COMPARATIVE PERFORMANCE OF *PLEUROTUS* GENOTYPES AND MICROCLIMATE INTERACTIONS UNDER INDOOR AND OUTDOOR BANANA-INTEGRATED SYSTEM ACROSS SEASONS IN KAKAMEGA, KENYA

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DEDICATION

This thesis is dedicated to my husband, my parents, my siblings and my sons whose unwavering support, encouragement, and love have been the foundation of my academic journey. To my mentors and friends, thank you for inspiring me and walking beside me every step of the way. And to anyone who dares to dream, may this work be a small light on your path.

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ABSTRACT

Growing of mushrooms is a sustainable opportunity that enhances availability of food and improving nutrition in Kenya. Its productivity however has since remained limited due to over reliance on controlled indoor systems. Deficient knowledge regarding how environmental conditions influence its performance under different cultivation settings is also another common challenge. Oyster (*Pleurotus* spp) is the most common edible grown mushrooms because of its high nutritional content and short cropping cycles. However, variations in temperature, humidity, carbon iv oxide and light often affect its performance in terms of yield, growth and quality. Banana plantations, which are widely grown in western Kenya is a common crop that provide shade and humid microclimates to other crops such as mushrooms when grown together. Their suitability that could potentially support mushroom cultivation compared to indoor systems however remains underexplored. This study evaluated the performance and microclimatic influence of *Pleurotus ostreatus* and *Pleurotus australis* under indoor and outdoor (banana-integrated) systems across wet and dry seasons at Masinde Muliro University Farm, Kakamega County. A quasi-split split plot design was used (season = main plot, cultivation condition = subplot, genotype = sub-subplot). Data collection (ODK, field notes) covered: mycelial growth (size and growth rate), incubation/spawn-run duration and time to pinhead appearance, length of the fruiting (fructification) phase, morphological traits (stalk/stipe length, cap/pileus diameter), number of fruit bodies, number of deformed fruits, contamination level (%), biological efficiency (%), total yield, and microclimate variables (relative humidity, temperature, light intensity in lux, and CO_2), with microclimate readings taken thrice daily. Data were analyzed using univariate ANOVA and least-square means comparisons (SAS 9.4). From the results, the treatments of season, cultivation condition and genotype interactions significantly influenced overall performance ($p < 0.05$). Notably, the interactions of wet season, outdoor (banana-integrated) condition, and *P. ostreatus* genotype performed the best. The interaction resulted to largest mycelial expansion (~32.3 cm), shortest incubation period (~22 days), widest caps (~13.1 cm), highest biological efficiency (~694%), and greatest yield (~2,582 g). These results coincided with favorable microclimate values (high humidity, moderate temperature, balanced light and lower CO_2). The findings indicate that outdoor banana-integrated cultivation creates a favorable microclimate that, through season × condition × genotype interactions, markedly improves mushroom growth and productivity relative to indoor systems.

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DEFINITION OF TERMS

Biological Efficiency (BE): A measure of productivity in mushroom cultivation, calculated as the ratio of fresh mushroom weight to the dry weight of the substrate, expressed as a percentage. It indicates how efficiently the substrate is converted into edible yield.
Cap Diameter: The width of the mushroom pileus measured in centimeters at full maturity. It serves as a key morphological trait influencing quality and market value.
Contamination Level: This refers to the percentage of infected substrate showing mold, bacteria, or other undesirable growth.
Cultivation Condition: The specific environmental setting used for mushroom production.
Season: The distinct climatic periods that influence temperature, humidity, and substrate moisture.
Fruiting Phase: The period between the appearance of pinheads and full maturation of fruiting bodies.
Genotype: The specific genetic makeup of mushroom species. In this study, it refers to *Pleurotus ostreatus* and *Pleurotus australis*, which differ in growth rate, morphology, and environmental adaptability
Incubation (Spawn-Run) Period: The time, measured in days from the mycelium appearance to complete colonization of the substrate before fruiting begins.
Microclimate: Localized atmospheric conditions surrounding the mushroom substrate, including temperature, relative humidity, light intensity, and carbon dioxide concentration, which directly influence mushroom development.
Mycelial Growth: The expansion of fungal hyphae through the substrate during the vegetative phase.
Outdoor (Banana-Integrated) System: A cultivation method where mushroom bags are suspended under banana canopies, utilizing natural shade, humidity, and temperature regulation from the banana plants to support mushroom growth.
Indoor System: A controlled-environment method of mushroom cultivation within enclosed structures (mud or iron-sheet houses) where humidity, light, and temperature are manually regulated.
Number of Deformed Fruits: Count of physiologically defective fruiting bodies per treatment.
Number of Fruits: Count of mature fruiting bodies harvested per treatment, representing the productivity of a given condition and genotype.
Season × Condition × Genotype Interactions: The combined effect of seasonal variation, cultivation environment, and genetic differences on growth, yield, growth, morphological, and production parameters analyzed using ANOVA.
Stalk Length (Stipe): The vertical distance between the base of the mushroom and the cap (pileus), measured in centimeters.
Substrate: The growth medium prepared from agricultural residues that provides nutrients for mushroom mycelium development.
Yield: The total fresh weight of mushrooms harvested per treatment across all flushes, used to assess productivity under different environmental and genetic conditions.

ABBREVIATIONS AND ACRONYMS

ANOVA	–	Analysis of Variance
BE	–	Biological Efficiency
BOD	–	Biological Oxygen Demand (incubator)
CFD	–	Computational Fluid Dynamics
CL	–	Contamination Level
CO_2	–	Carbon Dioxide
FAO	–	Food and Agriculture Organization
g	–	Gram(s)
hr(s)	–	Hour(s)
Kg	–	Kilogram(s)
LSM	–	Least Square Means
Lux	–	Unit of Light Intensity
MMUST	–	Masinde Muliro University of Science and Technology
mm	–	Millimeter(s)
ODK	–	Open Data Kit
PAR	–	Photosynthetically Active Radiation
P. australis	–	*Pleurotus australis*

P. ostreatus	–	*Pleurotus ostreatus*
RCBD	–	Randomized Complete Block Design
RH	–	Relative Humidity
SAS	–	Statistical Analysis Software
SHG	–	Self-Help Group
°C	–	Degree Celsius

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Mushrooms are the spore-bearing fruiting bodies of diverse fungi, mainly in the phylum Basidiomycota and class Agaricomycetes, with hundreds of genera such as *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia*, and *Termitomyces (*Katoch et al., 2023). Globally, about 14,000 macrofungal species have been described, of which roughly 2,000 are edible, and 30–40 are cultivated commercially (Mafe et al., 2025). Edible mushrooms are nutrient-dense, rich in protein, fibre, vitamins, and bioactive compounds, and play important ecological roles as decomposers (Fitsum et al.,2025).

Worldwide mushroom production has grown rapidly over the past four decades, reaching tens of millions of tonnes annually, with market growth estimated at 8–10 % (FAO, 2024). Asia, particularly China, accounts for over 90 % of cultivated mushrooms, while demand is rising steadily in Europe, North America, and Africa (Muhammad & Suleiman,2015). In Africa, production is modest (about 40–45 thousand tonnes per year), led by South Africa, Morocco, and a few other countries (Itubochi et al.,2025).

In Kenya, national output is estimated at ~500 tonnes per year against a domestic demand of ~1,200 tonnes (Gyalai et al.,2023), creating a persistent supply gap partly filled by imports from Rwanda, China, and South Africa. According to Sonnenberg,(2011), commercial cultivation is dominated by button mushrooms **(***Agaricus bisporus***)**, with increasing interest in oyster mushrooms **(***Pleurotus* spp.) and small pilot projects for shiitake (*Lentinula edodes*) and paddy-straw

(*Volvariella volvacea*). Wild edible species such as *Termitomyces* and *Cantharellus* contribute to local diets but are not farmed at scale (Sileshi,2023).

Mushroom farming offers advantages over many conventional crops. Short production cycles (4–8 weeks) allow multiple harvests and high returns per unit area (Pilz, 2002). Start-up costs can be modest, and the sector generates employment across spawn production, cultivation, processing, and marketing particularly benefiting women and youth (Maurya,n.d). Environmentally, mushrooms require little land or water, can be grown on agricultural wastes such as maize stalks, coffee husks, and banana residues, and generate a low carbon footprint (Madusanka et al., 2024). Spent mushroom substrate can be used as organic fertiliser or animal feed, closing nutrient loops and reducing pollution (Jasińska,, 2018).

In Kenya, cultivation systems are mainly indoor and outdoor (or semi-outdoor). Indoor systems; climate-controlled grow rooms, insulated "bag houses," or retrofitted containers permit year-round production and high quality but require high capital investment, energy for cooling and humidification, and technical expertise (Cotter, 2014; Kenya News, 2025).

Outdoor innovations such as thatched huts and shaded net-houses exploit natural humidity (Procházka et al., 2023) but remain relatively costly for smallholders. Banana-shaded plots, on the other hand, can provide a low-cost alternative by naturally moderating temperature and humidity, though limited research exists on their performance under drought, pest, and contamination pressures. Some other common shortcomings in mushroom growing include difficulty in finding reliable spawns, substrate contamination, pest and disease outbreaks, limited technical

expertise, poor cold-chain facilities, market fluctuations, and weak regulation (Farmbiz Africa, 2023; OICC, 2025).

Given these challenges, it is important to consider site-specific solutions that make use of local resources and favorable microclimates. Masinde Muliro University farm in Kakamega County, Western Kenya, is one such place. The region receives between 1,500 and 2,000 mm of rainfall each year, with moderate temperatures ranging from 18 to 28 °C and high humidity levels that are ideal for mushroom cultivation (County Government of Kakamega, 2023). Kakamega is also a major banana-growing area (Karanja et al., 2008), where large banana plantations provide shade, retain moisture, and produce organic residues that can be used in integrated mushroom–banana farming systems (EAJSTI, 2024)

1.2 Statement of the Problem

Mushroom farming in Kenya is increasingly recognized as a valuable source of nutritious food and extra household income. Yet, most growers still rely on indoor cultivation systems that are expensive to set up and maintain. Many smallholder farmers struggle with high input costs, lack of proper facilities, and unstable yields, particularly during the dry season when humidity levels drop. Meanwhile, large portions of farmland in western Kenya remain underused beneath banana plantations, where the natural shade and moisture could support mushroom growth. Although this potential has been noted, few studies have compared indoor production with outdoor cultivation under banana canopies. In particular, little is known about how different oyster mushroom varieties (Pleurotus ostreatus and P. australis) perform under varying seasonal conditions. The lack of such information limits farmers' ability to shift toward affordable and climate-resilient systems that

make better use of land and provide consistent harvests throughout the year. This study therefore aimed to assess the growth and yield of the two Pleurotus genotypes under indoor and banana-shaded outdoor setups during wet and dry seasons, in order to identify a more sustainable and cost-effective production approach for western Kenya.

1.3 Objectives of the Study

1.3.1 General Objective

To evaluate the interactive effects of season, cultivation condition, and genotype on the performance and microclimatic responses of oyster genotypes (*Pleurotus* spp.) for sustainable production.

1.3.2 Specific Objectives

i. To compare the growth, morphological parameters, contamination level (CL) and biological efficiency(BE) of *Pleurotus* genotypes cultivated under indoor and outdoor (banana-integrated) systems across the wet and dry seasons
ii. To evaluate the influence of season, cultivation conditions and genotypes interactions on the yield of *Pleurotus*.
iii. To determine how variations in season, cultivation condition, and genotypes influence key microclimate parameters associated with oyster mushroom growth.

1.4 Hypotheses

This study was guided by the following hypotheses;

H_{01}: There is no significant influence of season, cultivation condition and genotype, or their interactions on the growth, morphological, CL and BE parameters of oyster mushrooms.

H_{02}: There is no significant influence of season, cultivation condition, genotype, or their interactions on total yield.

H_{03}: There is no significant impact of season, cultivation condition, genotype, or their interactions on microclimate variables associated with oyster mushroom cultivation.

1.5 Scope of the Study

This study was conducted at the Masinde Muliro University farm, located in Lurambi Sub-County, Kakamega County, Kenya. The site was selected to ensure uniform soil characteristics, microclimate, and management practices. This helped minimize environmental variation so that any observed differences could be linked mainly to the experimental treatments rather than location effects. The work focused on integrating oyster mushroom production within existing banana stands at this single site.

Two oyster mushroom genotypes, *Pleurotus australis* and *Pleurotus ostreatus*, were used in the experiment. These species were chosen because they are widely cultivated in Kenya and are known for their high nutritional and market value. Limiting the research to these two strains made it easier to compare their biological and economic performance under the integration system. Mushrooms were grown under two production conditions: indoor and outdoor (beneath banana shade), during the two main climatic seasons of Kakamega County; the dry and the rainy seasons. The growth substrate was a uniform mixture of sugarcane bagasse, chick mash, molasses, and lime. Treatments were arranged in a randomized complete block design (RCBD) with two replications to balance available resources with statistical reliability. The study followed a quasi-split-split plot approach because

of the multiple factors involved (season × condition × genotype). Each treatment combination required substrate preparation, spawn running, and close monitoring, which made the experiment both labour- and resource-intensive. Since this was a new area of investigation, the focus was exploratory to test the concept rather than to make broad generalizations.

The experiment ran for twelve months, from January to December 2023, covering both the dry and rainy periods that strongly influence temperature, humidity, and substrate moisture. Conducting the study over a full year made it possible to assess how each genotype performed under different environmental conditions. Data were collected on growth behaviour, sporophore characteristics, yield, and weather conditions. These parameters provided a clear picture of how the two oyster mushroom genotypes responded to changes in production systems and seasonal variations.

