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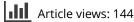
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Bioremediation and sustainable mushroom cultivation: harnessing the lignocellulolytic power of *Pleurotus* species on waste substrates

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ABSTRACT

Mushrooms, a diverse group of fungi, has captivated human interest for centuries due to their intriguing characteristics and versatile application. The nutritional profile, coupled with unique organoleptic qualities, renders mushrooms not only a culinary delight, but also a valuable component of a balanced diet. In addition, mushrooms have gained recognition for their medicinal properties due to certain compounds found within them that exhibit promising pharmacological activities. Among the edible mushrooms, the Oyster mushroom (*Pleurotus* spp.) is commercially important in the global mushroom market and is widely cultivated and consumed in various parts of the world. Beyond the culinary appeal and medicinal properties of Pleurotus species, their cultivation holds an important environmental significance. These mushrooms are efficient decomposers of lignocellulosic biomass, contributing to agricultural and forestry waste recycling. Many researchers have demonstrated how their cultivation can serve as a sustainable practice, reducing the environmental impact of organic waste while generating a valuable food resource. In Sri Lanka, oyster mushroom cultivation is practiced on a small scale as a self-employed cottage industry. Four main species of oyster mushrooms, namely P. djamor, P. eous, P. ostreatus, and P. cystidiosus, are commercially cultivated in Sri Lanka. Obtaining an efficient, cost-effective, and sustainable substrate is a key factor in promoting the mushroom industry within the country, ensuring export-quality mushroom production. Recently, there have been some attempts to use unconventional substrate materials, such as used diapers, as an innovative and eco-friendly approach, where the high cellulose content of diapers serves as a substrate for fungal growth, while, repurposing problematic non-biodegradable waste streams for agricultural practices. a.

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Introduction

Mushrooms are primarily classified in the taxonomic division Basidiomycota. They are characterised as macro-fungi with a discernible fruiting body, which may either be hypogenous or epigeous. These fruiting bodies are large enough to be visible to the unaided eye and can be manually harvested (Chang and Miles 2004; Ogidi et al. 2020).

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Mushrooms have been consumed throughout human history, with the ancient Greeks attributing them to imparting power to heroes in combat, while the Romans regarded them as divine sustenance (Zázik and Daňová 2022) The Chinese culture, mushrooms have long been highly esteemed as a health-promoting meal, often referred to as an 'elixir of life'. Throughout the course of human history, these entities have played a significant role in various civilisations, garnering substantial attention due to their sensory attributes (Jordan 2015; Rahi and Malik 2016).

There are a total of 14,000 distinct species of mushrooms that exist worldwide, with approximately 50%, or 7000 species, recognised for their varying levels of edibility. Furthermore, within this group, more than 3000 species belonging to 31 different genera are acknowledged as highly edible mushrooms, while approximately 200 species are classified as medicinal mushrooms (Sharma and Gautam 2015; Qwarse et al. 2021). According to Chang and Wasser (2017), there are 200 experimentally developed specimens, with an additional 100 being cultivated for economic purposes. Moreover, approximately, 60 species are under commercial cultivation, and a smaller subset of around 10 species has achieved industrial-scale production in numerous nations. Commonly cultivated species include *Agaricus bisporus* (button mushrooms), *Lentinula edodes* (shiitake mushrooms), and *Pleurotus* spp. (oyster mushrooms) (Mahari et al. 2020).

Mushrooms can be broadly classified into four distinct categories (Chang and Miles 2004.) Firstly, there are edible mushrooms, such as *Agaricus bisporus*, characterised by their fleshy nature and suitability for consumption. Secondly, there are medicinal mushrooms, like *Ganoderma lucidum*, recognised for their potential therapeutic applications. Thirdly, there are poisonous mushrooms, such as *Amanita phalloides*, scientifically proven or suspected to be toxic. Lastly, there is a miscellaneous category that includes numerous mushrooms with poorly defined properties (Chang and Miles 2004; El Sheikha and Hu 2018).

