jmbfs.2017/18.7.3.254-264

Adeniran, A. A. (2018). Histopathological Tissue Demonstration of Common Microorganisms in Resource-Limited Centre. *International Journal of Scientific and Research Publications (IJSRP)*, *8*(6). https://doi.org/10.29322/ijsrp.8.6.2018.p7831

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. M. (1996). *Introductory Mycology*. Wiley. https://books.google.com.np/books?id=7wAVAQ AAIAAJ

Alkoaik, F., Khalil, A., Fulleros, R., & Reyes, R. G. (2015). Cultivation of Oyster Mushroom (Pleurotus Florida) on Date Palm Residues in an Environmentally Controlled Conditions in Saudi Arabia. *Advances in Environmental Biology*, *9*(3), 955–962.

Ashraf, J., Ali, M. A., Ahmad, W., Ayyub, C. M., & Shafi, J. (2013). Effect of Different Substrate Supplements on Oyster Mushroom (Pleurotus spp.) Production. *Food Science and Technology*, *1*(3), 44–51. https://doi.org/10.13189/fst.2013.010302

Bano, Z., Srinivasan, K. S., & Srivastava, H. C. (1963). Amino Acid Composition of the Protein from a Mushroom (Pleurotus sp.) . *Applied Microbiology*, *11*(3), 184–187. https://doi.org/10.1128/am.11.3.184-187.1963

Barh, A., Sharma, V. P., Annepu, S. K., Kamal, S., Sharma, S., & Bhatt, P. (2019). Genetic improvement in Pleurotus (oyster mushroom): a review. *3 Biotech*, *9*(9), 322. https://doi.org/10.1007/s13205-019-1854-x

Baysal, E., & Peker, H. (2001). An alternate to waste paper recycling; mushroom cultivation. *Technology Sayi*, *34*, 9–13.

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., Júnior, A. M., & Ribani, R. H. (2019). Factors affecting mushroom Pleurotus spp. *Saudi Journal of Biological Sciences*, *26*(4), 633–646. https://doi.org/10.1016/j.sjbs.2016.12.005

Bhatti, M. A., Mir, F. A., & Siddiq, M. (1987). Effect of different bedding materials on relative yield of oyster mushroom in the successive flushes. *Pakistan Journal of Agricultural Research (Pakistan)*.

Clarke, D., & Crews, C. (2014). Natural Toxicants: Mushrooms and Toadstools. *Encyclopedia of Food Safety*, *2*, 269–276.

https://doi.org/10.1016/B978-0-12-378612-8.00429-7

Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2016). Biotechnological, nutritional and therapeutic uses of Pleurotus spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends in Food Science and Technology*, *50*, 103–117. https://doi.org/10.1016/j.tifs.2016.01.012

Custodio, C. D. (2004). *Coco Lumber Sawdust. Mushroom Growers Handbook*.

Deepalakshmi, K., & Mirunalini, S. (2008). Pleurotus. *Westcott's Plant Disease Handbook*, *5*, 516–516. https://doi.org/10.1007/978-1-4020-4585-1_845

Devkota, S., & Aryal, H. P. (2020). Wild Mushrooms of Nepal. *Plant Diversity of Nepal*, *February 2020*, 41–54.

Dhakal, P., Pokhrel, A., Bista, A., Shah, K., Acharya, B., & Shrestha, J. (2020). Growth and yield performance of oyster mushroom (Pleurotus ostreatus) on different substrates. *Agriways*, *08*(01), 1–8. https://doi.org/10.38112/agw.2020.v08i01.001

Dhar, B., & Shrivastava, N. (2012). *(PDF) Mushrooms and Environmental Sustainability* (Issue July 2012). https://www.researchgate.net/publication/325120361_Mushrooms_and_Environmental_Sustainability

Dubey, D., Dhakal, B., Dhami, K., Sapkota, P., Rana, M., Poudel, N. S., et al. (2019). Comparative study on effect of different substrates on yield performance of oyster mushroom Keywords : *Global Journal of Biology,Agriculture and Health Sciences*, *7*(7), 1–7. https://doi.org/10.24105/2319-CITATIONS

E., A. A., & D., M.-C. (2015). Oyster mushrooms (Pleurotus) are useful for utilizing lignocellulosic biomass. *African Journal of Biotechnology*, *14*(1), 52–67. https://doi.org/10.5897/ajb2014.14249

Elkhateeb, W. A. (2020). What Medicinal Mushroom Can Do? *Journal of Chemical Research*, *5*(1), 106–118.

Ferdousi, J., Riyadh, Z. Al, Hossain, M. I., Saha, S. R., & Zakaria, M. (2020). Mushroom Production Benefits, Status, Challenges and Opportunities in Bangladesh: A Review. *Annual Research & Review in Biology*, *March*, 1–13. https://doi.org/10.9734/arrb/2019/v34i630169

Ganguly, A., Nad, S., Singha, K., Pathak, R., Hazra, P., Singha, P., Dhua, P., Mohapatra, P. K. Das, & Mandal, A. (2021). Diversity and distribution of wild mushrooms in diff erent forest areas of Bankura district, WB, India. *Acta Biologica Szegediensis*, *65*(2), 185–198. https://doi.org/10.14232/abs.2021.2.185-198