1.6 Justification of the Study

Growing of mushrooms present a promising approach to addressing food insecurity that results from shrinking agricultural land. It also helps manage agricultural waste while offering nutritious and medicinal food products. Findings from this study show that oyster mushrooms can grow successfully both indoors and outdoors, provided they receive sufficient shade and suitable environmental conditions. In western Kenya, where banana farming is common among smallholder farmers, the crop's large leaves and dense canopy naturally provide shade and moisture. These conditions make banana fields a practical and low-cost alternative to expensive indoor mushroom houses. Integrating mushrooms with bananas allows farmers to make better use of their land, increase food variety, and boost household income without investing heavily in infrastructure.

The benefits of this research go beyond the farm level. For smallholder farmers, it offers a simple and affordable production method that improves land use and profitability. Extension officers can also draw on these findings to promote integrated systems that combine staple crops with high-value mushrooms, supporting climate-smart and diversified agriculture. For researchers and academic institutions, the study contributes new insights into how different *Pleurotus* genotypes perform under varying environmental conditions and production systems. It also provides a useful foundation for further research on other mushroom species and management practices. Overall, the study supports the development of sustainable food systems and highlights mushroom farming as a practical solution for improving food and nutrition security in Kenya and other regions with similar conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Chapter Two presents a review of existing literature on oyster mushroom (*Pleurotus* spp.) cultivation. It explores their biological features, nutritional and economic significance and potential for adoption by smallholder farmers. The chapter also examines different agricultural residues used as substrates and compares the performance of mushrooms grown under indoor controlled environments with those cultivated outdoors, including systems integrated with banana plants. It concludes by identifying gaps in previous studies on genotype and microclimate interactions, which the present research seeks to address

2.2 Edible Mushrooms Cultivated in Kenya

Edible mushrooms are macrofungi valued for their high protein content, essential amino acids, vitamins, and minerals. Globally, more than 2,000 species of edible mushrooms are known, with about 20 cultivated commercially (Kaliyaperumal et al.,, 2019). In Kenya, commercial production is dominated by oyster mushrooms (*Pleurotus spp*.), button mushrooms (*Agaricus bisporus*), and, to a smaller extent, shiitake (*Lentinula edodes*) and wood ear (*Auricularia spp*.) (Waiganjo et al., 2008). Among these, oyster mushrooms have become the most popular because they grow quickly, tolerate a wide range of substrates, and require relatively simple technology compared to button mushrooms, which demand climate-controlled houses and composted substrates (Besufekad et al.,2020). Recent surveys indicate that smallholder farmers and community groups increasingly prefer oyster

mushrooms. This is because they have short cropping cycles, can produce all year-round, and a ready strong market in urban centres (Wachira et al., 2022).

2.3 Substrates and Cultivation Materials for Oyster Mushroom

Successful mushroom cultivation depends on the use of substrates that contain high amounts of cellulose, hemicellulose, and lignin (Suwannarach et al., 2022). In Kenya, most producers rely on readily available and low-cost agricultural residues such as sugarcane bagasse, wheat or rice straw, maize cobs, coffee husks, sawdust, and banana leaves or pseudostems. To improve mycelial development and yield, these materials are often supplemented with nutrient sources like wheat bran, chick mash, or molasses (Njeru et al., 2023). Additives such as lime or gypsum may also be included to balance pH and minimize contamination (Khan et al., 2013).

The spawn, which serves as the vegetative seed material for mushrooms, is typically developed from pure cultures grown on sterilized grains (Moreaux, 2017). Farmers can obtain spawn from commercial laboratories, universities, or private producers. Once the substrate is prepared and inoculated, the bags or beds are kept in incubation until the mycelium completely colonizes the material. Fruiting then follows under conditions of high humidity and filtered light (Zervakis et al., 2001).

2.4 Methods of Cultivation

Mushroom production in Kenya mainly takes place under two systems: indoor and outdoor (or shaded) cultivation. Indoor production involves growing mushrooms in enclosed spaces such as rooms or greenhouses, where temperature, humidity, and lighting are regulated (Chong et al., 2023). Although this method provides

stable and often higher yields, it demands considerable investment in construction, ventilation, and humidity control (Jones, 2021).

In contrast, outdoor or semi-outdoor systems rely on natural shade provided by trees or simple shelters covered with materials such as banana leaves, grass, or polythene. This approach is cheaper and more accessible to smallholder farmers but tends to be affected by changes in temperature and moisture levels (Egerton University Study, 2023).

Recently, integrated methods that combine mushroom cultivation with shade-providing crops have drawn interest because they help lower production costs while improving land use efficiency.

2.4.1 Indoor Cultivation

Recent studies on indoor mushroom cultivation in Kenya and beyond provide useful background for the present work.

Myronycheva et al. (2017) examined how temperature affected growth and fruiting of *Pleurotus ostreatus* and *Pleurotus pulmonarius* in indoor setup in Ukraine. Their results showed that both temperature and strain influenced performance, suggesting that different genotypes respond uniquely to environmental changes. However, the authors pointed out several knowledge gaps including limited understanding of interactions between genetic and environmental factors, the lack of long-term adaptability data, and the absence of comparisons between indoor and more natural cultivation systems. These observations support the need for studies like this one, which compare *Pleurotus* genotypes under both indoor and banana-integrated systems across different seasons.

Chen et al. (2022) who analyzed advanced indoor cultivation facilities which use automated monitoring and control systems to improve production efficiency,

demonstrated major progress in controlled-environment farming. The study in contrast, focused mainly on costly, energy-intensive indoor setups. From the project, lack of standard cultivation protocols, insufficient attention to energy-saving methods, and few studies comparing indoor systems with low-cost or outdoor alternatives was also noted. These gaps align with the objectives of the current research, which seeks to evaluate sustainable and resource-efficient production approaches, that are capable of achieving comparable yields under varying environmental conditions.

The study of Lee et al. (2017) used computational fluid dynamics (CFD) modeling to simulate air movement and temperature in an indoor mushroom house. Their findings revealed uneven distribution of air velocity, humidity, and temperature when all control equipment operated simultaneously. Although environmental uniformity improved with the use of coolers and circulation fans, the study noted that even optimized ventilation rates did not always guarantee uniform mushroom growth unless air was evenly distributed across the room. This limitation has led some researchers to recommend exploring alternative cultivation systems, including outdoor production.

In the article *"Foster Phenomenal Fungi,"* Padilla-Brown (2024) discussed the use of grow tents as affordable indoor environments for small-scale mushroom producers. The work offered practical guidance on selecting tent types, regulating temperature and humidity, and maintaining hygiene to minimize contamination. While the findings highlighted grow tents as flexible and accessible tools for improving mushroom yields, the study was mainly experiential. It did not provide a detailed scientific comparison between controlled indoor systems and more natural, integrated setups. This gap emphasizes the importance of further

comparative research such as assessing *Pleurotus* genotypes grown both indoors and under banana-based systems across seasons to better understand how environmental factors influence performance and yield.

In another indoor study conducted by Sobieralski et al. (2011) in Poland where mycelial growth and yield of wild *Pleurotus ostreatus* strains with the commercial strain HK35 were compared using lignocellulosic substrates, results showed that the commercial strain had the fastest growth. The study confirmed that agricultural and textile by-products are suitable as substrates. The study however calls for further optimization of substrate formulations and a need to evaluate environmental sustainability. These findings highlight the importance of testing such substrates under alternative systems, including outdoor and integrated models.

In another study done at Egerton University in Nakuru County (Egerton University, 2023), researchers investigated the effects of different grain spawn types and substrate combinations on oyster mushroom yield in indoor shelters. They reported high biological efficiencies often above 90 percent when sugarcane bagasse was supplemented with wheat bran. They also managed to identify shelter designs that maintained stable humidity. Although these findings were significant, the research remained confined to indoor setups and did not examine combined cropping with bananas or the effect of seasonal variation on productivity

2.4.2 Outdoor Mushroom Cultivation

Although outdoor mushroom cultivation has received less research attention in East Africa compared to indoor systems, there are few existing studies which provide valuable insights into how land can be used more efficiently through integration with other crops.

The research done by Mohapatra and Niranjan (2014) explored the cultivation of straw mushrooms (*Volvariella volvacea*) beneath coconut plantations along the coastal region of Odisha. Their findings showed that the semi-shaded and humid environment created under coconut canopies supported healthy mushroom growth and good yields, as well as improving land-use efficiency. Their findings conclude that combined mushroom production with perennial crops increases productivity and farmer income in a sustainable manner. Still, the authors pointed out several areas needing further study, including the evaluation of high-yielding, climate-resilient strains and the need for multi-season experiments to better understand microclimate interactions in integrated systems.

Another work done by Hamed et al. (2021) where integration of oyster mushroom production with field-grown cabbage and cauliflower were examined, showed that both vegetables and mushrooms performed better under this mixed system compared to when each was grown separately. In this approach, mushrooms were cultivated between vegetable rows during head and curd formation stages. The researchers concluded that such integration enhances overall food productivity and makes land use more efficient. However, their research did not assess how different *Pleurotus* genotypes might respond to varying field conditions and how the microclimate within intercropped plots changes through the growing period.

In another example, Sipasi (2024) studied the integration of King Stropharia (*Stropharia rugosoannulata*) with asparagus and tomato crops using mulch-based systems. The inclusion of mushrooms led to higher asparagus yields and earlier harvests, while certain mulching materials boosted tomato fruit numbers. Interestingly, mushrooms also appeared in plots that had not been intentionally inoculated, showing the species' adaptability to field environments. This work

demonstrated that mushroom intercropping can improve crop performance and food diversity. A gap in knowledge was noted where exploration on how different mushroom genotypes behave under these conditions and how seasonal variations influence growth and yield were not done, leaving useful ground for future research.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the research design, data collection procedures, and analytical methods that were applied in this study. It will explain ways in which information was gathered, organized, and analyzed to explore the relationships among treatments. The chapter will go ahead to discuss the methodology applied their underlying rationale, and how factors such as reliability and validity were achieved. Finally, the chapter concludes by highlighting the practical and contextual limitations encountered during the research process.

3.2 Study Site

The research was carried out at the Masinde Muliro University of Science and Technology (MMUST) farm.The farm is situated in Lurambi Sub-County, Kakamega County, in western Kenya. This site lies at approximately 0°17′ N and 34°45′ E, with an average elevation of about 1,520 meters above sea level. The location was thought to be most suitable for this experiment because of the presence of established mushroom production units and mature banana stands. In

addition, the university environment has security, technical support, and controlled access which helped to ensure the study was implemented smoothly.

Kakamega County was chosen because of its favourable climatic conditions that favours mushroom cultivation. The region is known for its humid tropical rainforest climate characterized by two distinct rainy seasons. The long rains normally occur around April, with an average monthly rainfall of about 240 mm, while a shorter rainy period peaks in August with approximately 180 mm. The driest months extend from December to February, when rainfall declines to between 60 and 80 mm. Average daily temperatures range from 18 °C to 28 °C, and relative humidity generally remains high, which mostly fluctuate between 70 % and 90 % throughout the year. Figure 2 illustrates the location of Kakamega County and the specific site of the study.



Plate 1: Map of Kakamega County indicating the precise study location

3.3 Materials

The experiment utilized a variety of materials, tools, and equipment necessary to create suitable conditions for oyster mushroom cultivation and to facilitate accurate comparison between the two genotypes.

A large metal drum was used to sterilize the substrate through boiling or steam treatment, a process that eliminates competing microorganisms and ensures a clean growth medium. Sugarcane bagasse served as the main substrate, supplying cellulose and lignin that support mushroom nutrition. To enhance nutrient content, chick mash was incorporated as a supplement, enriching the substrate with proteins and minerals that stimulate mycelial development and fruiting. Molasses was incorporated to provide easily accessible sugars that encouraged rapid colonization by the mushroom mycelium. Lime on the other hand, was applied to adjust the substrate pH to a slightly alkaline range (approximately 6.5–7.0). This is normally done to help reduce contamination risk and promote optimal growth conditions.

Adequate water was essential to help maintain proper substrate moisture (around 65–70%) and to sustain humidity during incubation and fruiting stages. Oyster mushroom spawns of *Pleurotus ostreatus* and *P. australis* acted as the inoculum, introducing vigorous, pure mycelium of the two genotypes under investigation. Sorghum grains functioned as the carrier material for the spawn, providing both nutrition and a porous texture that supported rapid colonization.

The inoculated substrate was packed into polypropylene bags. The special bags are made to offer a sterile and durable environment with a potential to retain moisture while permitting gas exchange. The tops of the bags were fastened with rubber bands to prevent contamination and maintain internal humidity. Wooden racks were used to elevate the bags, improving air circulation, drainage, and overall management efficiency. Finally, a mud-walled mushroom unit served as the indoor cultivation chamber, providing natural insulation and stable humidity levels necessary for the controlled-environment treatment.

Nets installed on doors and windows of the mushroom unit were used to exclude flies and other pests, protecting the developing fruit bodies.

Cartons were purchased to be used during harvesting, sorting, and transporting fresh mushrooms while preventing bruising.

Mature banana plants in the outdoor plots, these provided natural shade and a humid microclimate, reducing direct sunlight and temperature fluctuations to mimic indoor conditions.