The genus *Pleurotus* stands as a globally renowned culinary delight, highly prized for its exceptional flavour and versatility in various cuisines (Bulam et al. 2022). *Pleurotus* comprises about 40 species distributed in a wide range of tropical and temperate regions (Raman et al. 2021). *They are* one of the most cultivated mushroom species, contributing to more than 19% of global mushroom production (Moshtaghian et al. 2022). The nutritional value of *Pleurotus* species renders them a favourable dietary choice. This mushroom is a nutrient-rich food source, abundant in protein, fibre, carbohydrates, vitamins, and minerals (Raman et al. 2021). Additionally, it is characterised by a pleasant aroma and possesses culinary attributes along with medicinal and nutraceutical properties (Kadam et al. 2023). The cultivation of the oyster mushroom was first initiated by Flank in Germany in 1917. This genus is widely known as a highly diversified collection of cultivated mushrooms, exemplifying the characteristic life cycle of Basidiomycetes (Adebayo and Oloke 2017).

Nutritional value of edible mushrooms: a culinary phenomenon

Mushrooms have been popular as a prominent component in gourmet cuisine worldwide, primarily due to their distinctive flavour profile, and remarkable culinary value (Valverde et al. 2015; Rahman et al. 2022). Mushrooms possess significant nutritional value due to their high protein content, substantial presence of vital amino acids and fibre, and low-fat level coupled with favourable fatty acid composition (Valverde et al. 2015). The protein content of four commonly consumed edible mushrooms; *Agaricus bisporus, Lentinula edodes, Pleurotus* spp., and *Flammulina velutipes*, has been reported in published literature. These mushrooms are commercially cultivated in different countries. The reported protein content ranges from 1.75% to 3.63% of their fresh weight (Reis et al. 2012).

Certain researchers have argued that the amino acid contents of mushrooms resemble those found in animal proteins (Mattila et al. 2001). Leucine, valine, glutamine, glutamic acid, and aspartic acid are among the most abundant amino acids found in mushrooms (Lee et al. 2011). In addition, studies by Mattila et al. (2001) and Heleno et al. (2010) have emphasised that edible mushrooms serve as a significant source of various vitamins, such as B1, B2, B12, C, D, and E. Mushrooms are low-calorie food sources due to their relatively low content of fat, often ranging from 20 to 30 grams per kilogram of dry matter (Assemie and Abaya 2022). The primary fatty acids found in mushrooms include linoleic acid (C18:2), oleic acid (C18:1), and palmitic acid (C16:0) (Sande et al. 2019). Edible mushrooms possess significant levels of ash, ranging from 80 to 120 g/kg of dry matter. This ash mostly consists of essential minerals such as calcium, copper, iron, magnesium, phosphorus, potassium, and zinc (Valverde et al. 2015).

The nutritional contents of many *Pleurotus* species have been extensively documented in the scientific literature (Khan et al. 2008; Khan and Tania 2012; Galappaththi et al. 2021; Raman et al. 2021) and are well-acknowledged as a valuable protein source. The protein content of dried fruit bodies from several species of Pleurotus has been shown to range from 11 to 42 g per 100 g, as documented in studies (Raman et al. 2021; Lesa et al. 2022). Hence, the high levels of protein make oyster mushrooms an alternative source of dietary protein. Natural lipids play important roles in energy storage, the formation of cell membranes, and in intracellular signalling regulation (Lesa et al. 2022). The lipid content of Pleurotus species varies among different species within the genus, typically ranging from 0.2 to 8 g per 100 g of dried fruit bodies (Lesa et al. 2022). While Pleurotus species are generally low in fat, they do contain several important fatty acids such as oleic acids and linoleic acids (Raman et al. 2021). Furthermore, *Pleurotus* mushrooms are widely recognised as a valuable dietary resource owing to their high content of carbs and dietary fibre. Polysaccharides or glycoproteins are the primary forms in which carbohydrates are predominantly found (Lesa et al. 2022). The polysaccharides found in the highest quantities include chitin, α - and β -glucans, along with various hemicelluloses (Synytsya et al. 2008; Lesa et al. 2022). Pleurotus sp. contains a specific glucan called pleuran, which serves as a source of antitumour polysaccharides (Khan and Tania 2012). The mineral analysis data from several research indicate that the Pleurotus species can serve as a valuable source of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc, thus, including these mushrooms in the diet could be one of the strategies for combating iron, zinc, and other micronutrient deficiencies (Ezeibekwe et al. 2009). Pleurotus mushrooms are not widely regarded as being highly abundant in all vitamins. Nevertheless, there have been publications documenting the vitamin composition of *P. ostreatus*, particularly the presence of vitamin B complex (Caglarirmak 2007; Lesa et al. 2022).

