CULTIVATION OF *Pleurotus tuberregium* (Fr.) SINGER ON WHEAT BRAN AND *Greenwayodendron suaveolens* SEED POWDER SUPPLEMENTED SAWDUST

BY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY

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This is to certify that this project work was carried out by Osayande Solomon IGBINOSA in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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Prof. B. IKHAJIAGBE Date

(Head of Department)

DEDICATION

I dedicate this work to Almighty God for His unconcealed guidance and strength, to my parents for their unlimited love, support, and encouragement, and to all the individuals who have inspired and motivated me throughout this endeavour.

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ABSTRACT

This study examined the effect of different levels of *Greenwayodendron suaveolens* seed powder and wheat bran supplementation on the growth and yield performance of *Pleurotus tuberregium*. *Pleurotus tuberregium*, commonly known as the king tuber oyster mushroom, is a tropical species valued for its nutritional, medicinal, and economic importance. Unlike plants, mushrooms lack chlorophyll and depend on decomposing organic matter for nourishment, making them suitable for cultivation on agricultural wastes such as sawdust, rice husk, and maize cobs. The experiment was done using sawdust as substrate. The substrate was prepared and supplemented with wheat bran and *Greenwayodendron suaveolens* seed powder at 0,2,4,6,8 and 10% levels respectively. Supplemented substrate were inoculated with spawn of *Pleurotus tuberregium* and then incubated incubated for growth and fruiting. The results showed that supplementation with wheat bran and *Greenwayodendron suaveolens* seed powder improved the growth and yield of *Pleurotus tuberregium* compared to the 0% control. Substrates supplemented with *Greenwayodendron suaveolens* seed powder reached 50% mycelial colonization as early as 12 days and full colonization by about 18 to 20 days, while those with wheat bran reached 50% between 12 and 14 days. Fruiting bodies appeared between 22 and 30 days in both supplements, but only sclerotia formed in the 0% control. The highest yield was recorded at 10% *Greenwayodendron suaveolens* seed powder with 790 g fresh weight, 50.43 g dry weight, and

219.44% biological efficiency. The outcome of this study has illustrated that *Greenwayodendron suaveolens* seed powder supplementation greatly enhances growth and yield performance of *Pleurotus tuberregium* compared to wheat bran and the 0% (contro). *Greenwayodendron suaveolens* seed powder enabled rapid mycelial colonization, primordia initiation at an earlier stage, and greater biological efficiency, which were best performed at 10% supplementation. The outcome of the research is expected to guide the use of affordable organic additives to enhance yield performance in mushroom production. Since *Pleurotus tuberregium* is adapted to tropical weather and able to utilize farm waste, its optimization may guarantee food security, reduce environmental waste, and provide small-scale farmers in Nigeria and other developing nations with an opportunity to earn income.

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CHAPTER ONE

INTRODUCTION

1.1 Mushroom

Mushrooms are generally not considered plants because they do not contain chlorophyll and thus cannot conduct photosynthesis to produce food. Instead, they get their nutrition by breaking down organic matter. Mushrooms are being cultivated all over the world for food, medicine, and even for environmental management. For instance, they can be grown on organic waste like sawdust, rice husk, or other agro-residues, thus helping reduce such materials that contribute to pollution. The ability to turn this waste into useful products makes mushrooms important, not just for nutritional purposes, but also for sustainability.

Apart from their ecological function, mushrooms aren't just a part of nature; they are in a class unto themselves. Classified as fungi-not plant or animal-they also grow differently. They have no true roots, stems, or leaves. Mushrooms aren't really plants; they grow from delicate threads-mycelium-that reach out to get food. What people generally recognize as a mushroom, the part you might have on your plate, is only a fraction of the whole living thing.

To better understand them, it helps to look at how scholars have defined mushrooms. (Chang and Miles, 2004) described them as “a macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and to be picked by hand.” In words, a mushroom is the fleshy, spore-bearing structure of certain fungi, usually produced on soil, decaying wood, or other organic material.

Nutritional issues, for instance, reveal that mushrooms are rich in protein, vitamins, minerals, and fiber and have a low fat content. All these make them a healthy food ingredient, especially in areas where other sources of proteins are expensive. They are also reported to have medicinal bioactive molecules that can enhance immune responses and lower cholesterol levels. (Lindequist et al., 2005), showed that mushrooms are not only food but also a source of pharmaceutical compounds that could be used against cancer and diabetes.

Economically, mushroom cultivation has become a sure source of livelihood for many. As mushrooms can be produced based on agricultural wastes, farmers minimize wastes while still earning a profit. Research evidence in Nigeria indicates mushrooms enhance food security but at the same time ease poverty due to the fact that they provide rural people with various means of generating income (Oyetayo, 2011; Fasidi, 2008). In their argument, (Marshall and Nair 2009) reiterated that mushroom cultivation encourages local economies, employment opportunities, and also conservation of the natural environment, therefore alleviating communities' wellbeing in non-urban areas remarkably.

What started off as simply finding food has now grown into a feasible business, one that's good for both people and the planet. In essence, it means the cultivation of useful fungi either for food or health benefits, using such mediums as compost. Mushrooms are not like most plants; they do not necessarily require sunlight to grow. They thrive from leftovers of the farm, like wood chips, rice straw, maize cobs, and groundnut shells.. Because of this, growing mushrooms is good for the planet, turning trash into treasure alongside a livelihood for farmers.

Generally, the cultivation process involves substrate preparation, spawning (inoculation with mushroom mycelium), incubation, fruiting, harvesting, and post-harvest handling. Substrates must be properly sterilized to eliminate contaminants, and suitable environmental conditions—especially temperature, humidity, and aeration—must be maintained for successful fruiting. With these conditions met, mushrooms have relatively short growth cycles and can be harvested multiple times, providing quick and sustainable returns.