Ganjikunta, H. K., Simon, S., Lal, A. A., & Bhuvanesh, R. A. (2020). Cultivation of Oyster Mushroom (Pleurotus florida) on Wheat Straw Supplemented with Wheat and Rice Brans. *International Journal of Current Microbiology and Applied Sciences*, *9*(12), 2324–2328. https://doi.org/10.20546/ijcmas.2020.912.275

Girmay, Z., Gorems, W., Birhanu, G., & Zewdie, S. (2016). Growth and yield performance of Pleurotus ostreatus (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express*, *6*(1), 1–7. https://doi.org/10.1186/s13568-016-0265-1

Gould, A. B. (2009). Fungi: Plant Pathogenic. *Encyclopedia of Microbiology*, 457–477. https://doi.org/10.1016/B978-012373944-5.00347-3

Hawksworth, D. L., & Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *The Fungal Kingdom*, 79–95. https://doi.org/10.1128/9781555819583.ch4

Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus). *Mycobiology*, *43*(4), 423–434. https://doi.org/10.5941/MYCO.2015.43.4.423

Hossain, M. (2017). Effect of Different Substrates on Growth and Yield of Oyster Mushroom (Pleurotus sajorcaju). *International Journal of Current Microbiology and Applied Sciences*, *6*(12), 760–764. https://doi.org/10.20546/ijcmas.2017.612.080

Jegadeesh, R., Raaman, N., Hariprasath, L., Ramesh, V., & Srikumar, R. (2014). Hypolipidemic effect of Pleurotus djamor var. roseus in experimentally induced hypercholesteromic rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *5*(2), 581–588.

Jr, C. M. T. (2023). *Performance of oyster Mushroom (Pleurotus sp .) using different sources of substrates in*. *16*(2), 1–5.

Kant Raut, J. (2019). Current Status, Challenges and Prospects of Mushroom Industry in Nepal. *International Journal of Agricultural Economics*, *4*(4), 154. https://doi.org/10.11648/j.ijae.20190404.13

Khadka, R. B., & Parajuli, G. P. (2015). *Performance of Two Oyster Mushroom Species (Pleurotus ostreatus and P . florida) on Different Substrates Composition in Western Terai of Nepal Performance of Two Oyster Mushroom Species (Pleurotus ostreatus and P . florida) on Different Substrates Com*. *January 2013*.

Kimbonguila, A., Matos, L., Petit, J., Scher, J., & Nzikou, J.-M. (2019). Effect of Physical Treatment on the Physicochemical, Rheological and Functional Properties of Yam Meal of the Cultivar “Ngumvu” From Dioscorea Alata L. of Congo. *International Journal of Recent Scientific Research*, *10*, 30693–30695. https://doi.org/10.24327/IJRSR

Kumar, M., & Tripathi, A. (2017). Study of the Different Types of Sugar Cane Planter in India. *IOSR Journal of Agriculture and Veterinary Science*, *10*(7), 1–7. https://doi.org/10.9790/2380-1007030107

Kumar, V., Chandra, S., Shancy, S., Shabnam, V., & Lamya, T. (2015). Cultivation of Edible Mushroom in India: Precautions, Opportunities and Challenges. *Journal of Plant Development Sciences*, *7*(5), 409–413. https://www.researchgate.net/publication/279177262

Martínez-Ibarra, E., Gómez-Martín, M. B., & Armesto-López, X. A. (2019). Climatic and socioeconomic aspects of mushrooms: The case of Spain.

Sustainability (Switzerland), *11*(4), 27–29. https://doi.org/10.3390/su11041030

MdZulfekar,A.(2013).*Introduction*.https://doi.org/DOI:10.13140/RG.2.1.1063.5363

Melanouri, E.-M., Dedousi, M., & Diamantopoulou, P. A. (2021). Cultivating Pleurotus ostreatus and Pleurotus eryngii mushroom strains on agro-industrial residues in solid-state fermentation. Part I: Screening for growth, endoglucanase, laccase and biomass production in the colonization phase. *Carbon Resources Conversion*.

Mijan Hossain, M. (2017). Effect of Different Substrates on Growth and Yield of Oyster Mushroom (Pleurotus sajorcaju). *International Journal of Current Microbiology and Applied Sciences*, *6*(12), 760–764. https://doi.org/10.20546/ijcmas.2017.612.080

Mitsou, E. K., Saxami, G., Stamoulou, E., Kerezoudi, E., Terzi, E., Koutrotsios, G., Bekiaris, G., Zervakis, G. I., Mountzouris, K. C., Pletsa, V., & Kyriacou, A. (2020). Effects of rich in b-glucans edible mushrooms on aging gut microbiota characteristics: An in vitro study. *Molecules*, *25*(12), 1–25. https://doi.org/10.3390/molecules25122806

Money, N. P. (2016a). Fungal Cell Biology and Development. In *The Fungi: Third Edition* (Third Edit). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-382034-1.00002-5

Money, N. P. (2016b). Fungal Diversity. In *The Fungi: Third Edition* (Third Edit). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-382034-1.00001-3

Muswati, C., Simango, K., Tapfumaneyi, L., Mutetwa, M., & Ngezimana, W. (2021). The Effects of Different Substrate Combinations on Growth and Yield of Oyster Mushroom (Pleurotus ostreatus). *International Journal of Agronomy*, *2021*. https://doi.org/10.1155/2021/9962285

Nadir, H., Ali, A., & Muhammed, G. A. R. (2016). Determination of Yield and Quality of Oyster Mushroom (Pleurotus florida) Using Different Substrates in Halabja, Kurdistan Reign-Iraq. *Journal of Plant Production*, *7*(7), 787–790.