Bioactive compounds in mushrooms: a health-promoting elixir

Edible mushrooms possess the potential to serve as a valuable reservoir of diverse nutraceuticals, including unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids (Reis et al. 2012). These bioactive compounds, when consumed as part of the human diet, can collectively contribute to the promotion of overall health. This is attributed to the synergistic interactions among the various bioactive components found within mushrooms (Barros et al. 2008). Phenolics, flavonoids, and tannins are the key secondary metabolites of mushrooms that are responsible for their antioxidant activity (Azieana et al. 2017). Consumption of foods rich with phenolics and polyphenols can form protection against several ailments, including heart diseases and cancer. These compounds act as free radical scavengers with antioxidant properties (Jayakumar et al. 2009; Valverde et al. 2015). They also possess a diverse array of physiological characteristics, including antiallergenic, antiatherogenic, anti-inflammatory, antibacterial, antithrombotic, vasodilator, and antioxidant capabilities (Ferreira et al. 2009; Devi et al. 2024). Flavonoids are considered one of the most diverse and widespread groups of natural phenolics, exhibiting the highest degree of antioxidant activity, which can reduce the access of deleterious molecules and protect the structure and function of membranes from oxidants (Azieana et al. 2017). Tocopherols are commonly accepted as natural antioxidants with significant biological activity, protecting against degenerative disorders, cancer, and cardiovascular ailments (Ferreira et al. 2009).

The most important are polysaccharides, structural components of the fungal cell wall (Pérez-Bassart et al. 2023). The polysaccharides have a strong ability to carry biological information. More specifically, they exhibit antitumour, immunomodulatory, antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities (Venturella et al. 2021). β -glucans constitute the predominant polysaccharides present in mushrooms, comprising around 50% of the fungal cell wall mass (Bhambri et al. 2022). The average concentration of β -glucans varies between 0.21 and 0.53 g/100 g on a dry basis (Chen and Seviour 2007). Beta-glucans (β -glucans) have been identified as the active compounds contributing to the anticancer, immunomodulating, anticholesterolemic, antioxidant, and neuroprotective properties observed in several edible mushrooms. Furthermore, it has been known that they possess significant immunological stimulatory properties in the human body (Khan et al. 2013; Bai et al. 2019).

Multifaceted medicinal attributes of mushrooms: fungal pharmacopeia

The therapeutic properties of medicinal mushrooms have been popular in China, Korea, and Japan for centuries. The application of mushrooms for health maintenance was formally recorded as early as 100 AD in China (Khan and Tania 2012). Notably, these organisms possess antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protective, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects. Additionally, their therapeutic potential extends to preventing tumour formation and mitigating inflammatory processes (Chang and Wasser 2012; Hassan et al. 2023). Based on scientific research, it has been documented that extracts or powders derived from the fruit bodies or mycelium of *Pleurotus* mushrooms possess various beneficial medicinal properties, including

anticancer, antihypercholesterolemic, antihypertensive, antidiabetic, antiobesity, hepatoprotective, antiaging, antimicrobial, and antioxidant activities (Khan and Tania 2012; Golak-Siwulska et al. 2018; dos Reis et al. 2022). Jedinak and Sliva (2008) reported an anticancer effect of the methanol extract of P. ostreatus on some breast and colon cancer cells, suppressing their proliferation while not affecting the proliferation of epithelial mammary and normal colon cells. The antimicrobial activity of mushrooms may be attributed to the presence of various bioactive secondary metabolites, proteinpolysaccharide compounds, certain phenols, phytochemicals, and free fatty acids (Bhambri et al. 2022). The polysaccharide extract from P. australis showed antibacterial activity against Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis, Escherichia coli 916, and Enterobacter aerogenes (Ren et al. 2014). Pleurotus ostreatus could be used to reduce blood glucose, cholesterol, and triglycerides in diabetic patients without adverse effects on liver and kidney function (Khatun et al. 2007). Similarly, the hepatoprotective activity of *Pleurotus* mushrooms is thought to be mainly due to their antioxidant potential (Jayakumar et al. 2006). Lentinus sajor-caju, P. floridanus, and P. ostreatus, have been observed to mitigate lipid peroxidation in hepatic tissue in hypercholesterolemic conditions (Alam et al. 2009; Khan et al. 2011a, 2011b). In addition to the antioxidant phenolic and flavonoid compounds, *Pleurotus* species are also rich in vitamin E and selenium content, which are important natural antioxidants in the biological system (Fakoya et al. 2020). Several studies, both in vitro and in vivo, evaluated the capacity of mushroom fruit bodies or mycelium and their extract to protect cells and tissues from oxidative injury (Alam et al. 2008; Liuzzi et al. 2023).