1.2 General Cultivation of Mushrooms

Mushroom cultivation is a biological process that involves growing edible fungi on organic materials rich in lignin, cellulose, and hemicellulose. These materials, known as substrates, include sawdust, rice husk, maize cob, palm press fiber, and other agricultural wastes. The cultivation process can be carried out on a small or large scale, making it suitable for both rural and urban settings.

Preparation of the substrate involves drying, grinding, and supplementation with ingredients such as wheat bran, rice bran, or calcium carbonate to enhance their nutritional quality. The substrate is then moistened and undergoes either pasteurization or sterilization to eliminate competing microorganisms that may hinder the colonization of the mushroom mycelium.

The prepared substrate is then inoculated with mushroom spawn, which is the planting material. The bags, thus inoculated, are then placed for incubation in a dark and warm atmosphere, which allows mycelial colonization. During this phase, the mycelium grows throughout the substrate, breaking down the organic matter into simpler compounds.

After colonization is complete, the bags are taken to a fruiting room where environmental conditions such as humidity, temperature, and light are maintained to initiate fruiting body formation. Within days, small primordia appear, maturing into harvestable mushrooms.

Harvesting is done by twisting or cutting the mushrooms from their base to avoid damaging the mycelium, which allows for subsequent flushes. Afterwards, the spent substrate can be used as compost or animal feed, minimizing waste.

Mushroom cultivation is an environmentally friendly and cost-effective agricultural practice. It converts agricultural residues into nutritious food, provides employment, and contributes to sustainable waste management, especially in countries like Nigeria where raw materials are readily available.

1.2.1 Cultivation of *Pleurotus tuber-regium*

Globally, people grow many kinds of mushrooms – *Agaricus bisporus, Lentinula edodes, Volvariella volvacea*, and members of the *Pleurotus* genus. Oyster mushrooms especially thrive because they can grow on diverse plant waste; therefore, they're perfect for places such as Nigeria with warm climates.

Among the *Pleurotus* species, *Pleurotus tuber-regium*—commonly known as the king tuber oyster mushroom– stands out from others because due to its unique biological characteristics and high economic value, alongside its worth. Originating in African tropics, it grows a hidden, potato-shaped mass underground which stores food. People eat both this mass also the actual mushrooms; they're packed with goodness like protein, vitamins, minerals, plus things that boost well-being.

People typically start growing *P. tuber-regium* by gathering underground stems – sclerotia – from nature, or their propagation through pure culture techniques. These mushrooms thrive on things like dampened sawdust, rice leftovers, corn leaves, alongside various farm waste products.

First, these materials get cleaned then heated to kill anything living within, before introducing the mushroom spores or pure culture. To get things growing, it then keept in a dark room which encourages the growth of mycelium, after full colonization has occur then you can start seeing forms of primodia which then turn into the mushroom fruiting body, which can then be picked by hand during harvesting.

This species thrives in tropical climates and can be cultivated year-round with minimal inputs, making it particularly suitable for smallholder farmers. Because it uses farm leftovers, it cuts down on waste while getting the most from available resources. Therefore, growing *Pleurotus tuber-regium* could significantly boost food supplies, create jobs in the countryside, plus foster eco-friendly farming throughout Nigeria alongside similar areas.

1.2.2 Previous research on *Pleurotus tuberregium* cultivation

A study was conducted in 2018 to determine how composting affects the performance of sawdust as a substrate in cultivating *Pleurotus tuber-regium* (Adedokun and Thomas, 2018). Freshly collected sawdust was compared with composted sawdust for two weeks. In their experiment, they considered the production of sclerotia, which are underground food reserve structures for this mushroom species. Results depicted that composted sawdust was at a clear advantage. On this substrate, mycelium spread more rapidly, colonization of bags was even, time to sclerotia formation was shorter, and sclerotia produced were heavier, having an average fresh weight of 21.6 g compared to 15.6 g on fresh sawdust and an average dry weight of 11.6 g compared to 8.2 g. These findings would tend to indicate that composting improves substrate quality by the probable breakdown of complex lignin and cellulose into simpler forms that the fungus can access more easily. The practical implication is that simple pre-treatment of sawdust by composting may improve yield and speed of cultivation considerably.

Otunla and colleagues at the National Horticultural Research Institute, Ibadan, investigated the effects of sawdust type and composting period on *Pleurotus tuber-regium* yield. They used mango (*Mangifera indica*), cassia (*Cassia siamea*), neem (*Azadirachta indica*), and a mix of the three. Each was composted for 4, 8, and 12 weeks after which the substrates were inoculated and monitored for various periods. The results showed marked differences between treatments. Cassia sawdust composted for 8 weeks performed very well, producing sclerotia fresh weights of 35.34 g, biological efficiency (BE) of 33.66%, and production efficiency (PE) of 13.51%. However, mango sawdust composted for 12 weeks gave the highest overall yield, with sclerotia fresh weights of 37.44 g, BE of 35.66%, and PE of 18.28%. Mixed sawdust and neem were consistently less productive, especially when composted for only 4 weeks. The study therefore highlighted two important factors for successful cultivation: the specific tree species from which sawdust is derived, and the length of time the sawdust is allowed to compost. Longer composting improved performance in most cases, but the inherent chemical composition of the sawdust also played a role.

A later study by (Adedokun and Friday, 2020) expanded the scope to field conditions, evaluating the effect of different amendments on the fruiting of *Pleurotus tuber-regium*. Outdoor beds were set up with four treatments: topsoil only (control), topsoil mixed with sawdust, topsoil mixed with poultry droppings, and topsoil enriched with both sawdust and poultry droppings. Their results demonstrated that the mixture of topsoil and sawdust produced optimal early fruiting, recording the highest dry weight of mushrooms harvested in the first flush. In the second flush, yield was not significantly different between treatments. This seems to indicate that during the early growing stages, sawdust could act on the substrate and nutrient structure in such a way as to improve the productivity of this mushroom. Over time, as nutrients are depleted, the advantage wanes. From the farmer's perspective, this will mean that applications of sawdust into beds during cultivation can be an effective methodology to ensure higher yields in the early harvests, which are generally the most commercially valuable.

