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Exploring the progress and techniques of cultivating oyster mushrooms: A comprehensive review

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Abstract

Mushrooms are edible fungi which is suitable for wide range of age group. It produces high quantity of quality food which has high biological value grown on many substrates. Mushroom can supply a high protein diet and lower calorific value so it is suitable for heart patients as it also contains all kinds of amino acids needed by human body. And we all know that there are many different species of mushroom and among those species, oyster mushroom is most commonly cultivated. The steps involved in cultivating oyster mushroom are substrate preparation, spawning of substrate, incubation, fruiting and harvesting. Complete process takes 25-30 days to get a good and healthy yield but due to the lack of knowledge related to causes and outbreak of diseases, oyster mushroom production has decreased therefore, the main objective behind writing of this review article is to learn more about the causal agents or factors and to reach out the appropriate control measures for the diseases of oyster mushroom.

Keywords: Oyster mushroom, cultivation, substrate, composting

Introduction

Numerous societies and faiths have a long history of regular use of mushrooms. In Asia, they were first used as food and medicine around 600 A.D. They were first solely gathered in forests, but with time, man started to grow them. Shiitake, the first mushroom to be produced using wood logs, was created by the Chinese, who were forerunners in the development of fungi culture techniques (Bernardi *et al*., 2008; Subramani-an, 1995) [5]. Later, the culture migrated to numerous nations in Europe and North America (Kues and Liu, 2000) [23]. Mushroom gathering and consumption were traditional practices among indigenous tribes in Brazil, but this like the majority of their other traditions, practice was lost over time. Asian immigrants brought the growing of edible mushrooms from their home countries in the 1950s. Only the champignon (*Agaricus bisporus*), the gigantic mushrooms (*Pleurotus ostreatus* and *Pleurotus ostreatoroseus*), and the shiitake (*Lentinula edodes*) are among the most popular and widely farmed edible mushroom species today, despite the fact that there are more than 2000 species of edible mushrooms in existence. Mushrooms are saprophyte fungus that fall within the Basidio-mycetes class. They thrive in damp environments with decaying organic materials and play a significant role in the cycle of nutrients (Subramanian, 1995) [40].

Mushrooms are regarded as a food with a great flavor and high nutritional value because of their high levels of fiber (34.0 g/100 g), phosphorus (104.13 g/100 g), carbohydrate (63.17 g/100 g), and protein (23.22 g/100 g) while having low levels of fats (4.7 g/100 g). Due to their incredibly low calorie content, mushrooms are also excellent for use in dieting. A number of metabolites of pharmaceutical and therapeutic relevance are also produced by them, including anti-oxidants, anti-tumor agents, immunostimulants, and antimicrobials (Elmastas *et al*., 2007; Kitzberger *et al*., 2007; Moradali *et al*., 2007; Israilides *et al*., 2008) [13, 22, 28, 18].

The advancements in culture technology, which enable the use of agricultural and industrial leftovers by recycling them as substrates for cultivation, have enhanced the relevance of edible mushrooms by enabling low-cost production and a continuous market (Eira, 2004) [12]. They also offer a great substitute for getting rid of a variety of leftovers, which helps to lessen the environmental pollution these materials create (Pandey *et al*., 2000) [32].

According to FAOSTAT (2008) [14], there were approximately 3.4 106 tonnes of mushrooms produced worldwide in 2008. China and the United States were the top two producers, each contributing 1.5 106 tonnes. Although there are no official statistics available for Brazil's output, Mogi das Cruzes is thought to be the state of So Paulo's primary cultivation region, with a yield of 7 to 15 kg of fresh mushrooms for every kilogram of wet substrate, according to Eira (2004) [12]. However, only tiny ethnic groups or those with greater cultural and economic standing are allowed to consume (Dias *et al*., 2004) [10]. The subject of this study, *Pleurotus* spp., is also known as a huge mushroom in Asia and goes by the names shimeji and hiratake. *P. ostreatus*, *P. pulmonaris*, *P. sajor caju*, *P. cornucopiae*, and *P. ostreatoroseus* are just a few of the species that make up the *Pleurotus genus*. In its natural habitat, *Pleurotus* is found all over the world, primarily in

forested areas (Bononi *et al*., 1999) [7]. These fungi are also given lignin-degrading enzymes, which is why they are also referred to as "wood white rottenness fungi" (Abreu *et al*., 2007) [1]. They are among the mushrooms with the largest production in numerous parts of the world and have a very good flavor (Ibekwe *et al*., 2008) [17].