Parker, N., Schneegurt, M., Tu, A. H. T., Forster, B. M., & Lister, P. (2017). *Microbiology*. OpenStax. https://books.google.com.np/books?id=tSzPjwEACAAJ

Patel, R., Suthar, H., Modi, K., Patel, P., & Khunt, M. (2022). Growth Parameters of Pleurotus ostreatus (Jacq. Fr.) Kumm Cultivation Using Paddy Straw as a Substrate. *Plantae Scientia*, *5*(1), 12–16. https://doi.org/10.32439/ps.v5i1.12-16

Piska, K., Ziaja, K., & Muszynska, B. (2017). Significance and Biological Activity. *Acta Scientiarum Polonorum. Hortorum Cultus*, *16*(1), 151–161. https://www.researchgate.net/publication/314664059_Edible_ mushroom _pleurotus_ostreatus_Oyster_mushroom_-_Its_dietary_ significance_and_biological_activity

Ponmurugan, P., Sekhar, Y. N., & Sreesakthi, T. R. (2007). Effect of various substrates on the growth and quality of mushrooms. *Pakistan Journal of*

Biological Sciences, *10*(1), 171–173.

Poudel, S. &, & Bajracharya, A. (2011). Prospects and Challenges of Mushroom Cultivation in Nepal : A Case Study of Lakuri Bhanjyang , Lalitpur. *Www.Forestrynepal.Org*, *February*, 1–4.

Raman, J., Jang, K. Y., Oh, Y. L., Oh, M., Im, J. H., Lakshmanan, H., & Sabaratnam, V. (2021a). Cultivation and Nutritional Value of Prominent Pleurotus Spp.: An Overview. *Mycobiology*, *49*(1), 1–14. https://doi.org/10.1080/12298093.2020.1835142

Raman, J., Jang, K. Y., Oh, Y. L., Oh, M., Im, J. H., Lakshmanan, H., & Sabaratnam, V. (2021b). Cultivation and Nutritional Value of Prominent Pleurotus Spp.: An Overview. *Mycobiology*, *49*(1), 1–14. https://doi.org/10.1080/12298093.2020.1835142

Rathod, M. G., Gadade, R. B., Thakur, G. M.et al. (2021). Oyster Mushroom: Cultivation, Bioactive Significance and Commercial Status. *Frontiers in Life Science*, *II*(August). ISBN: 978-81-953600-8-6

Regmi, S., & Puri, C. (2022). Effects of different substrates on production of oyster mushroom in walling of syangja, Nepal. *Research in Agriculture Livestock and Fisheries*, *9*(1), 11–16. https://doi.org/10.3329/ralf.v9i1.59530

Sánchez, C. (2010). Cultivation of Pleurotus ostreatus and other edible mushrooms. *Applied Microbiology and Biotechnology*, *85*(5), 1321–1337. https://doi.org/10.1007/s00253-009-2343-7

Sanjel, P., Shrestha, R. K., & Shrestha, J. (2021). Performance of oyster mushroom (Pleurotus ostreatus) grown on different fingermillet husk substrates. *Journal of Agriculture and Natural Resources*, *4*(1), 291–300. https://doi.org/10.3126/janr.v4i1.33370

Sharma, V. P., Annepu, S. K., Gautam, Y., Singh, M., & Kamal, S. (2017). Status of mushroom production in India. *Mushroom Research*, *26*(2), 111–120.

Singh, D. R. (2017). A Review on Different Benefits of Mushroom. *IOSR Journal of Pharmacy and Biological Sciences*, *12*(01), 107–111. https://doi.org/10.9790/3008-120102107111

Sitaula, H. P., Dhakal, R., DC, G., & Kalauni, D. (2018). Effect of Various Substrates on Growth and Yield Performance of Oyster Mushroom (Pleurotus ostreatus) in Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology*, *6*(3), 215–219. https://doi.org/10.3126/ijasbt.v6i3.20859

Sivaprakasam, K., & Kandaswamy, T. K. (1981). Waste materials for the cultivation of Pleurotus sajor-caju. *Mushroom Journal.*, *101*, 178—179. http://europepmc.org/abstract/AGR/IND81055215

Thakur, M. P. (2020). Advances in mushroom production: key to food, nutritional and employment security: A review. *Indian Phytopathology*, *73*(3), 377–395. https://doi.org/10.1007/s42360-020-00244-9

Wasser, S. P. (2014). Medicinal mushroom science: Current perspectives,

advances, evidences, and challenges. *Biomedical Journal*, *37*(6), 345–356. https://doi.org/10.4103/2319-4170.138318

Watkinson, S. C. (2016). Physiology and Adaptation. In *The Fungi: Third Edition* (Third Edit). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-382034-1.00005-0

Yang, W. J., Guo, F. L., & Wan, Z. J. (2013). Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi Journal of Biological Sciences*, *20*(4), 333–338. https://doi.org/10.1016/j.sjbs.2013.02.006

Zhang, R. Y., Hu, D. D., Ma, X. T., Li, S. G., Gu, J. G., & Hu, Q. X. (2014). Adopting stick spawn reduced the spawn running time and improved mushroom yield and biological efficiency of Pleurotus eryngii. *Scientia Horticulturae*, *175*(February 2019), 156–159. https://doi.org/10.1016/j.scienta.2014.05.028

Zhang, Y., Mo, M., Yang, L., Mi, F., Cao, Y., Liu, C., Tang, X., Wang, P., & Xu, J. (2021). Exploring the species diversity of edible mushrooms in Yunnan, Southwestern China, by DNA barcoding. *Journal of Fungi*, *7*(4). https://doi.org/10.3390/jof7040310

Zied, D. C., & Pardo-Giménez, A. (2017). *Edible and medicinal mushrooms: technology and applications*. John Wiley & Sons.