Mushroom poisoning: a minority, but a serious concern

Consuming toxic mushrooms, mistakenly collected with edible ones, can lead to poisoning. The incidence of mushroom poisoning markedly varies among different parts of the world (Govorushko et al. 2019). Poisonous mushrooms comprise a minority, accounting for less than 1% of the global inventory of known mushrooms. Despite their relatively low prevalence, it is crucial not to disregard the presence of these particular species, given their hazardous properties that can occasionally lead to lethal consequences (Chang and Miles 2004; Jo et al. 2014). The chemical composition of toxins found in different poisonous mushrooms significantly varies, leading to varying consequences (Yin et al. 2019). Poisonous mushrooms are divided into three groups concerning their toxicity. The first group includes fungi that contain locally acting toxins, frequently present in Agaricus xanthodermus, A. moelleri, and Rubroboletus satana which disturb the gastrointestinal tract, and their toxicity develops in 1-2 h after consumption (Morel et al. 2018). The second group comprises mushrooms with toxins that affect the nervous centres, including some Amanita and Inocybe species. Nausea, vomiting, diarrhea, and sweating occur within 2 h after consumption, leading to a state of intoxication characterised by uncontrolled laughter/crying and hallucinations and in extreme cases, there is a possibility of a diminishing consciousness (Yildirim et al. 2016). The third group of toxic mushrooms such as, Amanita phalloides, and Hypholoma fasciculare, contains toxic substances affect the liver, kidneys, and other vital organs. The toxic effects appear very late, after 8–48 h, resulting in irreversible damage to the vital organs, cells, and the central nervous system of the human body (Barman et al. 2018). Instances of poisoning may not always be directly caused by mushrooms; rather, toxic substances can accumulate within edible mushrooms due to long-term storage, improper cooking methods and handling, and contaminations (Gawlikowski et al. 2015). Regardless of the circumstances, it is imperative to treat suspected cases of mushroom poisoning with utmost seriousness and promptly seek medical intervention.

Cultivating mushrooms: precision protocols for the cultivation of culinary mushrooms

The process of mushroom cultivation encompasses various distinct procedures, all of which necessitate meticulous execution. The substrate preparation, substrate sterilisation inoculation, incubation, and production conditions are contingent upon the specific mushroom species intended for cultivation (Sánchez 2009). Obtaining pure mycelium from the selected strain of mushroom is an additional crucial stage. Mycelium can be obtained through several means, such as spores, a tissue of the mushroom, or from diverse sources of germplasm (Zięba et al. 2021). Mycelium is cultivated on many cereal grains, including wheat, rye, and millet, commonly known as the 'spawn' to obtain inoculum (Reis et al. 2012). The grain that has been coated with mycelium is specifically engineered to rapidly establish itself within the appropriate bulk growth substrate. The quality of mushroom production is heavily influenced by the 'spawn', which must be produced in a sterile environment to minimise substrate contamination (Sánchez 2009).

The initial stage of mushroom production involves the preparation of the substrate. The substrate refers to a substantial material that serves as a source of energy and nutrients for mycelium. To facilitate growth and fruiting of mushrooms, it is imperative to have a suitable substrate (Hoa et al. 2015). Different mushroom species require specific substrates based on their (C: N) ratio. The relative characterisation of a substrate is frequently based on the substrate's C: N ratio (Chang and Miles 2004). The substrate is responsible for providing the essential nutritional components that are necessary for mushroom growth including a carbon source, a suitable nitrogen compound, significant quantities of specific inorganic ions (such as calcium, phosphate, potassium, sulfate, and magnesium), various trace elements (including iron, zinc, copper, manganese, and molybdenum), and occasionally specific organic compounds, particularly vitamins, required in small quantities (Chang and Miles 2004; Carrasco et al. 2018). The preparation of the solid substrate for mushroom cultivation involves essential steps, including the addition of water, potential nutrient supplementation, and subsequent processing through sterilisation or pasteurisation (Figure 1). Subsequently, following the process of pasteurisation, the substrate undergoes a cooling phase and is then inoculated with the preferred strain (Sánchez 2009).

