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OYSTER MUSHRROM CULTIVATION AT DIFFERENT PRODUCTION SYSTEMS: A REVIEW

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ABSTRACT
This paper aims to review historical perspectives, nutritional and medicinal benefits, biodiversity, cultivation, diseases and their control, pathogen detection systems, food safety concerns and use of spent mushroom compost of oyster mushrooms. The genus *Pleurotus* (oyster mushrooms) is a diverse group of mushrooms high in nutritional value and other useful properties applicable to the environmental. The production and consumption of oyster mushrooms has increased owing to its benefits. Over the past few years, there have been efforts to identify locally available substrates, improve spawn production and limit disease incidences. Pathogen detection systems have also been improved in an effort to develop rapid and more accurate assays leading to reduced production losses. The use of chemicals threatened by development of resistance by pathogens as well as new legislations, hence alternative treatment methods have to be fully implemented. Lastly, despite its huge potential, spent mushroom compost is underexploited sometimes utilized for land filling and in crop production.

KEYWORDS: Review, oyster mushrooms, cultivation techniques, molecular pathogen detection systems, spent mushroom compost.

INTRODUCTION
Oyster mushrooms belong to the genus *Pleurotus*, which is a diverse group of over twenty five species of saprotrophic fungi such as *P. columbinus*, *P. flabellatus*, *P.florida*, *P. ostreatus* and *P. sajor-caju*. These species are primary decomposers and they grow well under natural conditions on tree stumps or dead woody branches of trees.[1] Hence, oyster mushrooms are the easiest to cultivate, and require less production technology.[2] The popularity of oyster mushrooms emanates from its taste, nutrients, strong anti-inflammatory, immune-modulatory properties and other medicinal properties. The popularity of these mushrooms is increasing because people have realized their nutritional potential that could alleviate the deficiency of proteins and their possible antimicrobial activity. Thus, the increased production of oyster mushrooms presents a feasible solution to malnutrition experienced in most developing countries. (Khare *et al*).[1]

Oyster mushrooms grow on various sources of lingo-cellulosic material including paddy straw,[3] sawdust,[2] indigenous grasses,[4] wheat-straw[5] and other substrates.[6] The largest producer of oyster mushrooms in the world is China accounting for more than 46% of the global production. The United States of America (USA) and Netherlands account for about 20% of the global production of oyster mushrooms. The African continent remains the least producer of oyster mushrooms despite efforts to train farmers and increase awareness. However, production has increased in countries such as South Africa where commercial farms account for the majority of the yield.

Over time, cultivation methods of oyster mushrooms have evolved in an effort to maintain parity with the increasing demand. This has been evident with assessment of new substrates for better yield and lower costs.[7,8] Diseases such as cobweb and green mould still remain a challenge in oyster mushroom production at small to large scale. These diseases have the capacity to inflict huge economic losses, thus, controlling outbreaks remains priority for farmers. However, recent focus is also on the safety of disease treatment procedures such as fungicides due to maximum residue level (MRL). Hence, development and implementation of food safety management systems is priority especially in developed countries where most of commercial farmers in developing countries have market access. Therefore, this review focuses on the cultivation of oyster mushrooms at different production systems including the latest techniques, diseases affecting production, pathogen detection systems and future prospects of oyster mushroom production.

HISTORICAL PERSPECTIVE OF OYSTER MUSHROOM CULTIVATION

The production of oyster mushrooms and the research thereof, dates back to 1917 by Falck,[9] who described the cultivation of *Pleurotus* on tree stumps and logs. Subsequently, Kaufert[10] and Etter[11] produced sporocarps of *P. ostreatus* in artificial culture medium. In the late 1960's, Block *et al*.[12, 13] described requirements for this mushrooms using sawdust as substrates. The results of the study showed that *P. ostreatus* grew vigorously on most of the substrates and produced fruits more readily than the other spp. Industrial production of *Pleurotus* in Europe and USA was first established in 1974.[14] Due to increased demand, natural and synthetic substrates were used for oyster mushroom production with the latter leading to increased annual production. In India, Jandaik[15] introduced *P. sajor-caju* thereby laying the basis of its production at a commercial scale. Reports indicate that the production of *Pleurotus* was then spread to other countries such as Mexico,[16] Israel,[17] Tanzania,[18] and many more.