APPENDIXCES

APPENDIX A: Materials and equipment

List of materials

Metallic Drum

Electronic Balance

Poly Bag

Rubber Band

Spawn

Rice Straw

Wheat Husk

Maize Cob

Rice Bran

Plastic Sheet

70% Ethanol

Needle

Note Book

Fire Wood

Drum Lid

Black Plastic

Water Sprayer

Hygrometer

Polythene

$CaCo_3$

Chaff Cutter

APPENDIX B: Data record of Pleurotus ostreatus mushroom cultivation

Substrate: Paddy Straw

Substrate		Inoculation	Colonization		Primordial formation	
				Days from inoculation		Days from inoculation
Replication no.	**Wt. in gm**	**Date**	**Date**		**Date**	
1	**6000gm**	3-May-23	19-May-23	16	25-May-23	22
2	**6000gm**	3-May-23	20-May-23	17	23-May-23	20
3	**6000gm**	3-May-23	20-May-23	17	25-May-23	22
Average						**21.33**

Replication1

Harvest no	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
1	6-Jun-23	34	420	1779		
2	15-Jun-23	43	111	444.55		
3	26-Jun-23	54	104	440	2663.5	887.5

Replication 2

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
1	4-Jun-23	32	376	1500		
2	13-Jun-23	41	93	375		
3	24-Jun-23	52	90	380	2255	751.67

Replication 3

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
1	4-Jun-23	32	388	1550		
2	14-Jun-23	42	198	790		
3	26-Jun-23	54	78	300	2640	880

Substrate: Wheat Straw

Substrate		Inoculation	Colonization		Primordial formation	
Replication no.	**Wt. in gm**	**Date**	**Date**	**Days from inoculation**	**Date**	**Days from inoculation**
1	**6000gm**	5-May-23	7-Jun-23	33	11-Jun-23	37
2	**6000gm**	5-May-23	5-Jun-23	31	10-Jun-23	36
3	**6000gm**	5-May-23	4-Jun-23	30	11-Jun-23	37
Average						

Replication 1

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	19-Jun-23	45	100	400		
SECOND	29-Jun-23	55	60	240		
THIRD	12-Jul-23	68	38	150	790	263.33
			198	790		

Replication 2

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	18-Jun-23	44	99	394		
SECOND	29-Jun-23	55	63	250		
THIRD	11-Jul-23	67	30	111	755	251.67

Replication 3

Harvest No	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	19-Jun-23	45	105	420		
SECOND	30-Jun-23	56	65	240		
THIRD	8-Jul-23	64	30	120	780	260

Substrate: Maize cob

Substrate		Inoculation	Colonization		Primordial formation	
Replication no.	Wt. in gm	Date	Date	Days from inoculation	Date	Days from inoculation
1	**6000gm**	7-May-23	27-May-23	20	5-Jun-23	29
2	**6000gm**	7-May-23	28-May-23	21	6-Jun-23	30
3	**6000gm**	8-May-23	28-May-23	20	7-Jun-23	30
Average				**61**		**29.67**
				30.5		

Replication 1

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	16-Jun-23	40	38	152		
SECOND	28-Jun-23	52	26	100	**252**	**126**
THIRD						

Replication 2

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	15-Jun-23	39	43	165		
SECOND	26-Jun-23	50	22	87	**252**	**126**
THIRD						

Replication 3

Harvest no	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	16-Jun-23	40	45	170		
SECOND	27-Jun-23	51	25	90	**260**	**130**
THIRD						

Substrate: 50%Paddy Straw + 40%Wheat Straw+10% Rice bran

Substrate		Inoculation	Colonization		Primordial formation(Pinhead formation)	
Replication no.	Wt. in gm	Date	Date	Days from inoculation	Date	Days from inoculation
1	6000	9-May-23	1-Jun-23	23	6-Jun-23	28
2	6000	10-May-23	31-May-23	21	7-Jun-23	28
3	6000	10-May-23	2-Jun-23	23	6-Jun-23	27
Average	6000					

Replication1

Harvest no.	Date of Harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	12-Jun-23	34	96	376		
SECOND	21-Jun-23	52	60	210	746	248.66
THIRD	8-Jul-23	60	46	160		

Replication2

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	14-Jun-23	35	95	373.8		
SECOND	29-Jun-23	50	55	206.5	745.56	248.52
THIRD	7-Jul-23	58	45	165.26		

Replication3

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	13-Jun-23	34	100	390		
SECOND	27-Jun-23	48	65	250	820	273.33
THIRD	9-Jul-23	60	50	180		

Substrate: 25%MaizeCob + 75%Wheat Husk

Substrate		Inoculation	Colonization		Primordial formation (pinhead formation)	
Replication no.	Wt. in gm	Date	Date	Days from inoculation	Date	Days from inoculation
1	6000	12-May-23	9-Jun-23	28	15-Jun-23	34
2	6000	12-May-23	7-Jun-23	26	15-Jun-23	34
3	6000	12-May-23	9-Jun-23	28	16-Jun-23	35
Average	6000					