Together, these materials ensured that the study could reliably compare the performance of the two oyster mushroom genotypes in both indoor and banana-shaded outdoor systems across dry and wet seasons.

3.4 Cultivation Techniques

3.4.1 Mushroom Spawn

The Pure culture of Oyster mushroom strains *P. australis* and *P. ostreatus* were obtained from nature niche spawn sellers company based in Kileleshwa Nairobi Kenya. The cultures were sub-cultured and maintained on Potato Dextrose Agar medium in Bio Oxygen Demand incubator at 23 + 2ºC temperature in Masinde Muliro University laboratory. Cleaned and healthy sorghum grains were washed in clean water and boiled for 30 minutes until soft at a ratio of 1:2 water to sorghum grains. Excess water was removed on sieve after boiling and the grains were cooled in plastic tray in shade for 4 hours up to 60% moisture. Cooled grains were supplemented with calcium carbonate and calcium sulphate powder at a ratio of 10g/kg of sorghum grains to avoid clumping. The supplemented boiled wheat grains were filled (250g/ bottle) in clean 300ml saline bottle.

Small cubes of actively growing mycelium were inoculated using laminar flow into the substrate and the bottles were plugged with non-absorbent cotton plug. The

sorghum grain filled bottles were sterilized in autoclave at 15 lb. pressure (121˚C) for 45 minutes and then allowed to cool at room temperature for some time. The bottles were incubated in BOD at 23+2°C for 21 days. The bottles were shaken at 4 days' interval for proper spread of the mycelium between the grains. Plate 2 shows some of images taken in the lab during subculture process.



Plate 2: Sub-culturing of mother spawn

3.4.2 Substrate Preparation

Sugarcane bagasse which is known for its significant amount of hemicellulose, cellulose and lignin was obtained from Kabras Sugar Company. It was mixed with Chicken mash in a ratio of 90kg of bag:1kg of chick mash. The chicken feed contains various carbon rich which help in raising the temperature in the compost, activating the composting process and providing nutrients for the necessary microorganisms. Molasses was used as an additive whereby 2 litres of it were mixed in 40 litres of water. One percent of substrate lime was added to the mixture of water and molasses to bring about alkalinity nature in the substrate. The substrate was steamed in a drum of boiling water for 2 hours.

This process helped to reduce the risk of substrate contamination. The properly sterilized medium was left to cool overnight before it was inoculated with the mushroom spawns. Plate 3 displays substrate and sterilization activities carried out in the field.



Plate 3: Substrate preparation and sterilization

3.4.3 Spawning

Twelve tubes were filled with 4 kg of the substrate each. Substrate was filled inside the polypropylene cylindrical bags as a layer of few inch thicknesses and pressed tightly with help of palm. One kilogram of ready-made standard fungal spawn was sprinkled randomly over the layer followed by another layer of substrates for each tube. All tubes were then closed tightly with rubber bands ready for colonization. Plate 4 shows how spawning process was done.



Plate 4: Spawning

3.4.4 Cropping (Indoor and Outdoor Methods)

Six spawned bags were randomly suspended inside a home-based mushroom infrastructure made of iron sheets and mud covering the roof and walls respectively. The spawn-run was carried out in dark conditions. No watering was done during spawn running period. After the completion of the spawn run, loss of moisture through evaporation was compensated by moistening the compact twice a day, i.e., morning and in the afternoon. The temperature, humidity, light intensity and carbon iv oxide levels were recorded thrice per day (morning, afternoon and evening). Air in and out of the room was controlled by opening and closing the windows as needed.

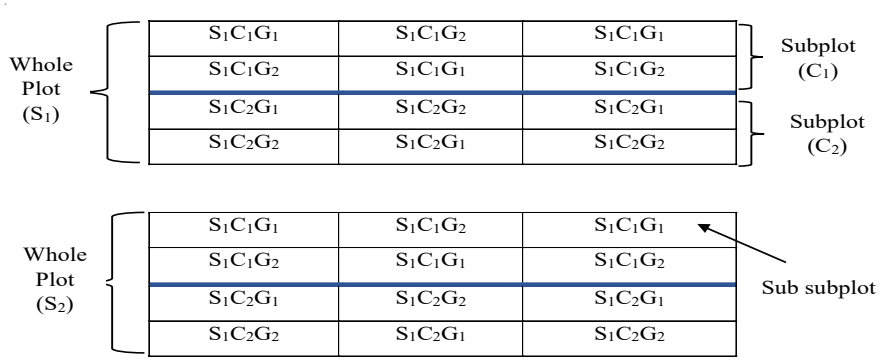
Three families of bananas were selected for the outdoor integration cultivation. A family of bananas chosen had six plants each. Two cylindrical bags of oyster spawned substrates were randomly suspended on wooden frames under each banana stand. The banana leave canopies were utilized to provide shaded environment for mushrooms. Plastic covers made to umbrella shaped and screen nets were wrapped around the bags to protect the substrate from heavy downpour and pests respectively. Plate 5 displays how indoor and outdoor cultivation activities were conducted.



Plate 5: Indoor and Outdoor cropping

3.5 Experimental Design

This experiment followed cross sectional quasi experimental research whereby a comparison of two different groups was done at the same time without control groups. The split-split plot design was applied with the wet and dry seasons representing whole plots, indoor and outdoor conditions representing the sub plot while *P. ostreatus* and *P.australis* genotypes represented the sub sub plot. The layout of the design was as shown in Figure one.



s_1= dry season s_2= wet season c_1= indoor condition c_2= outdoor condition g_1= *p.ostreatus* g_2= *p. australis*

Figure 1: Split-split plot layout design (Montgomery, 2020)

3.6 Data Collection

Open Data Kit (ODK) was used on each visit to collect data and series of geo-referenced pictures on the general field status and the mushroom conditions. Additionally, data sheets, field notes and sketches were used to record data. The following parameters were recorded:

Growth behavior and morphological characteristics: Different crop development stages such colonization, germination, fruiting, maturity and senescence were keenly monitored. Time was recorded in days for the completion of growth of mycelium, appearance of pinheads and maturation of fruiting bodies. Harvesting of mushrooms was done at maturity (when the pileus was fully opened).

The harvested mushrooms were thoroughly cleaned and data obtained for the following parameters; length of the stalk, cap diameter and number of fruit bodies per bag. Height of the stalk (from the base to the stipe of the pileus) and diameter of the cap were measured in centimeters using meter scale. Diameter of the pileus characterizes plant canopy structure and gives an idea of the amount of biomass available in a field. The number of fruiting bodies which validates the yield performance of the mushroom were counted for each treatment.

Total Yield: The fruiting bodies were weighed in grams(g) immediately after every harvest using the digital luggage scale. The average weight of the three flushes was calculated to arrive at the final mean weight of the oyster genotype as shown below;

$$\text{Yield} = \frac{1\text{ST flush} + 2\text{ND flush} + 3\text{RD flush}}{3}$$

Biological Efficiency: After fruiting phase was completed, the substrate was sun dried and its dry weight recorded. These records were used to calculate the biological efficiency using the following (Bandura et al.,2020)

$$BE = \frac{Fresh\ Weight\ of\ mushroom}{Dry\ weight\ of\ substrate} \times 100\%$$

Environmental Factors: Maximum and minimum levels of four environmental elements were measured and recorded. Humidity was measured using a hygrometer while a thermometer was used to measure the temperature. A carbon (iv) oxide sensor was used to measure carbon iv oxide and a light sensor (LUX meter) was used to record light levels.

3.7 Data Analysis

Univariate Analysis of variance followed by least square means comparisons were used to identify significant differences across treatment combinations using SAS 9.4 version. The model of this design as illustrated by Omotehinse et al., (2018) was given by;

Y= μ + A+B+(AB)+C+(AC)+(BC)+(ABC)+E(A) +E(B)+E(C) where (:

Y: The observed response variable

μ: The overall mean

A: The effect of the *whole plot* factor

B: The effect of the *sub plot* factor

C: The effect of the *sub sub plot* factor

(AB), (AC), (BC), (ABC): Interaction effects between the factors

E(A): Error term associated with the whole plot factor (whole plot error)

E(B): Error term associated with the sub plot factor (subplot error)

E(C): Error term associated with the sub subplot error (sub subplot error)

3.8: Presentation of analyzed data

Analyzed data were summarized using tables and charts to help in conveying patterns and insights from datasets.

3.9 Study Limitations and Justifications

Limited Replication:

This study adopted a quasi-experimental split–split plot design with a modest number of replicates, which may have reduced the statistical power to detect minor differences between treatments.

Justification: Despite the limited replication, the experiment incorporated numerous variables such as growth behavior, morphological traits, contamination levels, biological efficiency, yield, and microclimate factors across different seasons, production conditions, and genotypes. Expanding replication further would have required substantially more resources and labor. Therefore, the chosen level represented a practical balance between feasibility and the generation of meaningful data.

Single Study Site: This experiment was implemented exclusively at the Masinde Muliro University of Science and Technology (MMUST) farm.

Justification: Doing the trial at one controlled and secure location helped to minimize external variability. It also helped to ensure a uniform management across the indoor and banana-integrated systems. It also allowed close supervision of all experimental units throughout the study period.

Seasonal Coverage: The research spanned a single annual cycle encompassing both wet and dry seasons.

Justification: Although a multi-year investigation would have provided stronger

long-term insights, time and funding constraints limited the study duration. Nevertheless, inclusion of both major seasonal phases adequately captured the key climatic variations that influence mushroom growth, morphology, contamination levels, biological efficiency, yield, and microclimate interactions.

Microclimate Measurements: Environmental parameters temperature, relative humidity, light intensity, and carbon dioxide levels were measured three times daily rather than continuously.

Justification: While continuous monitoring would have provided more detailed records, factors such as equipment and logistical constraints restricted data collection to periodic intervals. The multiple daily readings, however, were sufficient to characterize representative microclimate conditions enabling subsequent analysis.

Limited Genotype Diversity: Only two *Pleurotus* genotypes were evaluated in this study.

Justification: This focused approach enabled a manageable and meaningful comparison of growth, yield, and environmental responses between genotypes. Given the number of variables and treatment combinations already involved, including additional genotypes would have made the experiment overly complex and resource-demanding.

Substrate Variability: Although standardized sugarcane bagasse and supplements were used, minor batch-to-batch differences in substrate composition were unavoidable.

Justification: Consistent procedures in substrate preparation, sterilization, and supplementation were followed to minimize such variation, ensuring reliable comparisons of growth and yield performance.

Manual Morphological Measurements: Traits such as stalk length, cap diameter, and fruit body number were measured manually.

Justification: Automated measurement systems could have reduced potential human error, but the use of trained personnel and repeated readings helped maintain accuracy and consistency in data collection.

CHAPTER FOUR
RESULTS

4.1 Introduction

This chapter presents the findings of the study derived from the experimental data. It describes how the investigated treatments influenced growth performance, morphological characteristics, contamination levels, and biological efficiency of the oyster mushrooms. The results are displayed in form of tables and figures which illustrate key patterns, trends, and statistically significant variations among the measured variables.

4.2 ANOVA Results for Main and Interaction Effects of Growth, Morphologically and BE

Summary of ANOVA results for measured traits which including the mycelium size, incubation time, fruiting phase, number of fruits, stalk length, cap diameter, number of deformed fruits, contamination level, and biological efficiency were as shown in table 1. The table contain p-values for the main factors (season, condition, genotype) and their interactions (season × condition and season × condition × genotype).

Table 1: ANOVA Interaction effects (-values and standard errors)

Variables	Season	Condition	Genotype	Season* Condition	Season*Condition*genotype
Mycelium size(cm)	<.00 ±1.4*	0.00 ± 1.4*	<.00 ± 1.4*	0.00 ± 1.4*	0.02 ± 1.4*
Incubation time(days)	<.00 ± 1.2*	0.00 ± 1.2*	0.37± 1.2	<.00 ± 1.2*	0.04 ± 1.2*
Fruiting phase(days)	<.00 ± 2.4*	0.00 ± 2.4*	0.12 ± 2.4	0.01 ± 2.4*	0.05± 2.4*
Number of fruits	0.02 ± 1.6*	0.01 ± 1.6*	0.61 ± 1.6	0.44 ± 1.6	0.02 ± 1.6*
Stalk length(cm)	0.45 ± 0.14	0.00 ± 0.14*	0.36 ± 0.14	0.04 ± 0.14*	0.01 ± 0.14*
Cap diameter(cm)	0.00 ± 0.42*	0.00 ± 0.42*	0.38 ± 0.42	0.02±0.42*	0.03 ± 0.42*
Number of deformed fruits	0.03 ± 0.83*	<.00 ± 0.83*	0.31 ± 0.83	0.59 ± 83	0.01 ± 0.83*
Contamination Level (%)	<.00 ± 1.43*	0.00 ± 1.43*	0.08 ± 1.43	0.001 ±.43*	0.04 ± 1.43*
Biological Efficiency(%)	<.00 ± 13.7*	<.00 ±13.7 *	0.02 ±13.7*	<.00 ±13.7*	0.01 ± 13.7*

Mycelium size: Results on analysis of variance (ANOVA) showed that season, growth condition, and genotype allowed a highly significant influence on mycelial size ($p < 0.001$). The interaction between season and growth condition was also statistically significant ($p < 0.001$), indicating that the effect of growing conditions on mycelial development varied across seasons. These findings show that the extent of mycelial growth varied markedly with wet versus dry season, indoor versus outdoor cultivation, and between the two *Pleurotus* genotypes, and that the advantage of particular growth conditions differed depending on the season. In

contrast, the season × genotype interaction was not significant ($p = 0.15$), indicating that the relative performance of the two genotypes was consistent across seasons. Similarly, the condition × genotype interaction ($p = 0.00$ after rounding to <0.001) was significant, showing that the response of each genotype differed by growth condition.The three-way season × condition × genotype interaction was also significant ($p = 0.015$). The significance for the three-way term means that combining all three factors produced additional effects beyond the significant main factors and the season–condition interaction already detected.