For the cultivation of *Pleurotus* spp., a variety of wastes including wheat straw, corn, cotton, cocoa nuts, crushed sugar cane, and sawdust may be employed. They produce lignocellulase enzymes, primarily laccase (LAC) and Mn-peroxidase (MnP), which turn these lignocellulosic residues into food in suitable circumstances (temperature, relative humidity, brightness). However, in order to achieve a satisfying development, it is advised to add supplements to these substrates, such as wheat bran, rice, and soy (Bononi *et al*., 1999; Eira, 2004; Bernardi *et al*., 2008) [7, 12, 5]. *Pleurotus* spp. have a very lucrative cultivation market due to their quick and efficient development and low production costs in the most diverse agro-industrial leftovers (Moda, 2003) [25]. The substrate to be employed for the cultivation of *Pleurotus* species must be carefully considered. Cost and feasibility need to be considered (Moda *et al*., 2005b) [27]. Additionally, each species' unique environmental, dietary, and genetic characteristics will influence how these mushrooms develop (Motato *et al*., 2006) [29]. The current work's goal is to conduct a bibliographic review of the primary cultivation methods used to produce *Pleurotus* spp. mushrooms in order to confirm the yield obtained from each of them.

The methods used to produce edible mushrooms

According to Eira (2004) [12], the manufacture of *Pleurotus* typically involves six steps: substrate preparation, composting, pasteurization, sowing, incubation, and harvest.

Substrate preparation

Small pieces of the mycelium from the target mushroom, which had previously been grown in culture medium, are transferred to tubes containing grains and/or cooked fibers or sawdust that have been thoroughly sterilized at 121 °C to create the matrix, or "spawn." These infected tubes are then normally incubated at 25 °C until the fungus have completely colonized the substrate (Bononi *et al*., 1999) [7].

Composting

The substrate is developed using composting, and contaminating microorganisms that might maintain a competitive interaction with the target fungus are removed using pasteurization (Eira, 2000) [12]. Composting typically takes between 15 and 20 days, depending on the substrate. The pH of the compost is adjusted by adding calcium carbonate, which may also be combined with other ingredients (such as bran, wheat bran, ammonia nitrate, etc.). Compost moisture must be kept under control and not go beyond 75%.

Incubation and pasteurization

Typically, pasteurization takes place in tunnels with water steam circulating through the compost for two to three days at temperatures above 55 °C. However, other growing systems use stoves to sterilize the substrate for about two hours at 121 °C (Moda, 2003) [25]. The substrate needs to be cooled to 25 °C and stored in plastic bags after that operation. The previously prepared spawn has to be vaccinated next. Finally, the bags are placed in a controlled incubation stove for around 50 days (Bononi *et al*., 1999) [7].

Cultivation Techniques

In numerous experimental experiments aiming at increasing yield, various techniques, including variations in composting, pasteurization, types of substrates (Table I), and supplements employed, have been tried. Coffee dregs were added to washed pangola grass (Digitaria de-cumbens) as a substrate in amounts of 70% and 30%, respectively, by Hernández *et al*. 2003 [16] Composting was conducted in both ventilated and unventilated boxes. According to storage and frequency of compost mixing, the experiment was divided into five treatments: T_1 was the control (compost was taken out of the box and mixed once a day for five days), T_2 was without aeration and without mixture, T_3 was with aeration and without mixture, T_4 was without aeration and with mixture, and T_5 was with aeration and mixture.. *P. ostreatus* matrixes were injected into the substrate after the experiment's five days were up till the substrate had fully colonized. According to the findings, BE was less prevalent in composts without mixing and aeration.