1.3 Economic importance

Mushroom cultivation, especially the economically important species such as *Pleurotus tuber-regium,* therefore forms one of the innovative and ecologically sustainable biotechnological means of converting agricultural and forestry wastes into useful resources. The mushrooms represent an environmentally friendly method of waste disposal as the mycelia have the capability to produce enzymes that can degrade lignocellulosic wastes, which cause pollution (Kulshreshtha, 2010; Adenipekun & Lawal, 2012). Technologies such as myco-filtration or mycoremediation illustrate that mushroom cultivation holds an important place in the restoration of ecosystems, including forest and degraded ecosystems, degrading toxic waste, and control of pests.These methods contribute significantly to environmental restoration, creating cleaner ecosystems with minimal residual impact (Osemwegie et al., 2013; Girmay et al., 2016; Kuforiji & Fasidi, 2007).

Beyond environmental benefits, mushroom cultivation, including *Pleurotus tuber-regium*, is a viable agro-industrial activity that generates income and employment opportunities, particularly for women and youth in both developing and developed regions (Aditya and Bhatia, 2020).

Unlike traditional agriculture, which often requires extensive land, mushroom farming is space-efficient, utilizing vertical stacking or shelf-like systems (Chang et al., 2008; Oei, 2003). The rapid growth cycle of mushrooms makes them an ideal short-term agricultural venture, offering immediate economic benefits to communities.

As a cottage industry, mushroom cultivation supports integrated rural development by providing opportunities for small-scale farmers, landless laborers, and other economically disadvantaged groups (Flores, 2006). Additionally, it addresses pressing global challenges such as food insecurity, declining human health, and environmental degradation caused by population growth, resource depletion, and climate change (Sher, 2006; Oseni et al., 2012; Valverde, 2015).

The benefits of mushroom farming extend beyond food production. After harvesting, the spent substrate, rich with mycelial networks, can be repurposed as animal feed, bio-fertilizer to enhance soil fertility, or feedstock for biogas production (Chang & Miles, 2004; Shah et al., 2004). This agricultural waste recycling encourages circular economy principles. Besides, mushroom production offers jobs both in rural and urban areas and reduces unemployment, making the production of nutritious food at low cost possible. (Kuforiji and Fasidi, 2009)

Pleurotus tuber-regium is a highly prized edible mushroom belonging to the genus *Pleurotus*, native to tropical regions of Africa, Asia, and Australia. This species is unique among the *Pleurotus* species in that it produces both a sclerotium-an underground nutrient-rich structure-and fruiting bodies, both edible and of high cultural value in most regions where the species is found (Cohen et al., 2002). The fruiting bodies have a fleshy, shell-like cap with a lateral or eccentric stipe and range in color from white to cream, brown, or gray, depending on environmental conditions.

Pleurotus tuber-regium is especially valued for its ability to produce high-quality protein from farm waste, hence an economical and sustainable crop (Shah et al., 2004). It does well on many substrates, including sawdust and rice bran, among other lignocellulosic materials; it is relatively amenable to culture even by resource-poor farmers. The mushroom reproduces sexually as well as asexually, adding to its hardiness and widespread culture (Cohen et al., 2002).

Particularly, *Pleurotus tuber-regium* is rich in minerals like calcium, manganese, zinc, and iron, which are very useful in the roles of acid-base balance and enzymatic functions for human and animal health (McDowell, 2003; Koyyalamudi, 2013). These nutritional properties, together with the fact that it is easily cultivated, make it a good candidate for nutritional intervention in resource-poor regions.

Pleurotus tuber-regium is not only a nutritious food source but also a powerhouse of medicinal properties. Extracts from its fruiting bodies and sclerotia have demonstrated anti-inflammatory, antioxidant, and antimicrobial activities (Avagyan et al., 2013; Vamanu, 2013; Chirinang and Intarapichet, 2009). Studies have also identified anticancer properties in *Pleurotus* species, with extracts increasing the expression of tumor-suppressor genes in breast and colon cancer cells (Jedinak & Silva, 2008; Wu et al., 2011; Khan & Tania, 2012).

The mushroom is a rich source of essential amino acids, vitamins (including B1, B2, niacin, biotin, and ascorbic acid), and dietary fiber, particularly beta-glucans (Chang, 2004; Musieba, 2013).However, these beta-glucans found in higher amounts in the *Pleurotus* spp. than in the Agaricus bisporus contribute toward cardiometabolic health through a decrease in insulin resistance, dyslipidemia, hypertension, and obesity (Khoury et al., 2012; Sari et al., 2017). The high viscosity and fermentability of these glucans in the gastrointestinal tract further enhance their health benefits (Adebayo et al., 2017).

In traditional medicine, *Pleurotus tuber-regium* has been used in Nigeria and other parts of Africa and Asia to treat various ailments (Osemwegie, 2010; Xu, 2011). Its dual role as both food and medicine has sparked increased research interest over the past two decades, with studies highlighting its potential in immune modulation, cholesterol reduction, and prevention of hypertension and atherosclerosis (Alam, 2008; Patel et al., 2012). The presence of bioactive compounds, such as phenols, flavonoids, and polysaccharide-peptide complexes, contributes to its antioxidant and antidiabetic properties (Jayakumar, 2011; Woldegiorgis, 2014).