Incubation Time: Analysis of variance showed that season, growth condition, and the season × condition interaction all had a highly significant influence on incubation time ($p < 0.001$). These results indicate that the number of days required for full substrate colonization varied markedly between the wet and dry seasons, between indoor and outdoor cultivation, and that the effect of growth condition depended on the season. In contrast, genotype did not have a significant main effect ($p = 0.37$), meaning the two *Pleurotus* species required similar incubation periods overall. The three-way season × condition × genotype interaction was also significant ($p = 0.04$), showing that combining all three factors produced additional variation beyond the strong main influences of season and condition

Fruiting Phase: Analysis of variance showed that the season ($p < 0.001$) and growth condition ($p = 0.001$) had a highly significant influence on the length of the fruiting phase. This indicates that the number of days required for mushrooms to initiate and complete fruiting differed markedly between wet and dry seasons and between indoor and outdoor cultivation. Variation due to genotype was not significant ($p = 0.12$). However, both the two-way season × condition interaction ($p = 0.01$) and the three-way season × condition × genotype interaction ($p = 0.05$)

was significant, indicating combined effects of these factors on the fruiting duration.

Number of Fruits: Analysis of variance (ANOVA) revealed that both season ($p = 0.02$) and growth condition ($p = 0.01$) had statistically significant effects on the number of fruiting bodies produced. This finding indicates that fruit production varied between the wet and dry seasons as well as between indoor and outdoor cultivation systems. Genotype, however, showed no significant effect ($p = 0.61$), suggesting that the two *Pleurotus* species exhibited comparable fruiting capacities under the tested conditions. The interaction between season and condition was not significant ($p = 0.44$), whereas the three-way interaction among season, condition, and genotype was significant ($p = 0.02$). This result implies that the combined influence of these factors affected fruiting performance beyond their individual effects.

Stalk Length: The length of mushroom stalks varied significantly with growth condition, as shown by the analysis of variance ($p < 0.001$). Stalks grown indoors differed markedly from those cultivated outdoors, highlighting the strong influence of environmental settings on mushroom morphology. The interaction between season and condition was also significant ($p = 0.04$), suggesting that the impact of growth environment was not uniform across the wet and dry periods. In contrast, the effects of season ($p = 0.45$) and genotype ($p = 0.36$) alone were not statistically significant. Notably, a significant three-way interaction among season, condition, and genotype ($p = 0.01$) revealed that stalk elongation depended on the combined interplay of these factors rather than on any single factor in isolation

Cap Diameter: The ANOVA results indicated that cap diameter was significantly influenced by both season ($p < 0.001$) and growth condition ($p < 0.001$), with a

notable interaction between season and condition ($p = 0.02$). This suggests that cap size varied considerably across seasons and cultivation environments, and that the effect of growth condition depended on the prevailing seasonal conditions. Although genotype alone did not significantly affect cap diameter ($p = 0.38$), a significant three-way interaction among season, condition, and genotype ($p = 0.03$) revealed that the combined influence of these factors contributed further to the observed variation in cap size

Contamination Level: Analysis of variance indicated that contamination rates were significantly influenced by season ($p < 0.001$), growth condition ($p = 0.001$), and their interaction ($p < 0.001$). This means that contamination varied notably between wet and dry periods and between indoor and outdoor growing environments, with the effect of each condition shifting depending on the season. Although the genotype effect was not statistically significant ($p = 0.08$), a significant three-way interaction among season, condition, and genotype ($p = 0.04$) showed that these factors together contributed additional variability in contamination levels beyond what was explained by individual factors alone.

Biological Efficiency: The analysis of variance revealed that biological efficiency was significantly affected by season, growth condition, and genotype (all $p < 0.05$). A strong interaction was also observed between season and growth condition ($p < 0.001$), suggesting that the effect of indoor versus outdoor cultivation varied across wet and dry periods. Moreover, the three-way interaction among season, condition, and genotype ($p = 0.01$) was significant, indicating that the combined influence of these factors further contributed to variations in biological efficiency. Overall, the findings suggest that yield performance was shaped by a complex interaction

between environmental conditions and the genetic makeup of the mushroom strains.

4.3 LSM Comparison Effects on Growth, Morphological, CL and BE

A summary of least-square mean comparisons for three primary growth parameters; mycelium size, incubation period, and fruiting duration under the combined influence of season, cultivation condition, and genotype are summarized in table 4. The table presents adjusted mean values (± standard error) for each interaction level, accompanied by letter groupings that denote statistical differences among treatments. Means sharing the same letter do not differ significantly at the 5% level, whereas those with different letters indicate significant variation.

Table 2: LSM comparisons (± standard error)

Season× Condition × Genotype	Mycelium Size (cm)	Incubation Time (days)	Fructification Phase (days)
Wet×Indoor×*P.ostreatus*	4.0 ± 1.4^{b}	44.7 ± 1.2^{b}	94.6 ± 2.4^{ce}
Wet×Indoor×*P.australis*	8.3 ± 1.4^{b}	46.6 ± 1.2^{b}	97.5 ± 2.4^{bcd}
Wet×Outdoor×*P.ostreatu*	32.3 ± 1.4^{a}	22.2 ± 1.2^{c}	71.3 ± 2.4^{ef}
Wet×Outdoor×*P.australis*	28.0 ± 1.4^{a}	24.3 ± 1.2^{c}	68.5 ± 2.4^{de}
Dry×Indoor×*P. ostreatus*	4.2 ± 1.4^{b}	112.8 ± 1.2^{a}	126.4 ± 2.4^{a}
Dry×Indoor×*P. australis*	0.1 ± 1.4^{b}	110.9 ± 1.2^{a}	123.6 ± 2.4^{ab}
Dry×Outdoor×*P.ostreatus*	2.4 ± 1.4^{b}	112.4 ± 1.2^{a}	114.8 ± 2.4^{abc}
Dry×Outdoor×*P.australis*	6.7 ± 1.4^{b}	114.3 ± 1.2^{a}	117.6 ± 2.4^{abc}

Mycelium size: A clear and significant three-way interaction of season, condition, and genotype was seen from the least square means in the table. The wet-season × outdoor × genotype interaction produced the greatest mycelial diameters, with the *P. ostreatus* genotype reaching about 32.3 cm and *P. australis* about 28.0 cm, both belonging to the highest significance group ("a"). All other season × condition × genotype interactions yielded markedly smaller mycelial growth (generally 0–8 cm) and were grouped in the lower letter category ("b"), demonstrating that they were statistically different from the wet-outdoor interaction. This pattern indicates that optimal mycelial expansion occurs only under the specific interaction of wet season and outdoor cultivation, regardless of genotype, while any other interaction of these factors significantly reduces mycelium size. Figure 2 shows how mycelium size changed under the combined influence of season, growth condition, and genotype.

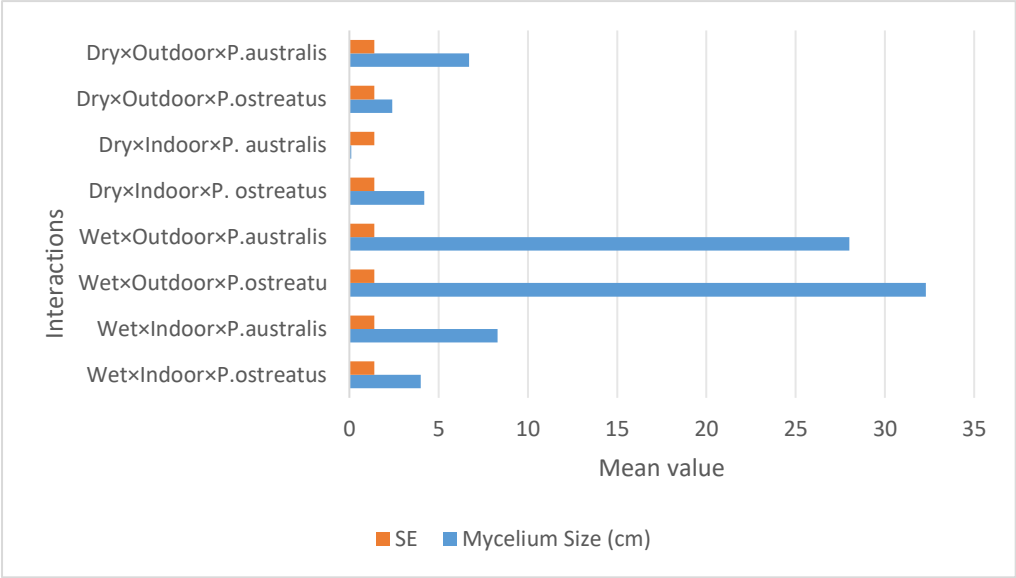


Figure 2: Mycelium size as influenced by the interaction of season, growth condition, and genotype.

Incubation Time (days): The least-square means for incubation time revealed a clear and statistically significant interaction among season, growth condition, and genotype. The combination of dry season, indoor environment, and genotype resulted in the longest incubation periods, exceeding 110 days for both strains and ranking in the highest significance group ("a"). Conversely, the wet season combined with outdoor cultivation produced the shortest incubation times, averaging about 22–24 days, and belonged to the lowest significance group ("c"). Intermediate incubation durations were recorded under the wet-season × indoor and dry-season × outdoor conditions, which fell within the mid-level significance group ("b"). These findings suggest that outdoor cultivation during the wet season markedly shortened substrate colonization, whereas indoor conditions in the dry season substantially delayed the process for both genotypes. The bar graph in figure 3 illustrate the incubation length across treatment interactions.

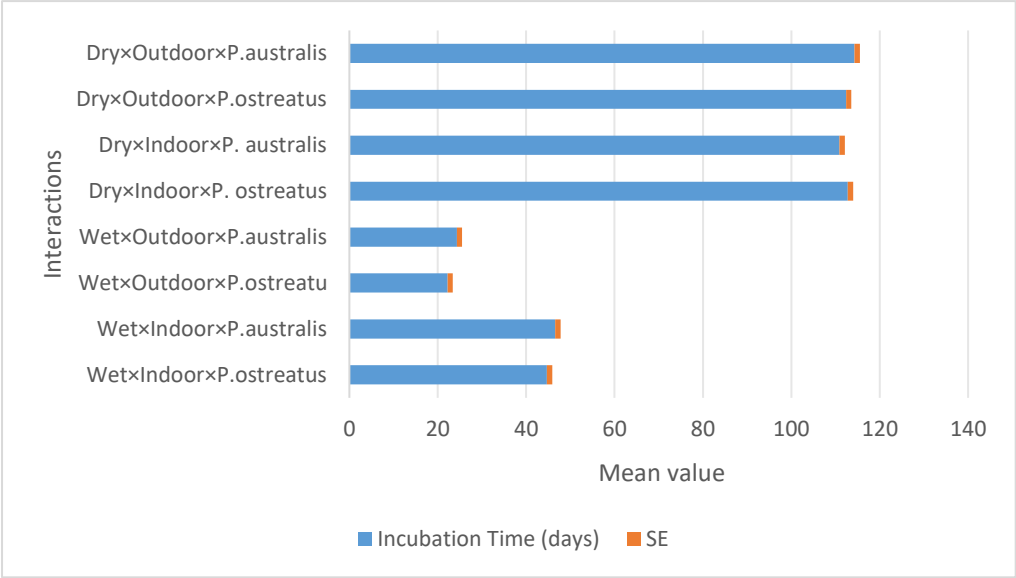


Figure 3: Incubation length across treatment interactions

Fruiting Phase: Analysis of the least-square means for the fruiting phase showed a significant interaction among season, growth condition, and genotype. The combination of dry season and indoor cultivation produced the longest fruiting

durations, with both genotypes requiring about 123–126 days to complete the cycle. Slightly shorter fruiting periods, averaging 115–118 days, were recorded under dry-season outdoor conditions. In contrast, mushrooms grown outdoors during the wet season exhibited the shortest fruiting times, generally between 68 and 71 days. Wet-season indoor treatments produced intermediate results, averaging 95–98 days. Overall, these patterns reveal that outdoor cultivation during the wet season promotes faster fruiting, whereas indoor production in the dry season extends the fruiting duration for both Pleurotus genotypes. Figure 4 illustrates the variation in fruiting phase across the different treatment combinations.