Replication1

Harvest no.	Date of Harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	29-Jun-23	48	113	454		
SECOND	4-Jul-23	53	75	303	1009	336.33
THIRD	12-Jul-23	61	63	252		

Replication2

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	27-Jun-23	46	112	450		
SECOND	5-Jul-23	54	77	310	1010	336.67
THIRD	14-Jul-23	63	62	250		

Replication3

Harvest no.	Date of harvest	Days from inoculation	No. of fruiting	Total wt. in gm	Total production	Mean production
FIRST	29-Jun-23	48	115	460		
SECOND	4-Jul-23	53	75	300	1010	336.67
THIRD	12-Jul-23	61	62	250		

Substrate: 75% Paddy straw+ 25% Maize cob

Substrate		Inoculation	Colonization		Primordial formation	
Sample no.	**Wt. in gm**	**Date**	**Date**	**Days from inoculation**	**Date**	**Days from inoculation**
1	**6000**	14-May-23	11-Jun-23	28	15-Jun-23	32
2	**6000**	14-May-23	11-Jun-23	28	14-Jun-23	31
3	**6000**	14-May-23	10-Jun-23	27	14-Jun-23	31
Average						

Replication1

Harvest no.	**Date of Harvest**	**Days from inoculation**	**No. of fruiting**	**Total wt. in gm**	**Total production**	**Mean production**
FIRST	25-Jun-23	42	195	783		
SECOND	1-Jul-23	48	152	609	**1740**	**580**
THIRD	6-Jul-23	53	87	348		

Replication2

Harvest no.	**Date of harvest**	**Days from inoculation**	**No. of fruiting**	**Total wt. in gm**	**Total production**	**Mean production**
FIRST	24-Jun-23	41	191	780		
SECOND	30-Jun-23	47	148	610	**1735**	**578.33**
THIRD	7-Jul-23	54	86	345		

Replication3

Harvest no.	**Date of harvest**	**Days from inoculation**	**No. of fruiting**	**Total wt. in gm**	**Total production**	**Mean production**
FIRST	25-Jun-23	42	195	783		
SECOND	1-Jul-23	48	151	605	**1728**	**576**
THIRD	7-Jul-23	54	85	340		

APPENDIX C: IBM SPSS Statistical Output

1. Effect of Substrate on colonization Day

ANOVA

Colonization Day

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	443.611	5	88.722	88.722	0.001
Within Groups	12.000	12	1.000		
Total	455.611	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Clonization Day
Tukey HSD

(I) SUBSTRATE	(J) SUBSTRATE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Paddy Straw	Wheat Straw	-14.66667*	.81650	.001	-17.4092	-11.9241
	Maize cob	-3.66667*	.81650	.007	-6.4092	-.9241
	Paddy straw + Wheat Husk +10% Rice bran	-5.66667*	.81650	.001	-8.4092	-2.9241
	Paddy Straw + Maize cob	-11.00000*	.81650	.001	-13.7425	-8.2575
	Maize cob +Wheat husk	-10.66667*	.81650	.001	-13.4092	-7.9241
Wheat Straw	Paddy Straw	14.66667*	.81650	.001	11.9241	17.4092
	Maize cob	11.00000*	.81650	.001	8.2575	13.7425
	Paddy straw + Wheat Husk +10% Rice bran	9.00000*	.81650	.001	6.2575	11.7425
	Paddy Straw + Maize cob	3.66667*	.81650	.007	.9241	6.4092
	Maize cob +Wheat husk	4.00000*	.81650	.004	1.2575	6.7425
Maize cob	Paddy Straw	3.66667*	.81650	.007	.9241	6.4092
	Wheat Straw	-11.00000*	.81650	.001	-13.7425	-8.2575
	Paddy straw + Wheat Husk +10% Rice bran	-2.00000	.81650	.214	-4.7425	.7425
	Paddy Straw + Maize cob	-7.33333*	.81650	.001	-10.0759	-4.5908
	Maize cob +Wheat husk	-7.00000*	.81650	.001	-9.7425	-4.2575
Paddy straw + Wheat Husk +10% Rice bran	Paddy Straw	5.66667*	.81650	.001	2.9241	8.4092
	Wheat Straw	-9.00000*	.81650	.001	-11.7425	-6.2575
	Maize cob	2.00000	.81650	.214	-.7425	4.7425
	Paddy Straw + Maize cob	-5.33333*	.81650	.001	-8.0759	-2.5908
	Maize cob +Wheat husk	-5.00000*	.81650	.001	-7.7425	-2.2575
Paddy Straw + Maize cob	Paddy Straw	11.00000*	.81650	.001	8.2575	13.7425
	Wheat Straw	-3.66667*	.81650	.007	-6.4092	-.9241
	Maize cob	7.33333*	.81650	.001	4.5908	10.0759
	Paddy straw + Wheat Husk +10% Rice bran	5.33333*	.81650	.001	2.5908	8.0759
	Maize cob +Wheat husk	.33333	.81650	.998	-2.4092	3.0759
Maize cob +Wheat husk	Paddy Straw	10.66667*	.81650	.001	7.9241	13.4092
	Wheat Straw	-4.00000*	.81650	.004	-6.7425	-1.2575
	Maize cob	7.00000*	.81650	.001	4.2575	9.7425
	Paddy straw + Wheat Husk +10% Rice bran	5.00000*	.81650	.001	2.2575	7.7425
	Paddy Straw + Maize cob	-.33333	.81650	.998	-3.0759	2.4092

*. The mean difference is significant at the 0.05 level.