The antimicrobial activity of *Pleurotus tuber-regium* is attributed to secondary metabolites, protein-polysaccharide complexes, and free fatty acids, which exhibit antibacterial, antifungal, and antiviral effects (Bala, 2012; Gyawali, 2014). These properties position the mushroom as a promising candidate for pharmaceutical applications, particularly in the development of natural antimicrobial agents. In fact, globally, *Pleurotus* species, including *P. tuber-regium*, are the second most cultivated edible mushrooms in the world, accounting for about 22% of the world's production of cultivated mushrooms, second to *Agaricus bisporus* (Royse et al., 2004; Khan et al., 2008). China is considered the world's leading producer and consumer of mushrooms, accounting for more than 70% of the global output(Martinez-Carrera, 1999). Other major producers are Japan, South Korea, and several Southeast Asian countries, including Indonesia and Thailand. Global demand for the *Pleurotus* species has continued to increase steadily due to their nutritional, medicinal, and ecological importance. The global demand for *Pleurotus* mushrooms has continuously increased due to their nutritional, medicinal, and ecological importance.

1.4 Challenges and Prospects of Mushroom Cultivation in Nigeria

In spite of an increasing awareness of nutritional and economic benefits accruing from mushrooms, the cultivation of mushrooms in Nigeria faces a number of challenges. Among these are limited technical knowledge on the part of farmers. Most potential cultivators have not been well trained in the preparation of substrates, sterilization, and handling of spawn, which often leads to contamination and low yields.

Other challenges include the scarcity and high costs of quality spawn due to the limited number of research centers producing it locally. This shortage makes it difficult for the majority of small-scale farmers to sustain their production. Poor storage and preservation facilities result in post-harvest loss because mushrooms are highly perishable and deteriorate rapidly under high temperatures.

Insufficient funding and support from the government are other serious drawbacks to the development of the mushroom industry. Many young farmers express their willingness to engage in cultivation, but lack capital, modern equipment, and reliable markets. There is also limited awareness amongst consumers, hence a low demand compared to other sources of protein such as meat and fish.

Mushroom cultivation in Nigeria nonetheless remains very promising. The warm and humid climate of the country favors several mushroom species, such as *Pleurotus tuberregium* and

Volvariella volvacea. Increasing demand for healthy, plant-based protein is also creating new market opportunities.

With advanced training, sensitization, and investment in research, mushroom cultivation would provide an important source of income and better food security. The utilization of inexpensive local materials such as sawdust and agricultural residues makes it a sustainable venture that helps reduce unemployment and promotes environmental conservation.

1.5 Justification of the Study

Mushroom cultivation largely depends on the nutrient composition of the substrate. While sawdust and other agricultural wastes can support the growth of mushrooms, their nutrient content alone is not sufficient for high yields. Supplementation is, therefore, necessary to enhance growth rate, fruiting, and overall yield performance.

Wheat bran is usually used as a supplement but has often proved to be expensive and not always in substantial amounts. This has therefore, created the need for other substitute supplements that are available locally at cheaper rates. One of such forest products, *greenwayodendron* seed, which is common in parts of Nigeria, is usually discarded despite its contents that could be useful for fungal growth.

The justification for this study will be based on the use of *Greenwayodendron* seed powder as a natural supplement in the cultivation of *Pleurotus tuberregium*. Comparing the effect with that of wheat bran and an unsupplemented control, this study determines whether *Greenwayodendron* seed enhances mycelial colonization, fruiting, and yield.

The results will give useful information to mushroom growers, researchers, and agricultural entrepreneurs. If effective, the utilization of *Greenwayodendron* seed powder could be an environmentally friendly low-cost supplement that will enhance resource utilization, income generation, and environmental management through waste reduction.

1.6 AIM AND OBJECTIVES:

Aim: The effect of different levels of *Greenwayodendron* seed powder and wheat bran supplementation on the growth and yield performance of *Pleurotus tuberregium* was assessed.

Objectives:

1. To determine the effect of the supplements on the colonization time and mycelial growth rate of *Pleurotus tuberregium*.

2. To assess the influence of *Greenwayodendron* seed powder and wheat bran on the yield and biological efficiency of the mushroom.

3. To compare the effectiveness of *Greenwayodendron* seed powder with wheat bran as substrate supplements for the cultivation of *Pleurotus tuberregium*

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of materials

The *Pleurotus tuberregium* culture used in this study was donated by the African Centre for Mushroom Research, Technology and Innovations (ACMRTI), University of Benin, Benin City. Potato Dextrose Agar (PDA) was used as the nutrient agar to cultivate the fungus. All the materials and media used were pre-treated and autoclaved under aseptic conditions to remove any possibility of contamination.

2.2 Preparation of Spawn

Grains of guinea corn were first washed in an abundant volume of water and then boiled, at a rate of 45 minutes to 1 hour, to soften the seed coat, which also helped in reducing possible contaminants. After boiling, the grains were drained, allowed to dry, and mixed with calcium carbonate ($CaCO_3$) to regulate pH, prevent clumping, and further discourage contamination. The grains thus prepared were dispensed into bottles, sterilized in an autoclave at 121 °C at 15 psi for 15 minutes to eliminate any remaining contamination. After cooling, the bottles were inoculated with pure culture of *Pleurotus tuberregium* under aseptic conditions. The inoculated bottles were gently shaken to ensure even distribution of the mycelium and then incubated at room temperature (about 23 °C) for approximately two weeks to allow complete colonization of the grains. The fully colonized grains served as the spawn for further use.

2.3 Preparation of Substrate

A mixture of substrates was prepared by mixing 14.5 kg of sawdust as the bulk material, 3.6 kg of wheat bran as a nutrient supplement, and 200 g of calcium carbonate ($CaCO_3$) for pH adjustment. All the components were mixed well until the wheat bran was properly dispersed in the sawdust. Adequate water was added to reach the ideal moisture content, as tested by the squeeze test—when some water was seen between the fingers, moisture content was presumed to be close to optimum 60%. The prepped substrate was allowed to rest for a few days to stabilize to facilitate uniform water and nutrient uptake. This also ensured that the substrate reached a stable pH range of 6.5 to 7.5 before bagging and sterilization.