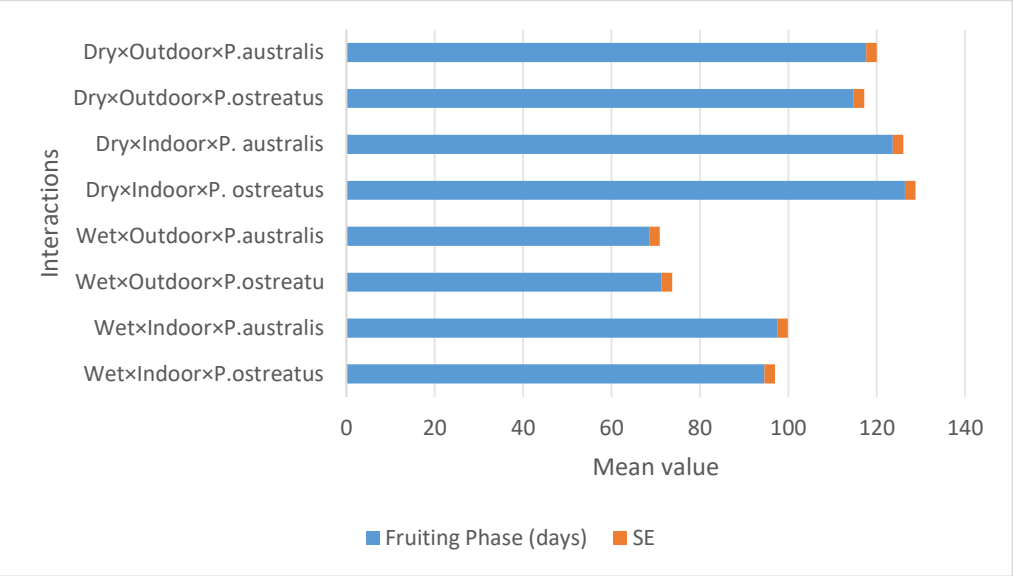


Figure 4: Fruiting phases across treatment interactions.

4.4 LSM Comparison Effects on Growth, Morphological, Contamination Levels and Biological Efficiency of *Pleurotus* Genotypes

Table 3 displays the least square means comparisons of all traits evaluated including stalk length, cap diameter, number of fruits, number of deformities, contamination levels, and biological efficiency. The table highlights the interaction

effects of season, growing condition (indoor and outdoor), and genotype (*P. ostreatus* and *P. australis*) on these parameters. Values are presented with standard errors, and superscript letters indicate significant differences among treatments.

Table 3: LSM comparisons (± standard error)

Season × Condition × Genotype	Stalk Length (cm)	Cap Diameter (cm)	Number of Fruits	Number of Deformities	Contamination Levels (%)	Biological Efficiency (%)
Wet × Indoor × *P.australis*	2.2 ± 0.2^{c}	7.2 ± 0.4^{b}	28.0±1.6^{ab}	2.3 ± 0.8^{b}	2.3 ±1.4^{b}	267.4±13.6^{b}
Wet × Indoor × *P. ostreatus*	2.5 ± 0.2^{bc}	6.1 ± 0.4^{b}	30.0±1.6^{ab}	0.4 ± 0.8^{b}	0.4 ±1.4^{b}	223.8±13.6^{ba}
Wet × Outdoor × *P. ostreatus*	2.5 ±0.2^{bc}	13.1 ± 0.4^{a}	38.2 ±1.6^{a}	14.0 ±0.8^{a}	0.3 ±1.4^{b}	693.8 ±13.6^{a}
Wet × Outdoor × *P. australis*	2.8 ±0.2^{abc}	12.8 ±0.4^{a}	31.3 ± 1.6^{ab}	12.3 ± 0.8^{a}	1.3 ±1.4^{b}	670.8 ±13.6^{a}
Dry × Indoor × *P. ostreatus*	1.8 ± 0.2^{c}	5.0 ± 0.4^{b}	16.8±1.6^{bc}	1.3 ± 0.8^{b}	14.0±1.4^{a}	133.3 ±13.6^{cd}
Dry × Indoor × *P. australis*	2.1 ± 0.2^{c}	4.7 ± 0.4^{b}	10.2 ±1.6^{c}	0.3 ± 0.8^{b}	12.3±1.4^{a}	110.4 ±13.6^{d}
Dry × Outdoor × *P. ostreatus*	3.3 ± 0.2^{ab}	7.44 ± 0.4^{b}	29.3±1.6^{ab}	11.4 ± 0.8^{a}	11.4±1.4^{b}	200.7±13.6^{bcd}
Dry × Outdoor × *P. australis*	3.6 ± 0.2^{a}	6.2 ± 0.4^{b}	31.5±1.6^{ab}	9.6 ± 0.8^{a}	9.6 ±1.4^{a}	157.1±13.6^{bcd}

Stalk Length: The least-square means for stalk length showed a clear and significant season × condition × genotype interaction. The dry-season × outdoor × genotype interactions produced the longest stalks, with values around 3.3–3.6 cm, and these were placed in the highest significance group ("a"). Wet-season × outdoor interactions were slightly shorter (about 2.5–2.8 cm) and fell into intermediate significance groups ("a–b–c"). Wet-season × indoor interactions averaged about 2.2–2.5 cm and were grouped lower ("b–c"), while the shortest stalks occurred under the dry-season × indoor × genotype interactions, measuring only 1.8–2.1 cm and occupying the lowest significance group ("c"). These results demonstrate that stalk elongation was greatest when the interaction of dry season and outdoor cultivation occurred, whereas the interaction of dry season and indoor conditions significantly limited stalk growth across genotypes. Figure 5 presents a bar graph illustrating the stalk length across treatment interactions.

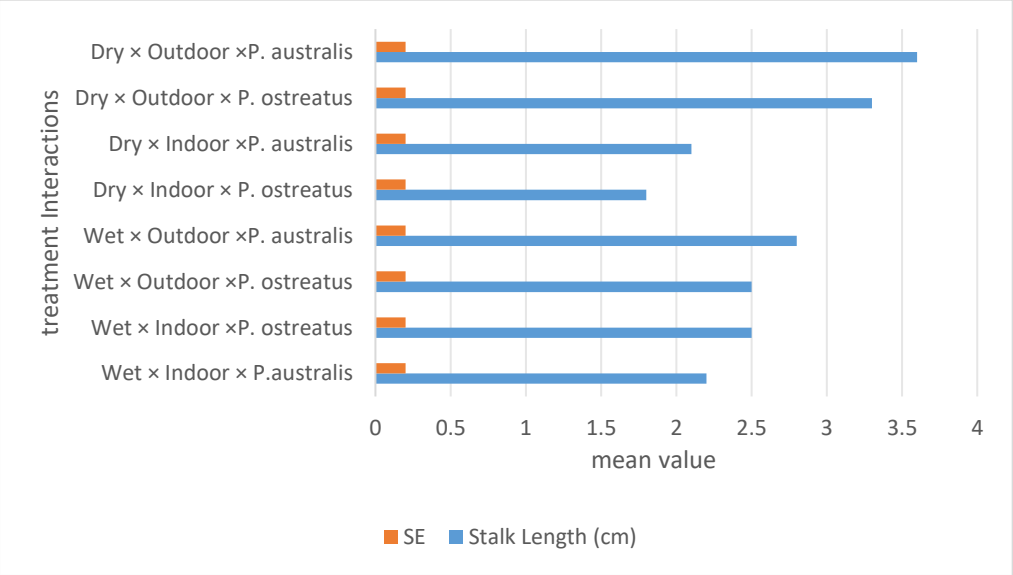


Figure 6: Cap diameter across seasons, conditions and genotypes interactions.

Number of fruits: The least-square means for the number of fruits exhibited a significant season × condition × genotype interaction. The wet-season × outdoor ×

genotype interactions produced the highest fruit counts, with *P. ostreatus* averaging about 38 fruits and *P. australis* around 31 fruits, and these values were grouped in the top significance category ("a"). Wet-season × indoor interactions yielded intermediate numbers (roughly 28–30 fruits, "a–b"), while the dry-season × outdoor × genotype interactions also showed moderate fruiting (around 29–32 fruits, "a–b"). The dry-season × indoor × genotype interactions had the lowest fruit numbers, typically 10–17 fruits, and fell into the lowest significance group ("c"). These results indicate that fruit production was maximized when the interaction of wet season and outdoor cultivation occurred, whereas the interaction of dry season and indoor conditions significantly reduced the number of fruits regardless of genotype. Figure 7 presents a bar graph illustrating the number of fruits across treatment interactions.

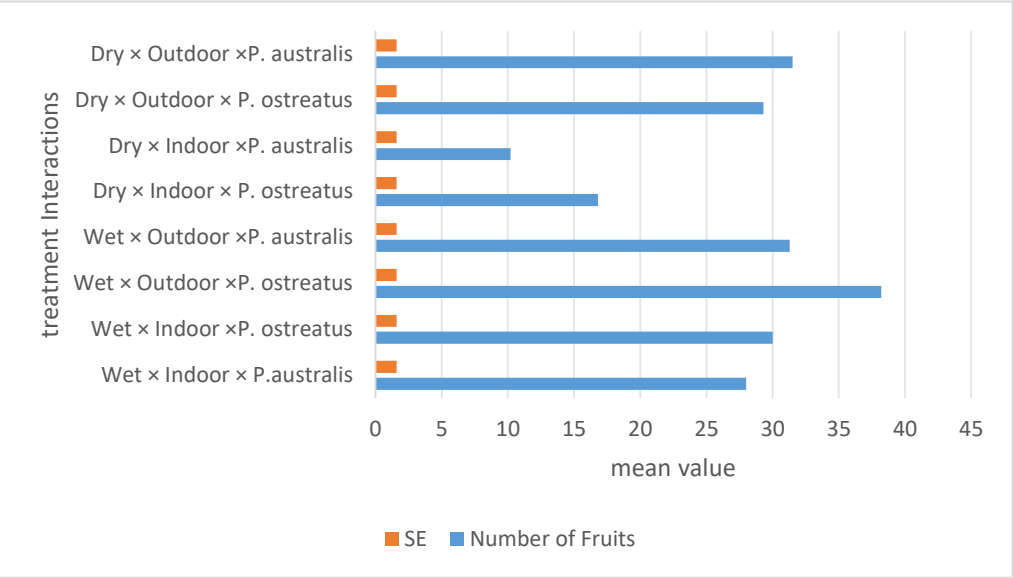


Figure 7: Number of fruits across treatment interactions

Number of deformed fruits: The least-square means for the number of deformed fruits showed a clear and significant season × condition × genotype interaction. The dry-season × outdoor × genotype interactions recorded the highest deformity counts, averaging about 9–14 deformed fruits, and these values belonged to the top

significance group (“a”). The wet-season × outdoor interactions also produced relatively high deformity numbers (around 12–14 fruits) and were placed in the same upper group. In contrast, both the wet-season × indoor and dry-season × indoor × genotype interactions had very low deformity levels (generally 0–2 fruits), occupying the lowest significance group (“b”). These results indicate that deformities were most pronounced when the interaction involved outdoor cultivation especially during the dry season whereas the interaction of indoor growth with either season greatly limited the number of deformed fruits across genotypes. Figure 8 presents a bar graph illustrating the number of deformed fruits across treatment interactions.

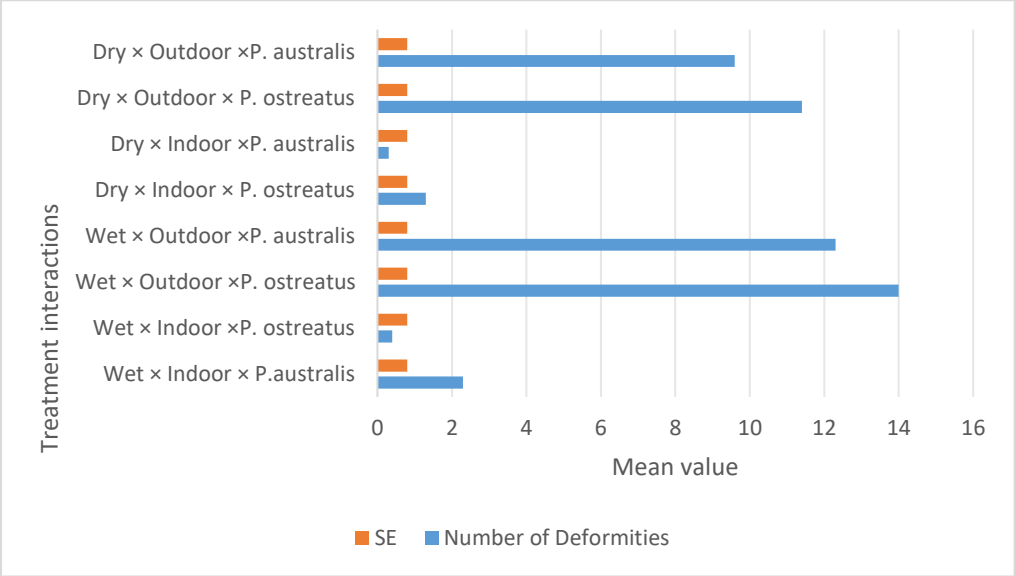


Figure 8: Number of deformed fruits across treatment

Contamination Level: The least-square means for contamination level demonstrated a pronounced and significant season × condition × genotype interaction. The dry-season × indoor × genotype interactions produced the highest contamination percentages, averaging about 11–14 %, and these values occupied the upper significance group (“a”). The dry-season × outdoor interactions showed moderately high contamination (around 9–11 %) and were grouped just below the

top category (“a–b”). In contrast, the wet-season × indoor and wet-season × outdoor × genotype interactions recorded the lowest contamination levels and were placed in the lowest significance group (“b”). This pattern indicates that contamination was most severe when the interaction involved the dry season and indoor cultivation, whereas the interaction of wet season with either indoor or outdoor conditions greatly reduced contamination across genotypes. Figure 9 presents a bar graph illustrating the level of contamination across seasons, conditions and genotype interactions