1. Effect of Substrate on primordia formation Day

ANOVA

Primordia

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	436.500	5	87.300	174.600	0.001
Within Groups	6.000	12	.500		
Total	442.500	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Primordia forming Day
Tukey HSD

(I) SUBSTRATE	(J) SUBSTRATE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Paddy Straw	Wheat Straw	-15.33333*	.57735	.001	-17.2726	-13.3941
	Maize cob	-8.33333*	.57735	.001	-10.2726	-6.3941
	Paddy straw + Wheat Husk +10% Rice bran	-6.33333*	.57735	.001	-8.2726	-4.3941
	Paddy Straw + Maize cob	-10.00000*	.57735	.001	-11.9393	-8.0607
	Maize cob +Wheat husk	-13.00000*	.57735	.001	-14.9393	-11.0607
Wheat Straw	Paddy Straw	15.33333*	.57735	.001	13.3941	17.2726
	Maize cob	7.00000*	.57735	.001	5.0607	8.9393
	Paddy straw + Wheat Husk +10% Rice bran	9.00000*	.57735	.001	7.0607	10.9393
	Paddy Straw + Maize cob	5.33333*	.57735	.001	3.3941	7.2726
	Maize cob +Wheat husk	2.33333*	.57735	.016	.3941	4.2726
Maize cob	Paddy Straw	8.33333*	.57735	.001	6.3941	10.2726
	Wheat Straw	-7.00000*	.57735	.001	-8.9393	-5.0607
	Paddy straw + Wheat Husk +10% Rice bran	2.00000*	.57735	.042	.0607	3.9393
	Paddy Straw + Maize cob	-1.66667	.57735	.109	-3.6059	.2726
	Maize cob +Wheat husk	-4.66667*	.57735	.001	-6.6059	-2.7274
Paddy straw + Wheat Husk +10% Rice bran	Paddy Straw	6.33333*	.57735	.001	4.3941	8.2726
	Wheat Straw	-9.00000*	.57735	.001	-10.9393	-7.0607
	Maize cob	-2.00000*	.57735	.042	-3.9393	-.0607
	Paddy Straw + Maize cob	-3.66667*	.57735	.001	-5.6059	-1.7274
	Maize cob +Wheat husk	-6.66667*	.57735	.001	-8.6059	-4.7274
Paddy Straw + Maize cob	Paddy Straw	10.00000*	.57735	.001	8.0607	11.9393
	Wheat Straw	-5.33333*	.57735	.001	-7.2726	-3.3941
	Maize cob	1.66667	.57735	.109	-.2726	3.6059
	Paddy straw + Wheat Husk +10% Rice bran	3.66667*	.57735	.001	1.7274	5.6059
	Maize cob +Wheat husk	-3.00000*	.57735	.002	-4.9393	-1.0607
Maize cob +Wheat husk	Paddy Straw	13.00000*	.57735	.001	11.0607	14.9393
	Wheat Straw	-2.33333*	.57735	.016	-4.2726	-.3941
	Maize cob	4.66667*	.57735	.001	2.7274	6.6059
	Paddy straw + Wheat Husk +10% Rice bran	6.66667*	.57735	.001	4.7274	8.6059
	Paddy Straw + Maize cob	3.00000*	.57735	.002	1.0607	4.9393

*. The mean difference is significant at the 0.05 level.

2. Effect of Substrate on Fruiting Number of *Pleurotus ostreatus*

ANOVA

Fruiting Number

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	586685.611	5	117337.122	227.667	0.001
Within Groups	6184.667	12	515.389		
Total	592870.278	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Fruiting Number
Tukey HSD

(I) SUBSTRATE	(J) SUBSTRATE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Paddy Straw	Wheat Straw	422.66667*	18.53625	.001	360.4049	484.9285
	Maize cob	553.00000*	18.53625	.001	490.7382	615.2618
	Paddy straw + Wheat Husk +10% Rice bran	415.33333*	18.53625	.001	353.0715	477.5951
	Paddy Straw + Maize cob	189.33333*	18.53625	.001	127.0715	251.5951
	Maize cob +Wheat husk	368.00000*	18.53625	.001	305.7382	430.2618
Wheat Straw	Paddy Straw	-422.66667*	18.53625	.001	-484.9285	-360.4049
	Maize cob	130.33333*	18.53625	.001	68.0715	192.5951
	Paddy straw + Wheat Husk +10% Rice bran	-7.33333	18.53625	.998	-69.5951	54.9285
	Paddy Straw + Maize cob	-233.33333*	18.53625	.001	-295.5951	-171.0715
	Maize cob +Wheat husk	-54.66667	18.53625	.098	-116.9285	7.5951
Maize cob	Paddy Straw	-553.00000*	18.53625	.001	-615.2618	-490.7382
	Wheat Straw	-130.33333*	18.53625	.001	-192.5951	-68.0715
	Paddy straw + Wheat Husk +10% Rice bran	-137.66667*	18.53625	.001	-199.9285	-75.4049
	Paddy Straw + Maize cob	-363.66667*	18.53625	.001	-425.9285	-301.4049
	Maize cob +Wheat husk	-185.00000*	18.53625	.001	-247.2618	-122.7382
Paddy straw + Wheat Husk +10% Rice bran	Paddy Straw	-415.33333*	18.53625	.001	-477.5951	-353.0715
	Wheat Straw	7.33333	18.53625	.998	-54.9285	69.5951
	Maize cob	137.66667*	18.53625	.001	75.4049	199.9285
	Paddy Straw + Maize cob	-226.00000*	18.53625	.001	-288.2618	-163.7382
	Maize cob +Wheat husk	-47.33333	18.53625	.183	-109.5951	14.9285
Paddy Straw + Maize cob	Paddy Straw	-189.33333*	18.53625	.001	-251.5951	-127.0715
	Wheat Straw	233.33333*	18.53625	.001	171.0715	295.5951
	Maize cob	363.66667*	18.53625	.001	301.4049	425.9285
	Paddy straw + Wheat Husk +10% Rice bran	226.00000*	18.53625	.001	163.7382	288.2618
	Maize cob +Wheat husk	178.66667*	18.53625	.001	116.4049	240.9285
Maize cob +Wheat husk	Paddy Straw	-368.00000*	18.53625	.001	-430.2618	-305.7382
	Wheat Straw	54.66667	18.53625	.098	-7.5951	116.9285
	Maize cob	185.00000*	18.53625	.001	122.7382	247.2618
	Paddy straw + Wheat Husk +10% Rice bran	47.33333	18.53625	.183	-14.9285	109.5951
	Paddy Straw + Maize cob	-178.66667*	18.53625	.001	-240.9285	-116.4049