OYSTER MUSHROOM CULTIVATION IN AFRICA

Reports show that there has been some work done on *Pleurotus* cultivation in Africa. *Pleurotus* species were introduced in Mauritius around 1982 and several trials were conducted.[19] *Pleurotus sajor-caju* was the most promising organism cultivated in Mauritius as it is adapted to the climatic conditions of the country. Mushroom production and research in South Africa are also advanced. Although *Agaricus bisporus* is the commonly cultivated mushroom,[20] alternative and locally available substrates for the production of oyster mushrooms have been studied.[21] Mohamed *et al*.[22] introduced different portions of compost in the substrate and evaluated the effect on the yield of *P. columbinus* in Egypt. As a result, the study proposed a formulation that included composted straw and raw rice straw or raw corn straw.

The use of local and affordable substrates for oyster mushroom production is common across the African continent. Cassava (*Manihot esculenta*) is a root crop that is cultivated in large quantities (commercial and subsistence) and used for edible and non-edible purposes in Nigeria. Peels of cassava have been used to produce spawn, replacing sorghum grains thereby reducing the cost of production for mushroom farmers.[7] In Ethiopia, substrates such straws of wheat, barley, sinar, as well as gabi wastes have been used.[8] The study also evaluated the effect of pore size, temperature, and relative humidity on growth of mushrooms. Thereafter, Tesfaw *et al*.[8] recommended pin hole sizes, temperature of 25°C and high relative humidity for oyster (*P. ostreatus*) growth in Ethiopia. In Botswana, research on cultivation of oyster mushrooms started in 1998 and several *Pleurotus* species and strains were screened on locally available crop residues.[23] A high temperature tolerant strain, *P. florida*, performed better than other species but currently a hybrid of *P. ostreatus* and *P. florida* has been found to be the most vigorous and well adapted for the conditions of Botswana. Subsequently, studies concerning affordable substrates and low cost technology were conducted in Botswana,[4] revealing that oyster mushroom production is a potential and viable enterprise for small-scale farmers.[24] A similar study was conducted in Swaziland following enactment by relevant stakeholders to increase awareness in oyster mushroom cultivation.[25] The cultivation of oyster mushrooms is at an advanced stage in Kenya due to the abundance of locally available substrates.[1] More recently, genetic diversity of *Pleurotus* using a molecular based approach was investigated in Kenya generating 293 polymorphic loci across 84 isolates.[26]

BIODIVERSITY OF *PLEUROTUS*

Over two decades ago, Guzmán[27] stated that over a thousand spp. of *Pleurotus* have been described globally despite about fifty valid spp. being recognized, highlighting a taxonomic challenge within the genus. These challenges emanate from several factors including misidentification that was based solely on morphological characterizations, and this was reiterated by other studies that also reported misidentifications.[28] However, the advent of biochemical and molecular protocols in recent years helped clarify taxonomic confusions within this genus[29] as well as other fungal spp.[30] *Pleurotus* spp. growing in association with *Apiaceae* plants have since been described based on polyphasic approaches and establishment of an identification key of the above mentioned spp.[31] As a result, the genus *Pleurotus* is now known to have about fourty spp.[32] including *P. florida*, *P. ostreatus*, *P. eryngii*, *P. pulmonarius* and *P. sajor-caju*, all of which are commonly edible mushrooms.