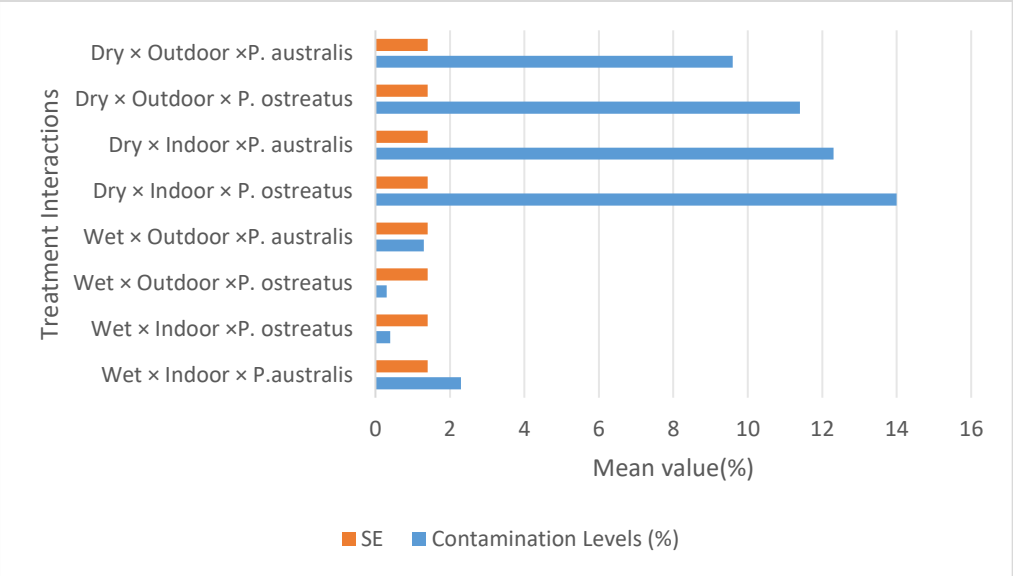


Figure 9: Level of contamination treatment interactions

Biological Efficiency: The least-square means for biological efficiency displayed a strong and significant season × condition × genotype interaction. The wet-season × outdoor × genotype interactions achieved the highest efficiencies, averaging about 670–694 %, and these values formed the top significance group (“a”). Wet-season × indoor interactions produced moderate efficiencies (roughly 224–267 %) and were placed in an intermediate group (“b”). The dry-season × outdoor interactions yielded lower efficiencies (around 157–201 %) and occupied a mid-to-low group (“b–c”), while the dry-season × indoor × genotype interactions

recorded the lowest efficiencies, generally 110–133 %, and were grouped in the lowest significance category ("c–d"). These results show that biological efficiency was maximized when the interaction of wet season and outdoor cultivation occurred, whereas the interaction of dry season and indoor conditions sharply reduced efficiency for both genotypes.Figure 10 presents a bar graph illustrating biological efficiency across seasons, conditions and genotypes interactions.

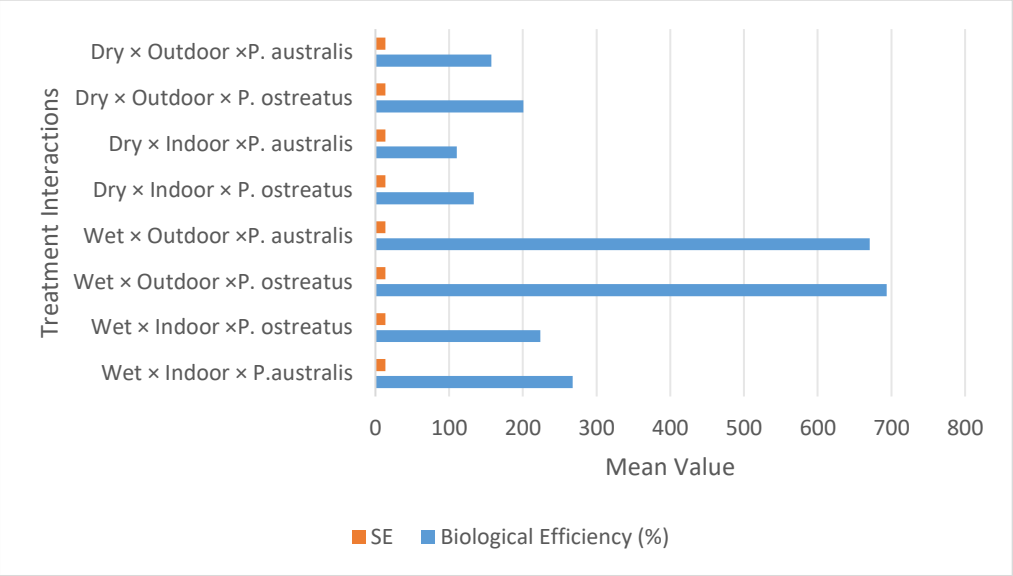


Figure 10: LSM of Biological efficiency across treatment Interactions

4.5 ANOVA Results for Main and Interaction Effects of Mushroom Yield

Table 4 summarizes the analysis of variance (ANOVA) results examining how season, growth condition, and genotype influenced mushroom yield. The table includes the degrees of freedom, sum of squares, F-statistics, and corresponding p-values for each source of variation, covering both main and interaction effects. Treatments showing p-values below 0.05 were considered statistically significant, indicating meaningful differences among the evaluated factors.

Table 4: ANOVA Table

Source	F Ratio	*P* value
Replication	0.1169	0.8905
Season	20.9858	0.0003*
Conditions	11.0514	0.0043*
Genotype	44.9396	<.0001*
Season×Genoype	23.317	0.001*
Condition×Genotype	10.285	0.009*
Season×Conditions	15.9452	0.0018*
Season×Conditions×Genotype	9.1790	0.0053*

Analysis of variance revealed that mushroom yield was significantly influenced by the main experimental factors. Season had a strong effect ($p = 0.0003$), showing clear yield differences between the wet and dry periods. Growth condition was also significant ($p = 0.0043$), indicating that production varied between indoor and outdoor systems. The most pronounced variation, however, was associated with genotype ($p < 0.0001$), demonstrating that the two *Pleurotus* species exhibited distinct yield potentials under the tested conditions. Additionally, all the interaction terms were significant at $p<0.05$. These significant results indicate that the effect of season on yield depend on whether mushrooms were grown indoors or outdoors, and that the interaction influence of season, condition, and genotype produced additional yield differences beyond the main effects.

4.6 LSM Comparison Effects on Mushroom Yield

Table 5 presents the least square means comparisons of mushroom yield for the interaction effects of season, growing conditions, and genotype. The table shows

how the interaction of factors such as wet or dry season, indoor or outdoor conditions, and the two *Pleurotus* genotypes affects yield. Values are presented with standard errors, and superscript letters indicate significant differences among treatments. This presentation of interaction effects allows for a clear comparison of how specific interactions between environmental factors and genotype influence mushroom productivity, highlighting the conditions that optimize yield

Table 5: LSM comparisons (± standard error)

Season × Condition × Genotype	Yields
Dry ×Indoor× *P.ostreatus*	276.50 ± 82.85^{bcde}
Dry× Indoor × *P.australis*	480.98 ± 82.85^{bef}
Dry × Outdoor× *P.ostreatus*	1291.25 ± 82.85^{bd}
Dry ×Outdoor × *P.australis*	689.22 ± 82.85^{cef}
Wet × Indoor × *P.ostreatus*	1620.12 ± 82.85^{b}
Wet × Indoor ×*P.australis*	970.96 ± 82.85^{bef}
Wet× Outdoor ×*P.ostreatus*	2582.01 ± 82.85^{a}
Wet ×Outdoor × *P.australis*	1411.88 ± 82.85^{abc}

The three-way interaction of season, growth condition, and genotype produced clear and highly significant differences in mushroom yield. The wet-season × outdoor × *P. ostreatus* interaction recorded the highest production, averaging 2582.01g, and belonged exclusively to the highest significance group ("a"), confirming that this interaction maximized yield. The wet-season × indoor × *P. ostreatus* interaction followed with 1620.12g (group "b"), significantly lower than the wet–outdoor interaction but higher than all dry-season treatments.

Moderate yields were observed under the wet-season × outdoor × *P. australis* interaction (1411.88g, group "a b c") and the dry-season × outdoor × *P. ostreatus* interaction (1291.25 g, group "b d"), which were statistically similar to each other and to the wet–indoor *P. ostreatus* interaction but lower than the top-yielding interaction.

Lower but distinct yields occurred for the wet-season × indoor × *P. australis* interaction (970.96g, group "b e f"), the dry-season × outdoor × *P. australis* interaction (689.22 g, group "c e f"), and the dry-season × indoor × *P. australis*

interaction (480.98g, group “b e f”). These interactions were not significantly different from one another but were clearly below the high-performing *P. ostreatus* interactions.

The lowest yield was obtained under the dry-season × indoor × *P. ostreatus* interaction (276.50g, group “b c d e”), which was significantly lower than all wet-season interactions and most outdoor setups. Figure 11 presents the least square means (LSM) of yield across the season × condition × genotype interactions

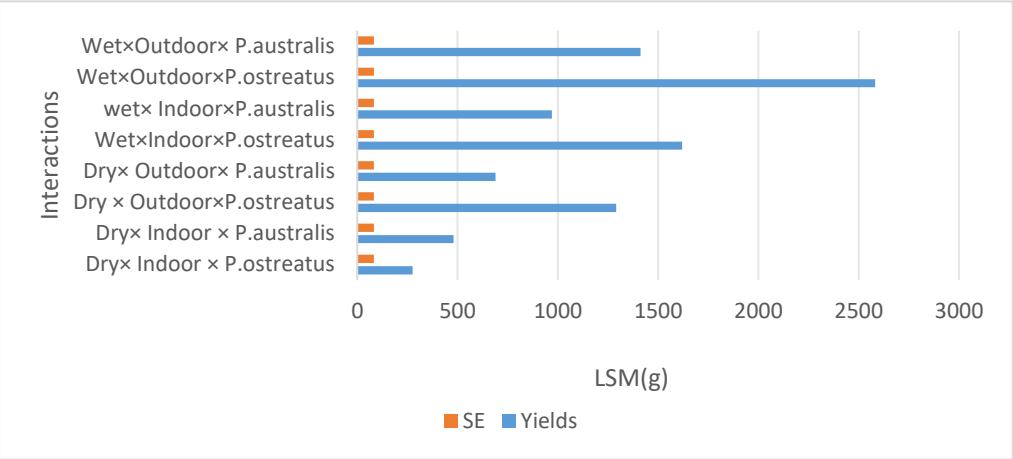
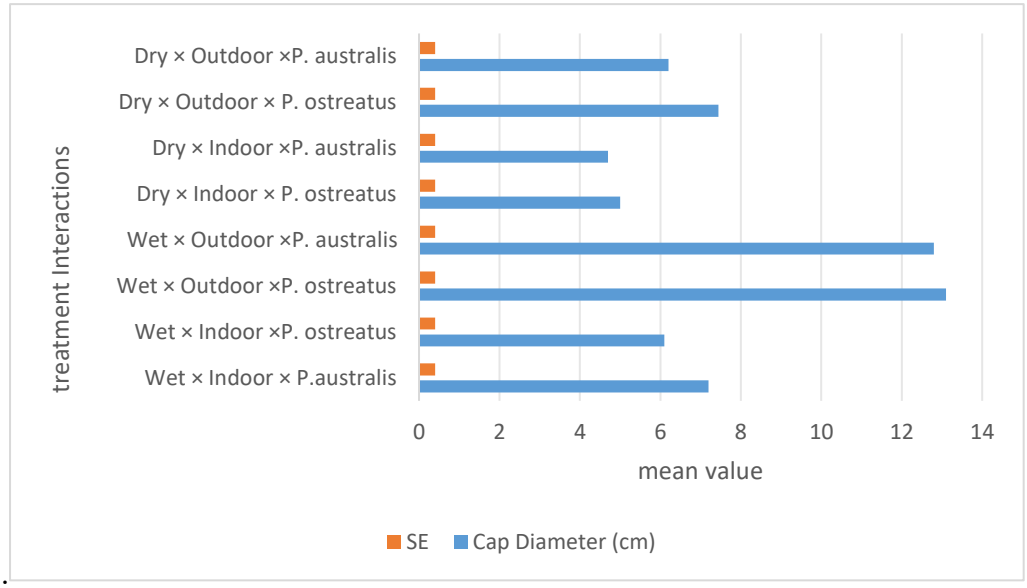


Figure 11: LSM of yield across seasons, conditions and genotypes interactions

Figure 5: Stalk length across treatment interactions.

Cap diameter: The least-square means for cap diameter revealed a pronounced and significant season × condition × genotype interaction. The wet-season × outdoor × genotype interactions produced the largest caps, with mean diameters of about 12.8–13.1 cm, and these values formed the highest significance group (“a”). Wet-season × indoor interactions yielded smaller caps (roughly 6–7 cm) and were placed in a lower significance group (“b”). Dry-season × outdoor interactions showed intermediate cap sizes (about 6–7.5 cm, groups “b”), while the dry-season × indoor × genotype interactions had the smallest caps, averaging only 4.7–5 cm,

and occupied the lowest significance group (“c”). These results indicate that cap expansion was greatest when the interaction of wet season and outdoor cultivation occurred, whereas the interaction of dry season and indoor conditions significantly restricted cap diameter across genotypes. Figure 6 presents a bar graph illustrating the cap diameter across seasons, conditions and genotypes interactions.



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4.7 ANOVA Results for Main and Interactions Effects of Microclimatic Parameters

Table 6 presents the analysis of variance (ANOVA) results showing the p-values and associated standard errors (SE) for the effects of season, cultivation condition, oyster mushroom genotype, and their interactions on the microclimate parameters of humidity, temperature, light intensity, and carbon dioxide.