Extensive research has been conducted globally about mushroom cultivation, encompassing investigations into the utilisation of various strains, diverse lignocellulosic substrates, different forms of spawn, as well as the influence of moisture levels and physicochemical conditions. Among the fungal species considered, the following are noteworthy: *Agaricus bisporus, Pleurotus ostreatus, L. sajor-caju, P. eryngii, P. pulmonarius, P. eous, P. floridanus*, and *Lentinula edodes*. The primary species of edible mushrooms cultivated globally are *Lentinula edodes, Ganoderma* spp., *Volvariella volvacea*, and *Grifola frondosa* (Sánchez 2009; Valverde et al. 2015). Saw dust (a) supplied with additional nutrients (b) Rice bran,(c) Magnesium sulphate,(d) Calcium oxide ,(e) Soya flour



Inoculation of cooled subtrate blocks (k) with mother spawn of preferred strain (j)



Mixing of the ingredients (f) and addition of water in required quantities(g)



Packing into polyproplylen bags (h) and processing through sterilization (i)



Figure 1. The preparation of the solid mushroom substrate utilised in mushroom growing subsequent processing through sterilisation or pasteurisation.

Oyster mushrooms in Sri Lanka: a lucrative endeavor in favorable climes

The late 1980s witnessed a surge in the agribusiness of mushroom cultivation in Sri Lanka, primarily propelled by the initiation of spawn production led by the Export Development Board. (EDB) (Thilakarathne and Sivashankar 2018). The current state of cultivation of edible wild mushrooms is relatively nascent due to limited knowledge encompassing only a few species from the *Agaricus, Ganoderma, Pleurotus*, (Rajapakse et al. 2010), *Schizophyllum* (Ediriweera et al. 2015; Dasanayaka and Wijeyaratne 2017) and species such as *Auricularia polytricha*, and *Lentinus squarrosulus* (Ediriweera et al. 2015).

The cultivation of mushrooms in Sri Lanka is considered to be a highly lucrative endeavour due to the favourable climatic conditions in different regions. The

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advancement of mushroom cultivation in Sri Lanka requires popularising the crop and fostering expertise in the field (Gamage and Ohga 2018). Relevant information and essential raw materials for mushroom cultivation can be acquired from reputable institutions such as the Mushroom Division of the Horticultural Crop Research and Development Institute located in Gannoruwa, the Mushroom Development and Training Center situated in Ratmalana, and the Regional Agricultural Research and Development Centre based in Makandura (Fernando et al. 2022). These entities are the primary service suppliers and also offer training programmes for mushroom production in Sri Lanka.

The cultivation of oyster mushrooms is a prevalent practice in Sri Lanka, especially among individuals involved in small-scale enterprises seeking self-employment and cottage industry. Oyster mushrooms are widely consumed in Sri Lanka as a vegetable and as an ingredient in soup, owing to their considerable nutritional value, distinctive taste, and therapeutic attributes. Therefore, they are highly favoured due to their adaptability for cultivation within the low-technology mushroom farming methods practiced in Sri Lanka (Gamage and Ohga 2018). In Sri Lanka, oyster mushroom cultivation primarily involves four distinct varieties, namely in general practice as the American oyster, Abalone, Bhutan oyster, and pink oyster (Fernando et al. 2022).

Pleurotus cystidiosus known as Abalone mushrooms (Figure 2a), is unique among oyster mushrooms, due to its large size, thickness, fleshiness, and buff-colored fruiting bodies (Krishnapriya et al. 2017). *Pleurotus djamor*, commonly known as the pink oyster mushroom (Figure 2b), could be easily recognised by its characteristic pink fruiting bodies and pink gills (Jegadeesh et al. 2018). *Pleurotus ostreatus*, also known as the American oyster mushroom (Figure 2c), is the most widely distributed species, characterised by its white and smooth-margined fruiting body. Bhutan oyster mushrooms (*Pleurotus eous*) (Figure 2d) are distinctive due to their light brown-coloured fruiting bodies with slightly corrugated margins (Khan et al. 2011b).

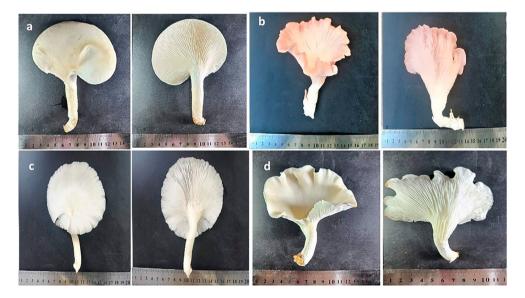


Figure 2. Most cultivated *Pleurotus* spp. in Sri Lanka. a. *Pleurotus cystidiosus*, b. *P. djamor*, c. *P. ostreatus*, d. *P. eous*.