2.4 Bagging and Pasteurization

This was achieved using different supplementation levels of 0%, 2%, 4%, 6%, 8%, and 10% using two types of supplements: wheat bran and *Greenwayodendron* seed powder . In each level of supplementation, 3 kg of the pre-prepared substrate was measured, supplemented according to the requirements, and then divided into three bags of 1 kg each.

Supplementation levels were calculated as follows:

0%(control): no supplement was added, and 3 kg of the pre-prepared substrate were divided straight into three bags of 1 kg each.

2%: The supplement was added to the substrate at a rate of 60 g to make 3 kg in total. Subsequently, it was divided up into three 1 kg bags.

4%: To arrive at the said 3 kg, 120g of supplement was added to the substrate, then divided into three 1kg bags.

6%: The 180 g of supplement was added to the 3 kg batch and divided into three 1 kg bags.

8%: To make 3 kg, 250 g of supplement was added to the substrate and then divided into three 1-kg bags.

10%: Added 300 g of supplement to make it 3 kg, then divided into three 1 kg bags.

This was done for both wheat bran and *Greenwayodendron* seed powder, therefore, a total of 36 bags were prepared. (6 supplementation levels × 3 replicates × 2 supplement types).

After bagging, substrate bags were subjected to pasteurization for 4 hours. This was done to remove contaminants and competitors of the mycelia, hence providing a conducive environment for its growth.

2.5 Inoculation of Bags

After pasteurization, the substrate bags were allowed to cool to room temperature. Inoculation was then carried out under strict aseptic conditions to prevent contamination. The procedure was performed while wearing a face mask and hair covering to maintain sterility. All equipment used, including scalpels and work surfaces, was cleaned with methylated spirit before iinoculation. The spawn was introduced into the cooled, pasteurized substrate bags and mixed to ensure even distribution for uniform colonization. The inoculated bags were then sealed and transferred to a dark room, where they were left to allow proper mycelial colonization under optimal conditions.

CHAPTER THREE

RESULTS

The mycelial colonization of *Pleurotus tuberregium* was clearly improved with the addition of wheat bran and *Greenwayodendron* seed powder as supplements. Between the two *Greenwayodendron* seed powder, supported faster and more complete colonization. The substrate supplemented with *Greenwayodendron* seed powder reached 50% colonization as early as 12.00 ± 0.00 days and full colonization between 17.67 ± 2.52 and 20.00 ± 0.00 days. For wheat bran, 50% colonization was attained between 14.00 ± 1.73 and 12.00 ± 0.00 days, though its 100% colonization was not attained within the period observed. This is contrary to what was obtained from the unsupplemented (0%) substrate, whose colonization was between 18.14 ± 0.20 and 21.15 ± 0.15 days. These results showed that supplementation enhanced nutrient balance in the substrate and mycelial spread, with *Greenwayodendron* having a more pronounced effect than wheat bran (Table 1).

	50% COLONIZATION		100% COLONIZATION	
	wheat bran	Seed powder	wheat bran	Seed powder
0%	18.41± 0.20	17.21± 1.15	18.41 ± 0.58	19.58 ± 1.73
2%	14.69± 0.58	12.33± 0.58	N. D	19.00± 1.73

4%	14.00± 1.73	12.00± 0.00	N. D	17.67± 2.52
6%	13.33± 1.15	12.00± 0.00	N. D	20.00± 0.00
8%	12.00± 0.00	12.00± 0.00	N. D	18.33± 2.89
10%	12.00± 0.00	12.00± 0.00	N.D	19.00± 3.61

Table 1: Effect of suppplementation on the mycelial colonization of sawdust substratesof *Pleurotus tuberregium*

The time required for the formation of primordia and fruiting bodies was also affected by the level and type of supplementation. Both supplements reduced the number of days needed for introduce compared to the control. wheat bran supported faster development, with primordia appearing from 25.67 ± 1.53 days at 2% to 21.67 ± 0.58 days at 10%, and fruiting bodies emerging from 30.00 ± 1.00 to 22.00 ± 2.00 days respectively. Similarly, *Greenwayodendron* seed powder supplement recorded primordia formation between 25.70 ± 0.58 and 22.00 ± 1.00 days, while fruiting occurred between 26.70 ± 0.58 and 23.00 ± 1.00 days. These results depict that both supplements stimulated faster fruiting, with W.B showing slightly earlier responses at higher levels. However, at 0% (control), primordia and fruiting bodies were not detected throughout the observation period but the formation of sclerotia, a typical survival structure for *P. tuberregium* under nutrient-limited conditions, was noted (Table 2).

Table 2: Effect of suppplementation on primodia and fruiting emergence of sawdust substrates of *Pleurotus tuberregium.*

	PRIMODIAL EMERGENCE		FRUITING EMERGENCE	
	wheat bran	Seed powder	wheat bran	Seed powder
0%	N.D	N.D	N.D	N.D
2%	25.67± 1.53	25.70± 0.58	30.00± 1.00	26.70± 0.58
4%	24.67± 0.58	23.67± 2.58	26.67± 0.58	25.00± 2.64
6%	23.67± 1.15	25.00± 0.00	25.70± 1.15	26.00± 0.00
8%	21.67± 0.58	22.00± 1.00	23.00± 1.00	23.00± 1.00
10%	21.67± 0.58	24.00± 1.00	22.00± 2.00	25.00± 1.00

The yield performance of *Pleurotus tuberregium* differed with supplement type and level. *Greenwayodendron* seed powder gave the best result for all parameters measured: fresh weight, dry weight, and biological efficiency increased with the increase in supplement level from 194 g at 2% to 790 g at 10%, 12.38 g to 50.43 g, and 53.89% to 219.44%, respectively. Wheat bran increased yield, but to a lesser extent than *Greenwayodendron*: fresh weight rose from 125 g to 670 g and dry weight from 7.98 g to 42.77 g, while biological efficiency increased from 34.72%

to 186.11%. At 8%, G.W gave 710 g fresh weight, 45.32 g dry weight, and 197.22% biological efficiency, while at the same rate, W.B yielded 560 g, 35.74 g, and 155.65%, respectively. At 0% (control), there was no fruit body formed, but there was an underground tuber formed from which fresh and dry weight was read. Both supplements improved yield, and their higher concentration supported higher yields. Best biological efficiency was recorded at 10% *Greenwayodendron* seed powder supplementation, indicating its superiority over other supplements in enhancing substrate utilization and mushroom yield (Table 3).