Table 6: ANOVA table (p-values and standard error)

Variables	Seasons	Conditions	Genotype	Season×Condition	Season×Conditions×Genotypes
Humidity	<.00 ±0.39*	0.021±0.3*	0.45± 0.39	0.02± 0.39*	0.01 ± 0.39*
Temperature	<.00 ±0.24*	0.02 ±0.24*	0.83±0.24	0.04±0.24*	0.00 ± 0.24*
Light Intensity	<.00±0.2*	<.00±0.2*	0.21±0.2	0.03±0.2*	0.02 ± 0.2*
Carbon (iv) oxide	0.03±0.4*	<.00±0.4*	0.71±0.4	0.03±0.4*	0.04 ± 0.4*

The ANOVA results show that season significantly influenced levels in humidity ($p < 0.001$), temperature ($p < 0.001$), light intensity ($p < 0.001$), and carbon dioxide ($p = 0.03$). Cultivation condition (indoor vs. outdoor) also produced significant effects on humidity ($p = 0.021$), temperature ($p = 0.02$), light intensity ($p < 0.001$), and carbon dioxide ($p < 0.001$). Genotype had no significant effect on any of the measured parameters (all $p > 0.05$). For the interaction terms, the season × condition interaction was significant for humidity ($p = 0.02$), temperature ($p = 0.04$), light intensity ($p = 0.03$), and carbon dioxide ($p = 0.03$), indicating that the effect of growth condition on each of these environmental parameters varied with season. The three-way season × condition × genotype interaction was also significant for all four parameters; humidity ($p = 0.01$), temperature ($p = 0.00$), light intensity ($p = 0.02$), and carbon dioxide ($p = 0.04$), showing that the combined influence of season, cultivation condition, and genotype produced further variations beyond the two-way interactions

4.8 LSM Comparison Effects on Microclimatic Parameters

Table 7 presents the least-square mean (LSM) values ± standard error for humidity, temperature, light intensity, and carbon dioxide for the season × condition ×

genotype interaction. Letters accompanying the means indicate statistically significant differences among the three-way interactions.

Table 7: LSM comparisons (± standard error)

Season× Condition× Genotype	Humidity	Temperature	Light Intensity	Carbon IV oxide
Dry× Indoor × *P.ostreatus*	65.4 ± 0.9^{c}	29.9 ± 0.6^{b}	211.5 ± 0.5^{c}	455.1 ± 0.9^{a}
Dry× Indoor × *P.australis*	63.8 ± 0.9^{cd}	30.8 ± 0.6^{ab}	211.4 ± 0.5^{c}	455.0 ± 0.9^{a}
Dry × Outdoor×*P.ostreatus*	59.2 ± 0.9^{e}	33.3 ± 0.6^{a}	246.5 ± 0.5^{a}	413.2 ± 0.9^{b}
Dry× Outdoor× *P.australis*	61.9 ± 0.9^{d}	32.2 ± 0.6^{ab}	245.5 ± 0.5^{a}	412.6 ± 0.9^{b}
Wet×Indoor×*P.ostreatus*	77.0 ± 0.9^{b}	21.9 ± 0.6^{c}	203.3 ± 0.5^{d}	455.3 ± 0.9^{a}
wet× Indoor×*P.australis*	79.8 ± 0.9^{a}	20.9 ± 0.6^{c}	202.3 ± 0.5^{d}	454.8 ± 0.9^{a}
Wet×Outdoor×*P.ostreatus*	79.2 ± 0.9^{ab}	21.2 ± 0.6^{c}	235.3 ± 0.5^{b}	416.8 ± 0.9^{b}
Wet×Outdoor× *P.australis*	77.7 ± 0.9^{ab}	22.0 ± 0.6^{c}	235.2 ± 0.5^{b}	416.7 ± 0.9^{b}

Humidity: The wet-season × indoor × *P. australis* interaction held the highest significance group "a," showing the greatest humidity among all interactions. It was followed by wet-season × indoor × *P. ostreatus* in group **"**b,**"** which was significantly lower than group "a" but higher than all dry-season interactions. Dry-season indoor interactions occupied group **"**c,**"** while the lowest humidity occurred in dry-season outdoor interactions, placed in groups "d" and "e."Figure 12 presents a bar graph illustrating the humidity levels across seasons, conditions and genotypes interactions.

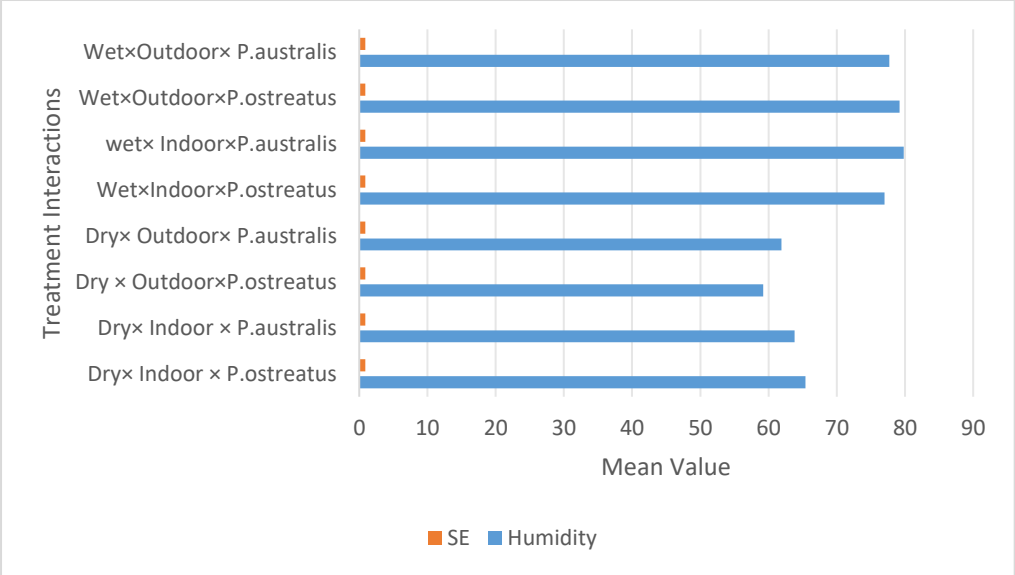


Figure 12: LSM of Humidity (%) across seasons, conditions and genotypes interactions

Temperature: The dry-season × outdoor × *P. ostreatus* interaction was in the top significance group "a," registering the highest temperatures. The dry-season × outdoor × *P. australis* interaction followed in group "ab," significantly cooler than group "a" but warmer than all wet-season interactions. All wet-season interactions were assigned lower groups such as "c," reflecting their markedly lower temperatures.Figure 13 presents a bar graph illustrating the LSM of temperature across seasons, conditions and genotypes interactions.

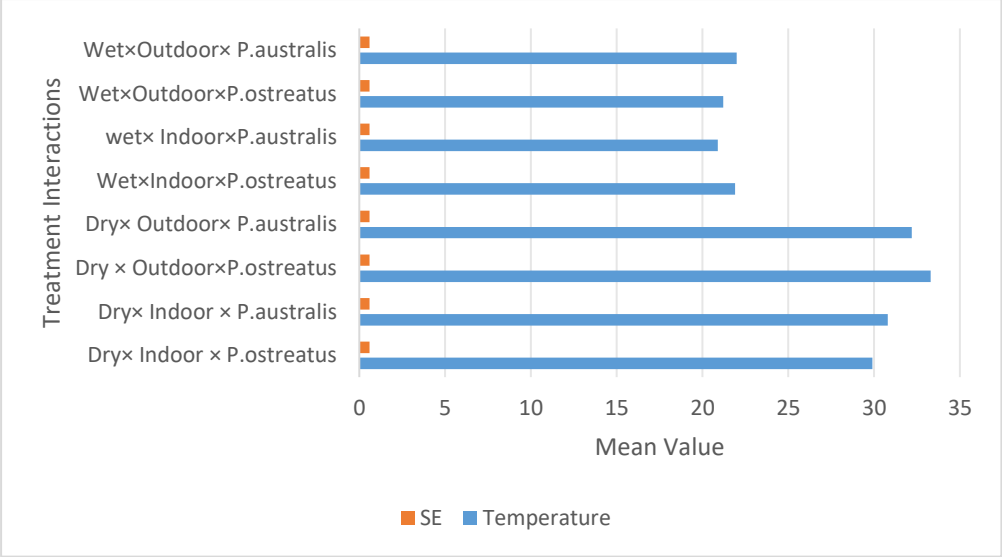


Figure 13: LSM of temperature (^{0}C) across seasons, conditions and genotypes interactions

Light Intensity: Both dry-season × outdoor interactions; *P. ostreatus* and *P. australis* were grouped together at the highest level "a," showing the greatest light intensity. Wet-season outdoor interactions were next in group "b," while all indoor interactions, regardless of season or genotype, clustered in lower groups "c" and "d."Figure 14 presents a bar graph illustrating the LSM of light intensity across seasons, conditions and genotypes interactions.

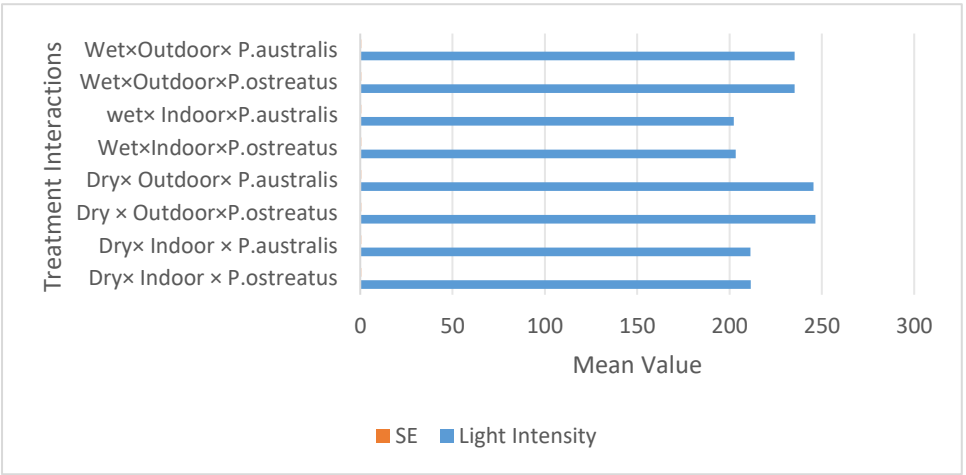


Figure 14: LSM of light intensity (LUX) across seasons, conditions and genotypes interactions

Carbon Dioxide: Indoor interactions for both seasons formed the top group "a," indicating the highest CO_2 concentrations. All outdoor interactions, wet or dry, were consistently lower and grouped under "b," confirming significantly reduced carbon dioxide levels compared with the indoor settings. Figure 15 illustrates how carbon dioxide levels varied with season, growth condition, and genotype, based on least-square mean comparisons.

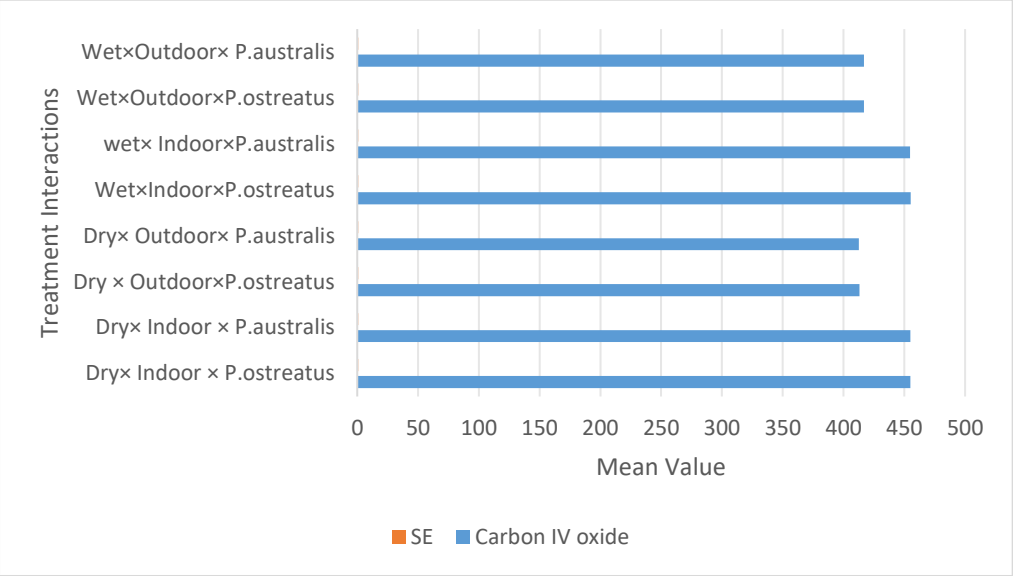


Figure 15: Variation in carbon dioxide concentration based on the interaction of season, cultivation condition, and genotype.

CHAPTER FIVE

DISCUSSIONS

5.1 Introduction

This Chapter provides a detailed discussion of the findings in relation to existing literature. It interprets the results, explains observed trends, and connects them to previous studies to highlight consistencies, differences, and possible explanations. The chapter emphasizes how the findings contribute to existing knowledge and their implications for mushroom production and environmental management.