The cultivation of oyster mushrooms does not require regulated environmental conditions, as it has a broad tolerance for temperature (18–28°C) and relative humidity (70– 90%) (Ramachela and Sihlangu 2017).

The cultivation process of oyster mushrooms is typically divided into four primary stages. Substrate preparation, substrate sterilisation, spawning of substrate, and crop management (Nongthombam et al. 2021). Mushroom spawn, often referred to as mushroom seed, is comprises the mycelium of the mushroom and a substrate acting as a nutritional base for the fungus in its early developmental phases. The method involves introducing a culture containing the targeted mycelium into a grain-based substrate, commonly termed 'spawning', while the resulting uncontaminated culture is known as spawn (Thakur and Rathod 2021).

Autoclaving is the physical procedure used to eliminate germs or microorganisms present in the media. This process facilitates the proliferation of the desired mycelium in the absence of nutritional competition from other microorganisms. Typically, sawdust medium applied for indoor cultivation systems undergo autoclaving, which is widely acknowledged as the most efficacious sterilisation technique. This method effectively eradicates a significant proportion of bacteria, bacterial spores, and pathogens present in the media (Sidik et al. 2015). In Sri Lanka, most mushroom farmers have traditionally employed large barrels containing water, which is heated using firewood as a means to generate heat and steam (Gamage and Ohga 2018). One significant limitation of this approach is the inability of the chamber to attain the recommended temperature of approximately 121°C at a pressure of 15 psi for 2 h. Consequently, the persistence of certain pathogenic spores becomes possible, leading to the potential contamination of the media during the incubation and fruiting process. To tackle this concern, extending the duration of steaming is a viable option. However, this approach comes with increased fuel costs and a prolonged overall duration of the procedure (Pathmashini et al. 2010; Gamage and Ohga 2018).

Waste as a sustainable substrate for Pleurotus cultivation

The study by Pathmashini et al. (2010) examined the effectiveness of using Kurakkan (finger millet) (*Eleusine coracana*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paddy (*Oryza sativa*) as substrates for oyster mushroom cultivation. Finger millet spawn demonstrated a higher rate of mycelium proliferation during the spawn run phase, while paddy spawns exhibited a slower pace of mycelium development compared to other spawn types.

Numerous government agencies and private agribusiness firms are engaged in commercial-scale spawn production. Paddy seeds serve as the primary substrate for spawn, with commonly used containers including polypropylene bags (200 gauges, measuring 37.5 cm in length and 17.5 cm in width), and discarded glass bottles often employed for alcoholic beverages are used for storage of spawns, often employed for alcoholic beverages (Gamage and Ohga 2018).

The successful cultivation of mushrooms relies on the optimal combination of substrates, reliable spawns, and favourable environmental conditions. Substrate preparation is particularly crucial, as it entails providing a nutrient-rich substrate to support the growth of mycelium and the development of fruiting bodies (Rajapakse et al. 2007a). In contrast to other types of mushrooms, species within the genus *Pleurotus* possess a distinctive ability to adapt to a diverse array of lignocellulosic substrates, such as straw, sawdust, and rice hulls, among others. Pleurotus ostreatus exhibited the highest level of biological efficiency when cultivated on sawdust, particularly softwood sawdust from mango and cashew trees, which is considered more appropriate than hardwood sawdust. Sawdust is currently widely utilised and is the favoured substrate on a commercial level in Sri Lanka (Pathmashini et al. 2010). In Sri Lanka, the formulation of media follows the guidelines of the Department of Agriculture (DOA). The recommended media preparation ratio includes 40 kg of sawdust, 4 kg of soft rice bran, 800 g of CaCO₃, and 80 g of MgSO₄ per 100 substrate packets. However, cost reduction is achievable by substituting traditional materials like animal manure as an economical nitrogen source, known for supplying ample major and secondary plant nutrients. (Rajapakse et al. 2007b). In addition to this, it has been observed that *Pleurotus* species can thrive on different types of agricultural wastes without the need for supplementary resources such as paddy straw, dried leaves, or shredded paper (Arulnandhy and Gayathri 2007; Anojanthy and Arulnandhy 2008). The production of artificial logs was recently started in Sri Lanka. The preparation of logs might involve the utilisation of a growing medium, like sawdust, compost, or straw, in combination with a binding agent, such as cement or plaster of Paris. This process typically entails the use of a mould, and the determination of the cost-effectiveness and practicality of this strategy remains to be established (Gamage and Ohga 2018).