Table 3: Effect of suppplementation on the freash weight, dry weight and biological efficiency.

	Fresh Weight		Dry Weight		B.E%	
	wheat bran	Seed powder	Wheat bran	seed powder	wheat bran	seed powder
0%	57	53	3.64	3.38	15.83	14.72
2%	125	194	7.98	12.38	34.72	53.89
4%	250	340	15.96	21.70	69.44	94.44
6%	470	510	30.00	32.55	130.56	141.67
8%	560	710	35.74	45.32	155.56	197.22
10%	670	790	42.77	50.43	186.11	219.44

Plate 1: Fully colonized spawn bottle of *pleuratus tuberregium*

Plate 2: Substrates begs of *pleurotus tuberegium* at 50% colonization

Plate 3: fruiting of 10% wheat bran as supplement

Plate 4: fruiting of 10% *Greenwayodendron* seed powder as supplement

Plate 5: fruiting of 2% *Greenwayodendron* seed powder as supplement

Plate 6: Primodial emergence of 10% wheat bran as supplement.

CHAPTER FOUR

DISCUSSION

The results of this study show that supplementation with *Greenwayodendron* seed powder and wheat bran significantly enhanced the growth and yield performance of *Pleurotus tuberregium* compared to the unsupplemented control. This agrees with earlier findings that nutrient-rich supplements improve substrate composition and stimulate faster mycelial development and higher biological efficiency in *Pleurotus* species (Chang and Miles, 2004; Kadiri and Fasidi, 1990). The faster and full colonization observed in the *Greenwayodendron* supplemented substrate indicates that *Greenwayodendron* seeds provide key nutrients which support vigorous mycelial spread. In this study *Greenwayodendron* achieved full colonization between 17 and 20 days, while the control took over 21 days. This agrees with (Adedokun and Thomas,2018), who reported that nutrient enrichment increases substrate digestibility, allowing quicker mycelial penetration. A better result was obtained from *Greenwayodendron* seed powder than from wheat bran, possibly because of its higher organic matter and oil content, which supplied more readily available carbon and nitrogen sources required for fungal metabolism and faster colonization. Supplementation further affected the emergence of primordia and fruiting bodies. Bags supplemented with *Greenwayodendron* seed powder and wheat bran formed primordia and fruited several days earlier than the control. wheat bran was slightly better at higher concentrations, probably because of its balanced carbon-to-nitrogen ratio, which supports fruiting initiation. In a similar vein, (Zadrazil 1980) also reported a related trend when he noted that a moderate level of nitrogen promotes fruit body formation in *Pleurotus* species. In contrast, the control bags produced no fruiting bodies but instead produced sclerotia, a survival structure characteristic of *Pleurotus tuberregium* under nutrient-deficient conditions (Osemwegie et al., 2013).The yield results further confirm the benefits brought about by supplementation. Thus, both *Greenwayodendron* seed powder and wheat bran increased fresh weight, dry weight, and biological efficiency of the mushrooms, with the best performance recorded at 10% supplementation. *Greenwayodendron* seed powder at 10% produced 790 g fresh weight, 50.43 g dry weight, and 219.44% biological efficiency, which were higher than those from W.B at the same level. These results agree with (Chang and Buswell, 1996), who stated that the nutrient composition of the substrate directly influences fruit body formation and, therefore, yield. The higher biological efficiency observed in *Greenwayodendron* seed powder-supplemented substrates supports the findings of (Kadiri and Fasidi 1990), who noted that organic supplements enhance substrate utilization by *Pleurotus* species. The steady increase in yield with higher supplement levels shows that nutrient enrichment promotes greater metabolic and enzymatic activity, improving the breakdown of lignocellulosic materials. However, supplementation levels above 10% may not result in increased performance, considering that too much nitrogen has already been reported to decrease fruit body formation (Zadrazil, 1980). While wheat bran continues to be an effective and popular supplement for mushroom culture, the results of this study indicate that the local *Greenwayodendron* seed powder performed better and could be a more suitable alternative. At 0% supplementation, fruiting could not be observed. The formation of underground tubers, however, enabled the measurement of fresh and dry weights. This agreed with the earlier reports that under a poor nutrient composition, *Pleurotus tuberregium* will form sclerotia rather than fruiting bodies, also confirmed by (Oso, 1977) and (Candy, 1990).

4.2 Recommendations:

On the basis of the findings of this research, it is here recommended that *Greenwayodendron* seed powder must be adopted as an effective supplement for the production of *Pleurotus tuberregium* as it significantly improves mycelial colonization, fruiting, and yield compared to wheat bran and the control without any supplement. For the best result, an optimal supplementation rate of 10% *Greenwayodendron* seed powder is suggested since it obtained the best biological efficiency and yield performance in this study. Anything above that must be shunned since too much nitrogen will lower the production of fruit bodies.

Further studies are encouraged to:

1. Analyze the nutrient composition of *Greenwayodendron* seeds to better understand the components responsible for the improved growth and yield.

2. Determine the optimal supplementation rate for large-scale cultivation.

3. Evaluate the economic feasibility of using *Greenwayodendron* seed powder compared to conventional supplements like wheat bran.

4. Explore the potential of other agricultural by-products as alternative supplements to reduce waste and promote sustainable mushroom cultivation.