5.2 Growth, Morphological, Contamination Levels and Biological Efficiency Parameters

Mycelium Size: The greatest mycelial diameters occurred under the wet-season × outdoor interactions, demonstrating that high ambient humidity, moderate temperatures and lower carbon iv oxide levels accelerate substrate colonization. Growers can shorten production cycles and increase the number of annual flushes by initiating crops during the rainy season in well-ventilated outdoor units. When cultivation must continue in the dry season, humidity-controlled shelters or regular misting are recommended to avoid suppressed growth. Similar patterns have been reported by other researchers, where higher rainy-season humidity promoted faster mycelial expansion (Aghajani et al.,2018; Kashangura, 2008; Rathore, n.d). Plate 5 shows how mycelium growth of oyster genotypes appeared in outdoor and indoor conditions.



Plate 5: Mycelium growth of oyster genotypes in outdoor and indoor conditions.

Incubation Time: In both genotypes, incubation was shortest in the wet-season × outdoor interactions and longest in the dry-season × indoor interactions, indicating that warm, moist outdoor conditions greatly reduce the time to full colonization. Scheduling spawn runs so that incubation coincides with the wet season can therefore cut labour and energy costs. Where dry-season production is unavoidable, humidification or insulated rooms are necessary to prevent incubation periods from exceeding three months. Comparable results from other authors were noted which highlight the need for high relative humidity to accelerate spawn run (FAO, 2021; Chhata, & Thakore, 2010; Rathore, n.d).

Fruiting Phase: The wet-season × outdoor interactions in both genotypes also produced the shortest fruiting phases, while the dry-season × indoor interactions prolonged fruiting by more than a month. These results reveal that aligning fruiting with the rainy season enables earlier harvests and the possibility of multiple flushes within a single season. Other researchers including; Büntgen et al.,, (2012) and Kauserud et al.,(2008) have reported similar results. .Plates 6 and 7 shows how fruiting of oyster genotypes appeared in outdoor and indoor conditions.



Plate 6: *Pleoretus ostreatus* genotypes



Plate 7: *Pleoretus australis* genotypes

Stalk Length: The dry-season × outdoor interactions in both genotypes produced the longest stalks, while the dry-season × indoor interactions yielded the shortest. This shows that fluctuating outdoor light and temperature favour stem elongation, whereas the more stable and shaded indoor conditions restrict it. Farmers targeting markets that prefer longer stalks can therefore benefit from partially open structures or outdoor production during the dry season. Similar effects of light and temperature on stipe elongation have been reported for *Pleurotus* genotypes in Africa and elsewhere (Mesele et al. 2024; Gebru et al. 2024).

Cap Diameter: The wet-season × outdoor interactions in both genotypes gave the largest cap diameters, whereas the dry-season × indoor interactions produced the smallest. High humidity and moderated outdoor temperatures during the wet season clearly enhance pileus expansion. To maximize cap size, a key market trait, growers should target wet-season outdoor cultivation or replicate high-humidity conditions indoors. Comparable relationships between humidity, temperature, and

cap size have been documented in oyster mushroom studies (Kortei et al.,2018; Popovych et al.,2019).

Number of Deformed Fruits: Deformities in both genotypes were most common in the dry-season × outdoor interactions, moderate in the wet-season × outdoor interactions, and very low in all indoor settings. Environmental stresses such as low humidity and wide temperature swings outdoors particularly in the dry season, are major contributors to these malformations. Pest activity adds to the problem: insects like sciarid flies (*Lycoriella spp*.) and mites can damage developing primordia, producing misshapen caps and stipes. Other field studies confirm that inadequate sanitation or open-air production increases fruit-body deformities (De Mastro et al., 2023; Bellettini et al., 2019;). Installing insect-proof netting, maintaining strict hygiene, and removing infested substrate promptly are practical measures to reduce such defects.

Contamination Level: The dry-season × indoor interactions showed the highest contamination levels, with dry-season × outdoor treatments slightly lower, while both wet-season treatments recorded minimal contamination. Warm, poorly ventilated indoor conditions during the dry season favour competitor moulds and bacteria. Extended incubation periods further increase the risk of contamination, because longer spawn runs leave more time for unwanted organisms to colonize the substrate. Similar observations connecting extended incubation periods with higher contamination risks have been reported in *Pleurotus* cultivation (Sánchez, 2010; Rodriguez & Pecchia, 2017). To minimize contamination, it is important for growers to maintain strict hygiene and adopt practices that reduce colonization time, such as regulating temperature and moisture levels appropriately and ensuring careful handling of substrates after pasteurization.

Biological Efficiency: Across both genotypes, the wet-season × outdoor treatments recorded the highest biological efficiency, followed by the wet-season × indoor combinations, whereas the dry-season × indoor setups yielded the lowest efficiency. These results emphasize the critical role of adequate humidity and ventilation in enhancing substrate conversion into mushroom biomass. Aligning the fruiting period with the wet season, or supplementing humidity during dry spells, can therefore help optimize production outcomes. Comparable improvements in biological efficiency under moist and well-aerated environments have also been documented in other studies examining substrate and environmental interactions (Ejigu et al., 2022; Munir et al., 2021).

5.3 Mushroom Yield and Microclimate Interactions

Within the season × condition × genotype framework, the highest yield was obtained under the wet-season × outdoor × *P. ostreatus* interaction, which ranked in the top significance group ("a"). This superior performance corresponded with the favorable microclimatic conditions recorded for that treatment characterized by elevated humidity, moderate temperature, adequate light, and balanced CO_2 levels. These observations are consistent with those of Morozova (2023), who reported that such conditions enhance metabolic activity and promote efficient substrate conversion. Conversely, the dry-season × outdoor treatments produced comparatively lower yields, reflecting their harsher microclimate with higher temperatures and reduced humidity, factors known to suppress fungal growth and productivity (Visvanathan et al., 2021). Similarly, indoor treatments exhibited limitations associated with restricted light availability and possible CO_2 accumulation, both of which can disrupt photosensory regulation during fruit body

formation (Yue et al., 2022). These combined microclimatic constraints help explain the placement of such interactions within lower statistical groups.

The superior performance of *P. ostreatus* in the top-yielding treatment highlights the presence of genotype × environment synergy. This species is frequently noted for its resilience under humid and moderately warm conditions compared to other *Pleurotus* genotypes (Gaitán-Hernández & Salmones, 2008). Under less favorable environments characterized by low humidity or high temperature, yields of *P. australis* and *P. ostreatus* tended to converge, though *P. ostreatus* often maintained a marginal advantage, reflecting its relative stability under suboptimal conditions.

Prolonged incubation periods observed in the less favorable combinations further contributed to yield reduction. Extended colonization increases the risk of contamination and substrate depletion, thereby reducing the biomass available for fruiting, a phenomenon widely reported in previous studies (Saputra et al., 2024). In interactions with delayed substrate colonization, yield losses likely accumulated progressively.

Overall, these findings underscore the integrated influence of moisture, temperature, light, and gas balance on mushroom yield. They further emphasize that genotype selection plays a crucial role, particularly under optimal microclimatic conditions. Cultivators are therefore encouraged to synchronize production cycles with favorable seasons or simulate similar environments within controlled cultivation systems to maximize productivity.

CHAPTER SIX

CONCLUSIONS ND RECOMMENDATIONS

6.1 Introduction

Chapter Six presents the summary, conclusions, and recommendations derived from the study. It restates the major findings in brief, draws conclusions based on the objectives, and provides practical and research-based recommendations for improving mushroom cultivation under different environmental conditions.

6.2 Conclusion

The present study set out to determine how the season × condition × genotype interaction influences the growth, morphological, CL and BE parameters, yields, and microclimate of oyster mushrooms cultivated in association with banana plants at Masinde Muliro University. The findings provide clear evidence that this three-way interaction is a critical driver of production outcomes and that the null hypotheses linked to each specific objective must all be rejected.

6.2.1 Growth, Morphological, CL and BE

Mycelial expansion, incubation time, and fruiting phase were each strongly shaped by the interaction effects of season × cultivation environment × *Pleoretus* genotypes. Wet-season × outdoor conditions × genotypes consistently promoted rapid colonization and shorter fruiting periods, while dry-season × indoor settings × genotypes prolonged both stages. Stalk length, cap diameter, number of deformed fruits, contamination level, and biological efficiency also showed significant three-way interaction effects. For example, dry-season× outdoor conditions × genotypes produced the longest stalks, whereas wet-season× outdoor conditions × genotypes yielded the widest caps and highest biological efficiency.

The results provide sufficient evidence to reject the null hypothesis, demonstrating that interactions among factors significantly affected growth, morphological characteristics, carbon loss, and biological efficiency.

6.2.2 Yield Performance

Yields varied considerably across the combined treatments. The highest production was obtained from the wet-season × outdoor × *Pleurotus ostreatus* interaction, which outperformed all other treatment combinations. In contrast, mushrooms grown during the dry season under indoor conditions recorded the lowest yields, suggesting that the moist and well-ventilated environments of the wet season are more favorable for production. The superior performance of *P. ostreatus* across most growing environments further demonstrates that genetic differences play a crucial role in determining yield potential. Based on these findings, the null hypothesis stating that there is no interaction effect on yield is rejected

6.2.3 Microclimate Conditions

Measurements of humidity, temperature, light intensity, and carbon dioxide showed clear differences that resulted from the interaction between season, cultivation condition, and genotype. Indoor environments during the wet season recorded the highest humidity and carbon dioxide concentrations, while outdoor setups in the dry season experienced elevated temperatures and stronger light intensity. These microclimate shifts mirrored variations observed in growth performance, morphological traits, contamination levels, biological efficiency, and overall yield. The findings highlight the critical role of environmental control in optimizing the integration of oyster mushrooms with banana plants. Hence, the null hypothesis proposing no interaction effect on microclimate is rejected.

Overall, the rejection of all three null hypotheses confirms that season, cultivation conditions, and genotype jointly influence growth behavior, morphological characteristics, contamination levels, biological efficiency, yield, and microclimate within integrated *Pleurotus* banana production systems.

6.3 Recommendations

The findings of this study demonstrate that growth, morphological, contamination levels and biological efficiency parameters of oyster mushrooms are strongly influenced by the interaction of season, cultivation condition, and genotype. Farmers are therefore advised to align mushroom production with the wet season and to cultivate outdoors under banana canopy, where natural humidity and moderated temperature favour rapid mycelial growth, shorter incubation periods, wider caps, and higher biological efficiency. *Pleurotus ostreatus* is recommended as the preferred genotype for integration with bananas because it consistently produced superior stalk length, cap diameter, and overall growth, morphological, and production parameters compared with *P. australis.* For growers who must produce during the dry season or indoors, careful control of humidity and carbon dioxide, together with strict sanitation, is necessary to prevent the contamination that becomes more likely when incubation times are long.

Yield performance also depended heavily on the season × condition × genotype interaction. To achieve maximum production, farmers should align their cropping cycles with the wet season and prioritize outdoor cultivation beneath banana canopies, which naturally provide a favorable microclimate. The combination of outdoor conditions and the *P. ostreatus* genotype yielded the best results, highlighting their compatibility for optimal productivity. Under less favorable environments, such as during the dry season or in indoor setups using higher spawn

rates and techniques that promote faster substrate colonization can help minimize contamination and reduce yield losses.

Effective microclimate management remains essential, as humidity, temperature, light intensity, and carbon dioxide levels were all significantly influenced by the interaction of season, growth condition, and genotype, and were closely linked to growth and yield performance. Integrating banana plants with mushroom beds enhances shading and moisture retention, helping to stabilize these factors in outdoor systems. For indoor production, growers can mimic the beneficial wet-season outdoor environment by installing automated humidifiers, improving ventilation, and providing shading to maintain optimal humidity, temperature, and CO_2 conditions.

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APPENDICES

Appendix A: Variables for Experimental Research

1.Time stamp of data collection Date () Time ()

2.Bag type

a. *Pleurotus ostreatus* (Outdoor) ( ) b. *Pleurotus australis*(Outdoor) ( )

c. Pleurotus ostreatus (Indoor) ( ) d. Pleurotus australis(Indoor) ( )

3. Growth behavior

a. Take picture/ Choose image b. Size of mycelium(cm) ( )

c. Percentage substrate contamination (cm) ( ) d. Bag sprouting Yes ( ) No ( )

e. Incubation period ( ) f. Any other comment

4.Sporophores Parameters

a. Take a picture/Choose image b. Color of fruits ( )

c. Number of fruits ( ) d. Length of the stalk(cm) ( )

e. Diameter of mushroom cap(cm) ( ) f. Fructification period ( )

f. Any other observation

5.Yield

a. Take a picture/Choose image b. Total number of fruits at flush/Harvest ( )

c. Number of mature fruits ( ) d. Number of deformed fruits ( )

e. Fresh weight yield (grams) ( )

6. Temperature

a. Outdoor ( ) b. Indoor( )

7.Humidity level

a. Outdoor ( ) b. Indoor ( )

8. Carbon iv oxide level

a. Outdoor ( ) b. Indoor ( )

9.Light Intensity

a. Outdoor ( ) b. Indoor ( )

Appendix B: MMUST-ISERC letter

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
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E-mail: ierc@mmust.ac.ke
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P. O. Box 190,
50100.
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01)

Date: March 14th, 2023

To: Ms. Gladwel Chepng'etich Ng'eno

Dear Ms.,

RE: EDIBLE MUSHROOM-BANANA INTEGRATION AS AN ALTERNATIVE PLANT SHELTER METHOD FOR INDOOR CULTIVATION IN KAKAMEGA COUNTY.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/141/2023.** The approval covers for the period ***March 14th, 2023 to March 14th, 2024.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)