The sustainable cultivation of oyster mushrooms is of interest in agriculture and environmental studies. An increase in mushroom output corresponds to a higher volume of commercial waste of mushroom substrate (WMS) (Pérez-Chávez et al. 2019). WMS exhibits elevated levels of extracellular lignocellulosic enzymes, fungal mycelium, organic components including carbohydrates, proteins, and lipids, inorganic nutritional compounds, and heavy metals (Meng et al. 2017; Gong et al. 2019). The existing methods of waste management, like open burning, incineration, burying, and landfilling, have been identified as posing significant environmental risks and contributing to environmental pollution. These risks involve contamination of water sources, eutrophication, and air pollution (Mahari et al. 2020). Considering the prevailing economic inflation in Sri Lanka, the scarcity and rising costs of essential substrate materials have emerged as significant issues within the local mushroom cultivation industry. These challenges have led to the adoption of a more environmentally friendly approach to substrate production for cultivation.

Mushroom production can be characterised as both a method of agricultural waste remediation and a financially viable crop for farmers. Agricultural residues have been identified as a valuable resource for the development of mushrooms. Table 1 illustrates the use of different agricultural waste materials as a sustainable source for mushroom cultivation. A significant proportion of agricultural wastes and forestry materials consist mostly of cellulose (40–60%), hemicellulose (15–35%), and lignin (10–30%), which serve as the primary source of C and N₂ (Cueva et al. 2017). The mushroom life cycle comprises mycelium running (vegetative phase) and fruiting (reproductive phase) phases. Initially, mycelia release enzymes, breaking down cellulose and lignin in the substrate. The carbon: nitrogen (C: N) ratio significantly influence the mycelium development and mushroom growth (Nanje Gowda and Chennappa 2021). *Pleurotus* species

Agricultural waste materials used	Mushroom cultivated	Yield	Reference	
Saw dust	Hericium erinaceus,	+	Hassan 2007	
	Auricularia polytricha along with stalks of	+	Liang et al. 2019	
	Panicum repens	_	Sardar et al. 2017	
	Pleurotus eryngii	_	Obodai et al. 2003; Hoa et al	
	P. ostreatus	-	2015	
	P. cystidiosus		Hoa et al. 2015	
Composted sawdust	P. ostreatus	+	Obodai et al. 2003	
Sawdust	Ganoderma lucidum	+	Roy et al. 2015	
(Swietenia mahagoni)			,	
Wheat straw	Hericium erinaceus	_	Hassan 2007	
	P. eryngii	+	Sardar et al. 2017	
	P. ostreatus	+	Fayssal et al. 2021	
	Agrocybe cylindracea	+	Koutrotsios et al. 2014	
	Lentinus sajor-caju	+	Patil 2013	
Composted wheat straw	Agaricus bisporus	+	Toker et al. 2007	
composica inical strain	A. subrufescens	+	Pardo-Giménez et al. 2020	
Rice straw	Volvariella volvacea	+	Biswas and Layak 2014	
	Lentinula edodes	_	Gao et al. 2020	
	P. eryngii	+	Sardar et al. 2017	
	P. ostreatus	+	Obodai et al. 2003	
	L. sajor-caju	+	Patil 2013	
	Flammulina velutipes along with sawdust	+	Harith et al. 2014	
Oil palm empty fruit bunch	V. volvacea	+	Triyono et al. 2019	
	A. polytricha supplied with sawdust	+	Razak et al. 2013	
Corn cobs	P. eryngii	+	Sardar et al. 2017	
	P. ostreatus	+	Hoa et al. 2015	
	P. cystidiosus	+	Hoa et al. 2015	
	Agrocybe cylindracea	+	Koutrotsios et al. 2014	
Banana leaves	V. volvacea	_	Hag et al. 2011	
	P. ostreatus	+	Obodai et al. 2003	
Sugarcane bagasse	L. edodes	+	Salmones et al. 1999	
	P. eryngii	+	Sardar et al. 2017	
	P. ostreatus	+	Hoa et al. 2015	
	P. cystidiosus	+	Hoa et al. 2015	
Sugarcane leaves	L. edodes	+	Salmones et al. 1999	
Plant stalks	P. eryngii	+	Xie et al. 2016	
	Ramie stalks	+		
	Kenaf stalks	_		
	Bulrush stalk			
Cotton waste	P. eryngii	+	Sardar et al. 2017	

Table 1. Utilisation of	⁻ agricultural	waste in	mushroom	cultivation.