*. The mean difference is significant at the 0.05 level.

3. Effect of Substrate on Yield of *Pleurotus ostreatus*

ANOVA

YEILD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9955366.299	5	1991073.260	217.891	0.001
Within Groups	109655.002	12	9137.917		
Total	10065021.301	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: YEILD
Tukey HSD

(I) SUBSTRATE	(J) SUBSTRATE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Paddy Straw	Wheat Straw	1744.50000*	78.05091	.001	1482.3332	2006.6668
	Maize cob	2264.83333*	78.05091	.001	2002.6665	2527.0002
	Paddy straw + Wheat Husk +10% Rice bran	1748.98000*	78.05091	.001	1486.8132	2011.1468
	Paddy Straw + Maize cob	785.16667*	78.05091	.001	522.9998	1047.3335
	Maize cob +Wheat husk	1509.83333*	78.05091	.001	1247.6665	1772.0002
Wheat Straw	Paddy Straw	-1744.50000*	78.05091	.001	-2006.6668	-1482.3332
	Maize cob	520.33333*	78.05091	.001	258.1665	782.5002
	Paddy straw + Wheat Husk +10% Rice bran	4.48000	78.05091	1.000	-257.6868	266.6468
	Paddy Straw + Maize cob	-959.33333*	78.05091	.001	-1221.5002	-697.1665
	Maize cob +Wheat husk	-234.66667	78.05091	.090	-496.8335	27.5002
Maize cob	Paddy Straw	-2264.83333*	78.05091	.001	-2527.0002	-2002.6665
	Wheat Straw	-520.33333*	78.05091	.001	-782.5002	-258.1665
	Paddy straw + Wheat Husk +10% Rice bran	-515.85333*	78.05091	.001	-778.0202	-253.6865
	Paddy Straw + Maize cob	-1479.66667*	78.05091	.001	-1741.8335	-1217.4998
	Maize cob +Wheat husk	-755.00000*	78.05091	.001	-1017.1668	-492.8332
Paddy straw + Wheat Husk +10% Rice bran	Paddy Straw	-1748.98000*	78.05091	.001	-2011.1468	-1486.8132
	Wheat Straw	-4.48000	78.05091	1.000	-266.6468	257.6868
	Maize cob	515.85333*	78.05091	.001	253.6865	778.0202
	Paddy Straw + Maize cob	-963.81333*	78.05091	.001	-1225.9802	-701.6465
	Maize cob +Wheat husk	-239.14667	78.05091	.082	-501.3135	23.0202
Paddy Straw + Maize cob	Paddy Straw	-785.16667*	78.05091	.001	-1047.3335	-522.9998
	Wheat Straw	959.33333*	78.05091	.001	697.1665	1221.5002
	Maize cob	1479.66667*	78.05091	.001	1217.4998	1741.8335
	Paddy straw + Wheat Husk +10% Rice bran	963.81333*	78.05091	.001	701.6465	1225.9802
	Maize cob +Wheat husk	724.66667*	78.05091	.001	462.4998	986.8335
Maize cob +Wheat husk	Paddy Straw	-1509.83333*	78.05091	.001	-1772.0002	-1247.6665
	Wheat Straw	234.66667	78.05091	.090	-27.5002	496.8335
	Maize cob	755.00000*	78.05091	.001	492.8332	1017.1668
	Paddy straw + Wheat Husk +10% Rice bran	239.14667	78.05091	.082	-23.0202	501.3135
	Paddy Straw + Maize cob	-724.66667*	78.05091	.001	-986.8335	-462.4998

*. The mean difference is significant at the 0.05 level.

APPENDIX D: Site Approval Letter

Bahumukhi Krishi Farm Pvt. Ltd.
बहुमूखी कृषि फार्म प्रा.लि.

To **02-May-2023**

Tribhuwan University
Central Campus of Technology
Hattisar, Dharan,Sunsari

Subject: Site Approval Letter

To whom it may concern:

This letter acknowledges that I have received and reviewed a request by **Prakash Bajagain** to conduct a research project entitled **"EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATION ON THE YEILD OF MUSHROOM. (*Pleurotus Ostreatus)*** " at **Bahumukhi Krishi Farm Pvt.Ltd** and I approve of this research to be conducted at our facility.