NUTRITIONAL AND MEDICINAL BENEFITS

There are several benefits including medicinal purposes that accrue from the chemical and nutritional composition of oyster mushroom spp. Oyster mushrooms are rich in protein (29%), dietary fiber (13%), vitamins and minerals, and have no cholesterol.[33] Of the total protein composition in oyster mushrooms, there is an abundance of essential amino acids such as leucine, glutamine and valine.[34] Some chemical compounds derived from *Pleurotus* spp. show activity against some chronic illnesses,[35] while others have antitumor properties and antibacterial.[36] Results of Schillaci *et al*.[36] show that an acid extract containing cationic protein from two spp. of *Pleurotus* (*P. nebrodensis* and *P. eryngii*) inhibited the growth of *Staphylococcus epidermidis* at minimum inhibitory concentration of

≤0.025% v/v. In addition, Hu *et al*.[37] stated that the compounds derived from *Pleurotus* spp. had protective capabilities against cardiovascular diseases. It is important to note that effectiveness of extracts against disease causing organisms and concentration of active compounds varies across different *Pleurotus* spp.

MUSHROOM CULTIVATION TECHNOLOGY

Substrates and their preparation

Substrates are organic materials such as lingo-cellulosic farm waste on which the mycelium grows to produce mushrooms. Some of the substrates that have been used for the production of oyster mushrooms include saw dust and rice bran or wheat bran, rice straw and rice bran, wheat straw and wheat bran. The preparation of these substrates is one of the most critical steps in mushroom production to ensure less disease incidences and better yield. Substrates such as grasses,[4] sugarcane bagasse[25] and others are pasteurized to eliminate potential competitors (e.g. *Trichoderma* spp.) of *Pleurotus* spp. either by dipping the substrate in hot water (±70°C) or using steam for a few hours. Prolonged soaking of substrates in water and treatment with benomyl (0.06g/L) have the ability to lessen/suppress the risk of contaminants such as *Trichoderma* spp.[38] Singh *et al*.[39] conducted an *in vivo* study using Neem (A*zadirachta indica*) oil and ashoka (*Saraca indica*) leaves extract for pretreatment of paddy straw and wheat straw to increase xylanase production required to break down xylan, a major constituent of lignocellulosic material. Most of the treatments described above are applicable and affordable to both commercial and small scale oyster mushroom farmers.

Spawn production

Spawn as mushroom seed is defined as a living ramified mycelium of a mushroom, multiplied on a suitable sterile base material or carrier under aseptic techniques. There are several carriers that can be used for spawn production including grains of wheat[40] and sorghum.[41] Available literature is varied on the choice of grains and amount of additives used in spawn production. Jongman *et al*.[41] concluded that grains of sorghum are better mycelium carriers than those of wheat and barley. The parameters tested in their study included mycelium growth vigor, spawn running time and yield of the oyster mushroom. Das *et al*.,[40] reported that an increase in the percentage of spawn carried with wheat grains resulted in better yields despite no effect on time of fruiting while Stoller[42] preferred rye grain and cotton seed meal for spawn production. The latter suggested the use of gypsum (6g) and chalk (1.5g) per kilogram of grain to inhibit clumping. Zhang *et al*.[43] developed a new type of solid spawn (stick spawn) to cultivate *P. eryngii*. The authors concluded that the adoption of the spawn reduced spawn running time while improving the yield and biological efficiency (BE). Abdullah *et al*.[44] also investigated the potential of producing liquid spawn for *P. pulmonarius* in a bioreactor under controlled conditions. The results of the study showed that the liquid spawn colonized the substrate in a shorter time and produced higher yield of sporophores compared to the traditionally used grain spawn. However, it is important to note that adoption of any new technologies by small scale farmers (especially in sub-Saharan Africa) depends on the accessibility and cost of such an innovation.