+ mark indicates the yield of mushrooms cultivated in the agriculture waste is significantly higher than the control (mostly the commercial growth substrate) and the – mark indicates the yield of mushrooms cultivated in the agriculture waste is not significant compared to the control treatments.

can synthesise lignocellulolytic enzyme complexes. Therefore, the mentioned assemblage encompasses the oxidative enzymes laccase and manganese peroxidase (MnP), which play a crucial role in the process of lignin degradation. Additionally, it comprises the hydrolytic enzymes xylanase and cellulase, which are responsible for the decomposition of hemicellulose and cellulose, respectively (Rodrigues et al. 2012). The utilisation of cellulolytic and hemicellulolytic enzymes can lead to the saccharification of substrates, with a substantial portion of these sugars allocated towards growth and metabolism, while the remaining sugars remain in the residual substrate (Kumla et al. 2020). Cultivation of *Pleurotus* species has been demonstrated with effective outcomes when utilising various agricultural waste materials, including banana leaves, date palm leaves, maize

shaft, olive cake, rice straw, and sawdust (Da Luz et al. 2012; Yang et al. 2013; Alananbeh et al. 2014; Marlina et al. 2015; Rezania et al. 2017; Yamauchi et al. 2019).

In Sri Lanka, sawdust is used as the main raw material, supplemented with rice bran as a protein supplement as the substrate for oyster mushroom cultivation., Commonly used protein supplements in substrate preparation include soya and green gram powder (Pathmashini et al. 2010). Many attempts and experiments have been taken to mitigate manufacturing expenses and to enhance the production efficacy. It has been suggested that the cost of production can be minimised by substituting cheaper nitrogen sources, such as animal manure, which can supply a substantial amount of major plant nutrients (Rajapakse et al. 2007a). A study investigated the success of using different ratios of poultry manure, *Gliricidia* leaf manure, and urea, combined with traditional substrates (Rajapakse et al. 2007b). Similarly, a study was carried out on the use of livestock animal dung in oyster mushroom cultivation (Ruhunuge et al. 2021).

The global production of disposable diapers is increasing exponentially, with the expectation that it will exceed US\$71 billion per year in 2022 (Khoo et al. 2019). Disposable diapers rank as the third-largest single-consumer item in landfills, contributing significantly to the accumulation of daily waste in the environment (Ntekpe et al. 2020). Disposable diapers are not easily biodegradable due to their durable plastics and superabsorbent polymers, contributing to long-term environmental pollution. The improper disposal of diapers can impede the unobstructed movement of runoff water and leach toxic substances into the soil and water (Rai et al. 2009; Maponga et al. 2013; Khoo et al. 2019). Typically, baby diapers consist of cellulose (50.2%), superabsorbent polymer (15.5%), and plastic components (like the back sheet, elastics, non-woven fabric, adhesives, and top sheet) that make up 33.0% (Espinosa-Valdemar et al. 2015).

Mushrooms, saprophytes that feed on cellulosic material, can convert the cellulosic components of diaper waste into glucose through enzymatic activities, using it as a food source. The potential valorisation of recycling food and diaper waste to generate Lingzhi mushroom growth, sustaining zero-waste production. has been studied (Khoo et al. 2022). Researchers have reported the use of diapers as a substrate for mushroom cultivation, with studies exploring the feasibility of diapers in mushroom cultivation and the use of gardening wastes such as withered leaves, grass, and wheat straw as co-sub-strates (Espinosa-Valdemar et al. 2011; Espinosa-Valdemar et al. 2015). In another study, a more effective method for growing mushrooms was discovered using a growth sub-strate made from used diapers and food wastes like coffee grounds, banana peels, sugar-cane bagasse, and eggshells (Ma et al. 2020). The utilisation of NMR metabolite profiling demonstrated the safety of the substrates for mushroom growing, as no harmful or unde-sirable biological components originating from the waste-derived substrate were detected (Ma et al. 2020).

The review underscores the dual significance of Oyster mushrooms and other edible mushrooms as both a medicinal resource and a nutrient-dense food. It also explores the cultivation practices of mushrooms in Sri Lanka, emphasising the sustainable aspect of cultivating *Pleurotus* mushrooms on waste substrates, including agricultural waste, as well as non-conventional substrates such as diapers. Such innovative approaches address waste management concerns and contribute to sustainable agricultural practices by converting waste into a valuable resource for mushroom cultivation.

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