Supplementing with *Greenwayodendron* seeds also reduces waste in the environment, as these seeds are usually discarded despite their nutritional value. Finally, farmers and researchers should also look into the promotion of the cultivation and use of *Greenwayodendron* trees, whose seeds will serve dual agricultural and environmental purposes while guaranteeing extra sources of income.

4.3 CONCLUSION

In conclusion, this study has illustrated that *Greenwayodendron* seed powder supplementation greatly enhances growth and yield performance of *Pleurotus tuberregium* compared to wheat bran and the 0% (contro). *Greenwayodendron* seed powder enabled rapid mycelial colonization, primordia initiation at an earlier stage, and greater biological efficiency, which were best performed at 10% supplementation. This enhanced growth is most likely a result of the high organic matter and nutrient content of *Greenwayodendron* seeds, which produced an available carbon and nitrogen source that facilitated enhanced substrate degradation and fungal metabolic processes. The control substrates that produced only sclerotia also confirmed that nutrient supplementation is a prerequisite for fruiting in *Pleurotus tuberregium*. Using *Greenwayodendron* seed powder not only improves mushroom productivity but also helps reduce environmental waste, as these seeds are often discarded despite their value. Therefore, adopting *Greenwayodendron* seed powder as a supplement offers an efficient, low-cost, and eco-friendly approach to sustainable mushroom cultivation.

REFERENCES

Adebayo, E. A., Oloke, J. K., Ayandemi, J. A., & Adegunlola, C. O. (2017). Phytochemical, nutritional, and antioxidant activity of *Pleurotus tuber-regium* (Fr.) Sing's sclerotia. *Current Research in Environmental and Applied Mycology*, **7(3):** 244–251.

Adedokun, O. M., & Friday, J. N. (2020). Evaluation of organic substrates for outdoor cultivation of *Pleurotus tuber-regium*. *The International Journal of Biotechnology,* **9(1):** 8–13.

Adedokun, O. M., & Thomas, B. T. (2018). Effect of nutrient supplementation on mycelial growth and yield of *Pleurotus* species cultivated on different agro-wastes. *Nigerian Journal of Mycology*, **10(2):** 45–53.

Adedokun, O. M., & Thomas, R. (2018). Adapting sawdust for *Pleurotus tuber-regium cultivation. Acta Horticulturae*, 1225, 447–454.

Adenipekun, C. O., & Lawal, R. (2012). Uses of mushrooms in bioremediation: *A review. Biotechnology and Molecular Biology Reviews*, **7(3):** 62–68.

Aditya, J. P., & Bhatia, A. (2020). Mushroom cultivation: A sustainable approach for income generation and waste recycling. *International Journal of Current Microbiology and Applied Sciences*, **9(6):** 164–173.

Alam, N. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh—*Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida and Calocybe indica. Mycobiology*, **36(4):** 228–232.

Avagyan, A., Trchounian, A., & Gabrielyan, E. (2013). Antioxidant and antimicrobial activity of extracts from fruiting bodies of *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. *European Journal of Pharmaceutical and Medical Research*, **2(3):** 23–27.

Bala, N. (2012). Antimicrobial potential of *Pleurotus tuber-regium* (Fr.) Sing. *African Journal of Microbiology Research,* **6(4):** 807–812.

Candy, R. (1990). Observations on the formation of sclerotia in *Pleurotus tuber-regium. Mycologia,* **82(2):** 234–239.

Chang, S. T., & Buswell, J. A. (1996). Biochemistry and molecular biology of mushroom development. *Fungal Genetics and Biology*, **21(2):** 147–161.

Chang, S. T., & Miles, P. G. (2004). *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact.* CRC Press, Boca Raton. 451p.

Chang, S. T., Lau, O. W., & Cho, K. Y. (2008). The cultivation and nutritional value of Pleurotus species (oyster mushrooms). *Food Reviews International*, **4(3):** 231–262.

Chirinang, P., & Intarapichet, K. O. (2009). Amino acids and antioxidant properties of the oyster mushrooms *(Pleurotus ostreatus and Pleurotus sajor-caju). ScienceAsia,* **35(4):** 326–331.

Cohen, R., Persky, L., & Hadar, Y. (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus. Applied Microbiology and Biotechnology*, **58(5):** 582–594.

Fasidi, I. O. (2008). Cultivation of edible tropical mushrooms. Ibadan University Press, Ibadan. 221p.

Flores, C. (2006). *Mushroom cultivation as a small-scale family enterprise*. FAO Diversification Booklet No. 7, Rome

Girmay, Z., Gorems, W., Birhanu, G., & Zewdie, S. (2016). Growth and yield performance of P*leurotus ostreatus* (Jacq. ex Fr.) Kumm on different substrates. *AMB Express*, **6(1):** 87.

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control*, 46, 412–429.

Jayakumar, T. (2011). Antioxidant and anti-inflammatory activity of *Pleurotus florida on paracetamol-induced liver injury in rats. Food and Chemical Toxicology*, **49(8):** 1618–1624.

Jedinak, A., & Silva, J. M. (2008). Cytotoxic activity of edible and medicinal mushrooms on colon cancer cells. *International Journal of Oncology*, **33(6):** 1307–1313.

Kadiri, M., & Fasidi, I. O. (1990). Studies on the cultivation of *Pleurotus tuber-regium* (Fr.) Sing. on different agro-industrial wastes. *Proceedings of the Nigerian Mycological Society*, **3(1):** 10–15.

Khan, M. A., & Tania, M. (2012). Nutritional and medicinal importance of *Pleurotus* mushrooms: An overview. *Food Reviews International*, **28(3):** 313–329.

Khan, N. A., Ajmal, M., & Iqbal, M. (2008). Cultivation of *Pleurotus* species and its role in recycling of lignocellulosic wastes in Pakistan. *Pakistan Journal of Botany*, **40(5):** 2005–2013.