Spawning and Incubation

Several factors affecting the quality of spawn include moisture (60-70%) and quality of carrier as these will determine the colonization and growth of the mycelium on the substrate. However, it is important to establish appropriate levels to use that are specific to locally available substrates. The spawned substrate is placed in dark room at temperature ranging from twenty five to thirty degrees Celsius (25 - 30°C), which is optimum for mycelia growth for most of *Pleurotus* spp. It takes three to five (3-5) weeks for the mycelium to completely colonize the substrate, depending on the substrate and *Pleurotus* spp. used. However, a recent study established an appropriate cold stimulation (12 hrs. cold stimulation at 5°C) as a strategy for improving the performance of *P. pulmonarius* and subsequent mushroom yield.[45]

MICROBIOLOGICAL SAFETY AND POSTHARVEST QUALITY

Despite global enactment by relevant stakeholders to develop more effective food safety protocols and improve the microbiological safety of fresh produce along the supply chain, the mushroom industry is still lagging behind in this regard. Unfortunately, there has been a report of *Listeria monocytogenes* isolated at a mushroom facility[46] and subsequent produce recalls from retail markets in the Canada. However, it is important to note that there have been no reports of foodborne disease outbreaks or illnesses linked to mushrooms. Kortei *et al*.[47] assessed the microbiological quality of gamma irradiated oyster mushrooms (*P. ostreatus*). In that study, *Bacillus cereus* was detected but *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and coliforms were not detected. Dzingirayi and Korsten[48] assessed primary production and horticultural safety management systems (HSMS) of mushroom farms in South Africa. The finding of the study was that farm size affects food safety performance and small farms lacked effective HSMS implementation systems. New technologies such as ozonation,[49] gamma irradiation[47] or associated procedures are not economically feasible for small scale farmers. Therefore, development and proper implementation of food safety management systems may assist such farmers to improve produce of microbiological quality.

DISEASES AND LOSSES OF OYSTER MUSHROOMS

There are several diseases such as green mould (*Trichoderma* spp.), brown blotch (*Pseudomonas* spp.) and cob web (*Cladobotryum* spp.) known to limit production of oyster mushrooms causing huge yield losses. An outbreak of green mould was reported at a farm producing *Pleurotus* and *Agaricus* mushrooms in Hungary.[50] This was the first confirmed report about the occurrence of *T. aggressivum* in oyster mushroom cultivation environments. The two species of Trichoderma (*T. pleurotum* and *T. pleuroticola*) were isolated for the first time on substrate samples collected from an oyster mushroom farm in Croatia.[51] Symptoms of cobweb (*C. mycophilum*) disease were noted on oyster (*P. eryngii*) mushrooms in Spain.[52] Up to 85% of the tested samples (fruit bodies) were positive for the causal agent. The economic losses inflicted by outbreaks of these diseases have been reported globally. For instance, brown blotch was responsible about 10% losses worldwide[53] while cobweb outbreaks in epidemic proportions in the mid-1990s led to yield losses of up to 40%.[54] Soković and van Griensven[55] stated that about 25% of production costs could be lost due to three main diseases including green mould. Thus, early detection of these pathogens can help avert massive losses in the mushroom industry.

PATHOGEN DETECTION SYSTEMS

Traditionally, culture techniques have been solely relied upon for the detection and identification of pathogens of mushrooms. However, in recent times, more accurate and sensitive methodologies such as molecular based protocols such as PCRs and matrix assisted laser desorption time of flight mass spectrometry (MALDITOF MS) have been adopted to improve pathogen detection and identification. Although many recent studies have reported on proteomic phenotyping of fungal pathogens using MALDITOF MS,[56] few have concentrated on those related to mushroom production.[57] However, proteomic profiles generated by the MALDITOF MS can help provide more information useful for pathogen intervention strategies in mushroom farms.