When the researcher receives approval for his research project from the Tribhuwan University, I agree to provide access for the approved research project.

Sincerely,

Devyani Basnet
Chief Executive Officer
bahumukhikrishifarm@gmail.com

Belbari-9, Morang
+ 977-9852083
info@bahumukhi
www.bahumukhi
bahumukhikrishifarm
mukhikrishifarm@gmail.com

Reg.No. : 191711/074/075 Ph.No.
Pan No. : 606682136

नेपाल ब्रदर्श मशरुम एण्ड एग्रो रिसर्च प्रा.लि.

APPENDIX E: Spawn of *Pleurotus ostreatus*

सुन्दरहरैचा न.पा.वडा नं. ४,मोरङ

स्थाः२०७५ मितिः 03 May, 2023

To

The campus chief

Central Campus of Technology

Hattisar, Dharan, Sunsari

Subject:-Authority for performing Thesis Research Work.

Dear Sir

We are pleased to confirm that your student namely Mr. Prakash Bajagainwho performing the thesis work for completion of Master's degree in Agricultural Microbiology from Central Campus of Technology is allowed to perform the research work entitled "**EFFECT OF DIFFERENT SUBSTRATES AND THEIR COMBINATION ON THE YEILD OF MUSHROOM. (*PleurotusOstreatus*)".** The organization has provided him Spawn of *Pleurotus Ostreatus* mushroomto perform his research work from 03- May -2023.

We wish for the successful completion of his work.Thank you

Saroj Nepal

Director

PHOTOPLATES

MAIZE COB

1.Maize cob hammering

3. **Primordia formation**

2. Clonization

4.Fruiting

5.Fruiting

7.Fresh Mushroom

6. Fruiting

8.Fruiting

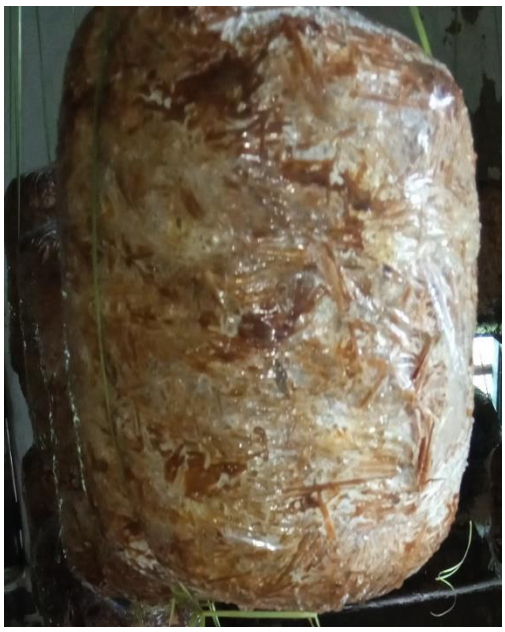
PADDY STRAW

1.Paddy Straw chopping

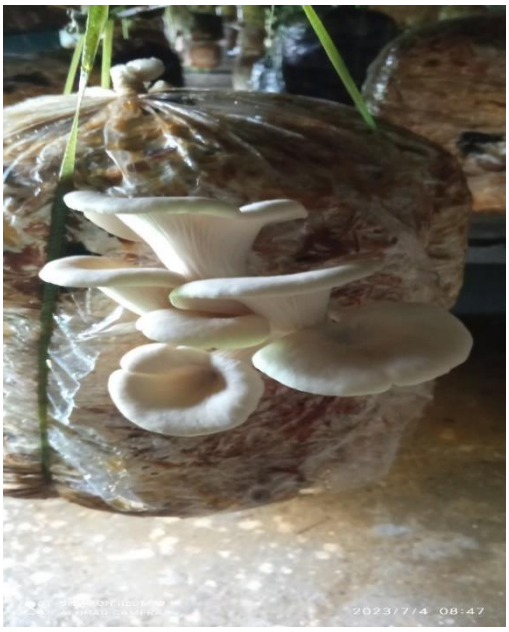
3.Sterlization

2.Soaking in Water

4.Inoculation



5. Full Clonization



7. Fruiting

6 .Primordia Formation

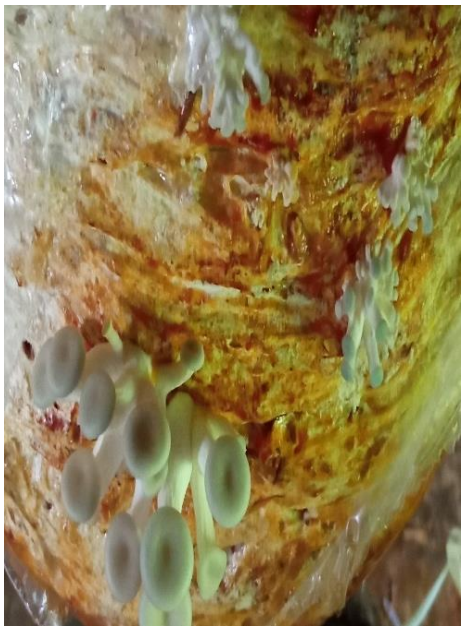
8. Fruiting

WHEAT HUSK

1.Wheat Husk

3.Primordia initiation

2. Colonization



4. Primordia

5. Fruiting

CRD OF SUBSTRATE