Khoury, D. E., Cuda, C., Luhovyy, B. L., & Anderson, G. H. (2012). Beta glucan: Health benefits in obesity and metabolic syndrome. *Journal of Nutrition and Metabolism*, 2012, 851362.

Koyyalamudi, S. R., Jeong, S. C., Song, C. H., Cho, K. Y., & Pang, G. (2013). Vitamin D_2 formation and bioavailability *from Agaricus bisporus mushrooms treated with ultraviolet irradiation. Journal of Agricultural and Food Chemistry*, **57(8):** 3351–3355.

Kuforiji, O. O., & Fasidi, I. O. (2007). Biodegradation of agro-industrial wastes by *Pleurotus tuber-regium* (Fr.) Sing, a Nigerian edible mushroom. *Advances in Food Sciences*, **29(2):** 70–73.

Kuforiji, O. O., & Fasidi, I. O. (2009). Nutritional value of *Pleurotus tuber-regium* (Fr.) Sing cultivated on different agricultural wastes. *African Journal of Biomedical Research*, **12(1)** 63–67.

Kulshreshtha, S. (2010). *Fungal biodegradation of lignocellulosic waste for environmental management and sustainable agriculture.* LAP Lambert Academic Publishing.

Lindequist, U., Niedermeyer, T. H. J., & Jülich, W.-D. (2005). The pharmacological potential of mushrooms. Evidence-Based Complementary and *Alternative Medicine*, **2(3)** 285–299.

Marshall, E., & Nair, N. G. (2009). *Make money by growing mushrooms*. FAO.Rome. 144p.

Martinez-Carrera, D. (1999). Mushroom biotechnology in tropical America: Challenges and opportunities. *Micológica Aplicada Internacional*, **11(2):** 73–82.

McDowell, L. R. (2003). *Minerals in animal and human nutrition* (2nd ed.). Elsevier Science.

Musieba, F. (2013). Comparative analysis of nutritional composition of *Pleurotus tuber-regium* (Fr.) Sing and *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm. *Journal of Food Research*, **2(3):** 93–102.

Oei, P. (2003). *Mushroom cultivation: Appropriate technology for mushroom growers* (3rd ed.). Backhuys Publishers.

Osemwegie, O. O. (2010). Diversity of macrofungi in Edo State, Nigeria. *Nigerian Journal of Mycology*, 3, 65–78.

Osemwegie, O. O., Ekanem, M., & Asemota, S. (2013). Biotechnological potentials of Nigerian macrofungi in environmental sustainability. *African Journal of Biotechnology,* **12(31):** 4930–4945.

Osemwegie, O. O., Okhuoya, J. A., & Oghenekaro, A. O. (2013). Physiological studies of sclerotia of *Pleurotus tuber-regium* (Fr.) Singer *in selected soil types in Nigeria. Mycosphere*, **4(6):** 1046–1054.

Oseni, T. O., Dube, J. K., Wahome, P. K., & Masarirambi, M. T. (2012). Cultivation of oyster mushroom (Pleurotus spp.) on different substrates. *International Journal of Agriculture and Biology*, **14(2):** 251–256.

Oso, B. A. (1977). *Pleurotus tuber-regium from Nigeria. Mycologia*, **69(2):** 271–279.

Otunla, C. A., Jonathan, S. G., Idowu, O. O., & Olawuyi, O. J. (2018). Mycelial growth and sclerotia production of *Pleurotus tuber-regium on four sawdust types at three composting intervals. Agronomy*, **80(2):** 79–90.

Oyetayo, O. V. (2011). Medicinal uses of mushrooms in Nigeria: Towards full and sustainable exploitation. *African Journal of Traditional, Complementary and Alternative Medicines,* **8(3):** 267–274.

Patel, Y., Naraian, R., & Singh, V. K. (2012). Medicinal properties of *Pleurotus* species (oyster mushroom): A review. *World Journal of Fungal and Plant Biology*, **3(1):** 1–12.

Royse, D. J., Baars, J., & Tan, Q. (2004). Current overview of mushroom production in the world. *Mushroom Growers' Handbook 1: Oyster Mushroom Cultivation*, 1–6.

Sari, M., Prange, A., & Lelley, J. (2017). Screening of beta-glucan content in commercial mushrooms from different regions. *Food Chemistry*, 216, 45–51.

Shah, Z. A., Ashraf, M., & Ishtiaq, M. C. (2004). Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Pakistan Journal of Nutrition*, **3(3):** 158–160.

Sher, H. (2006). *Mushroom cultivation for sustainable livelihoods: A case from NWFP, Pakistan*. IUCN Pakistan, 1-46.

Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology,* 2015, 376387.

Vamanu, E. (2013). Antioxidant and antimicrobial activities of ethanol extracts of *Pleurotus ostreatus and Pleurotus eryngii. Annals of Microbiology*, **63(2):** 471–478.

Woldegiorgis, A. Z., Abate, D., Haki, G. D., & Ziegler, G. R. (2014). Major, minor, and toxic minerals and anti-nutritional factors in *Pleurotus ostreatus and Pleurotus cystidiosus* mushrooms. *Food Science & Nutrition*, **2(5):** 386–391.

Wu, G. S., Lu, J. J., Guo, J. J., Huang, M. Q., Gan, L., Chen, X. P., & Wang, Y. T. (2011). Anti-cancer properties of *Trametes versicolor* (Yunzhi) extract and its major component polysaccharopeptide. *Biochemical Pharmacology*, **81(7):** 1136–1144.

Xu, X. (2011). Medicinal potential of the genus *Pleurotus* (oyster mushrooms). *World Journal of Microbiology and Biotechnology*, **27(2):** 270–275.

Zadrazil, F. (1980). Influence of ammonium nitrate on the yield of *Pleurotus* florida (Mont.) Singer. *European Journal of Applied Microbiology and Biotechnology*, **9(1):** 31–35.