A rapid molecular identification assay for improved and specific detection of *Trichoderma* spp.[58] and other fungal pathogens has been developed. Molecular approaches such as amplified fragment length polymorphism (AFLP) have also enabled diversity studies of *Pleurotus* spp.[26] Other characterization technologies include denaturing gradient gel electrophoresis which revealed higher diversity compared to other methods used to profile bacterial isolates associated with mushroom production.[20]

Recent studies have focused on a holistic approach of describing the entire microbial biomes rather than concentrating on individual or closely related species.[59] Next generation sequencing approaches such as Illumina and pyrosequencing analyzes the hypervariable regions of bacterial and fungal rDNA to describe population diversity. These high-throughput sequencing methods have been used to provide deeper knowledge of microbial community dynamics at different agricultural settings such as grapevines[60] and other fresh produce.[59] These novel technologies can be used in mushroom production environments to provide baseline information for a more targeted pathogen intervention approach and reduce postharvest losses. In addition, knowledge of variations of bacterial and fungal microbiota throughout the different production stages of mushrooms can be used to determine when control measures should ideally be applied. Potential bio-control applications against economic diseases can also be revealed by studying pathogen to pathogen interactions. However, such approaches in oyster mushroom production settings are yet to be reported.

CONTROL OF DISEASES

A common method of pathogen control on mushroom farms globally is the application of chemicals such as prochloraz because of their effectiveness.[61] Complete control of *Hypomyces rosellus* (anamorph *Dactylium dendroides*) has been reported using two plant (neem) based products applied in combination with three fungicides: carbendazim, sporgon containing procloraz or anthracol containing propineb.[62] However, recent legislation amendment proposals on the use of chemicals in food production systems in developed countries have exerted pressure to find alternative disease control measures. Simultaneously, other challenges of using chemicals include toxic fungicides against the mushroom mycelium[61] and development of resistance owing to misuse.[63] As a result, the use of more environmentally friendly applications such as essential oils,[64] plant based products[65] and other bio-control formulations have been studied in the recent past.

A native herb in Australia known for oil with antimicrobial properties has been reported to inhibit the growth of *T. harzianum* on oyster mushroom substrate.[66] Nagy *et al*.[67] recovered *Bacillus* isolates that effectively antagonizes *T. pleurotum* without negatively affecting the mushroom. More importantly, the biological applications should not only be effective against the target pathogen, but they should also be safe for human consumption.

UTILIZATION OF SPENT MUSHROOM COMPOST

The substrate from which mushrooms have been cultivated and harvested is called spent mushroom compost (SMC). Despite many studies outlining the potential of SMC as a source for fuel, animal feed and soil fertilizer, it has been underexploited in the past.[68] However, biotechnological applications have led to new SMC products for pesticide degradation[69] and heavy metal filtration.[70] Kwack *et al*.[71] used SMC as a growing media for transplant production of lettuce and reported that SMC had higher nutrient content than the commercial medium. The study also reported a high germination and early growth of lettuce grown on SMC.

A recent study recovered active enzymes such as total cellulase, β-glucosidase, dextranase, amylase and lactase from SMC of several mushrooms including *P. eryngii* and *P. ostreatus*. Applications of these enzymes are beneficial including the use of dextranase to prevent caries by inhibiting dental biofilm formation.[72] Other enzymes such as laccase are useful in wastewater treatment.[73] The utilization and development of applications to reuse SMC, a post production residue, is a fundamental benefit to the mushroom industry as it reduces environmental issues related to its disposal.

CONCLUSION

In recent years, several studies described most aspects associated with oyster mushroom cultivation. That includes the latest molecular techniques for early detection of diseases helps farmers to promptly apply the available control measures. Microbiome studies will also provide a better understanding of the pathogen and its relationship with other microbial epiphytes leading to improved disease control protocols and higher production yields. The advent of these new technologies has improved the cultivation of oyster mushrooms but questions around the economic feasibility still remain a concern. This is particularly true in developing countries where adoption of these technologies is below satisfactory. In future, more biotechnological assays should be implored to harness the potential of products such as SMC. Although chemicals extracted from *Pleurotus* spp. have potential to develop useful products, research is required to authenticate the safety of these compounds.

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