In contrast, Moda *et al*.'s (2005a) [27] investigation was split into two studies, each of which tested the use of crushed sugar cane as a substrate for the growth of *P. sajor-caju*. The first experiment consisted of two treatments: control, in which crushed sugar cane was heated to 80 degrees Celsius for two hours, and another, in which sugarcane culms were simply cleaned. Three different treatments—all employing the same straightforward substrate and wash—were used in the second experiment. Treatment 1 (control) included no supplements, Treatment 2 included broken corn as a supplement, and Treatment 3 included a solution of minerals as a supplement. The authors confirmed that neither treatment utilizing washed or pasteurized crushed sugar cane produced a significantly different BE. Nevertheless, a greater contamination rate was seen in the pasteurization-based therapy. In terms of supplementation type, mineral supplementation (30.03%) produced a higher BE in the substrate than organic supplementation (15.66%). These authors conclude that cleaning the material employed as the substrate can restore pasteurization. Castro *et al*. (2007) [8] assessed *P. sajor-caju* yield using cotton textile processing residue as substrate. Treatment 1 bran and Treatment 2 bran and bean straw were added as supplements to the cotton waste. The composting of the material took place over the course of 10 days, after which it underwent a 24-hour process of pasteurization to attain a temperature of 60 °C. Then, over the course of six weeks, inoculation and incubation were carried out. It was found that both treatments produced satisfactory BE (55.76% in T_1 and 55.39% in T_2), indicating that cotton textile residue is a great alternative for the growing of *P. sajor-caju*.

P. sajor-caju was grown by Mane *et al*. (2007) [24] in a variety of agro-industrial residues, including wheat straw, soy straw, pea stalk, and peanut stalk. Residues were cleaned before being pasteurized for two hours at 80 °C. Substrates were chilled after that and then injected with the fungus. Composts were kept in plastic bags and incubated at 27 °C for 15 days. Utilizing wheat straw, pea stalks, and

cotton waste as substrates produced the best results. Silva *et al*. (2007) [38] examined the cultivation of *P. sajor-caju* in coast-cross hay and crushed sugar cane with the addition of wheat bran and urea. The experiment was divided into five treatments, T_1 (control): coast-cross (500 g) + crushed sugar cane (500 g), T_2: coast-cross (450 g) + crushed sugar cane (450 g) + wheat bran (100 g), T_3: coast-cross (450 g) + crushed sugar cane (450 g) + wheat bran (100 g) + urea (10 g), T_4: coast-cross (450 g) + crushed sugar cane (450 g) + wheat bran (100 g) + urea (20 g), and T_5: coast-cross (450 g) + crushed sugar cane (450 g) + wheat bran (100 g) + urea (30 g). The compost was twice sterilized for one hour at 121 °C while being stored in bags. Following the fungus inoculation, each bag was allowed to incubate at room temperature until "frutification". The three treatments with the highest yields were T_1 (35.1%), T_2 (35.9%), and T_3 (34.0%). Therefore, the addition of urea did not result in an increase in the tested mushroom's production.

For the cultivation of *P. ostreatus*, Bernardi *et al*. (2008) [5] employed elephant grass as the substrate and added tannery leather sawdust in concentrations of 0, 5, 10, 15 and 20% relative to the wet mass of elephant grass. Glass bottles were first filled with compost, and then the inoculum was added. Bottles were closed, sterilized twice for 40 minutes at 121 degrees, and then incubated for 37 days at 26 degrees. The bottles were then left in the incubator for a further 60 days. Only the 0% and 5% tannery residue supplemented treatments achieved "frutification," with BE of 76 and 64%, respectively. The fungi did not colonize surfaces with a supplementing level of more than 5%. The authors suggested that research be done to examine the tannery residue's physical and chemical characteristics as well as the bromatological characteristics of the mushrooms grown in a substrate enhanced with tannery waste.

Pedra and Marino (2006) [33] used coconut bark combined with rice and wheat bran to assess *Pleurotus* spp. productivity. Six treatments made up their experiment. T_1: coconut sawdust (100%); T_2: coconut sawdust (80%) and rice bran (20%); T_3: coconut sawdust (80%) and wheat bran (20%); T_4: coconut sawdust (60%) and wheat bran (20%); T_5: coconut sawdust (60%) and rice bran (40%); and T_6: coconut sawdust (60%) and wheat bran (40%). The composts were stored in airtight containers and then subjected to two cycles of sterilizing at 120 °C for 40 minutes. Soon after being sterilized, the substrate was chilled before being injected. Thirty days of incubation at 25 °C were completed. Unlike treatments T_4, T_5, and T_6, which each had BE values of 14.32, 15.69, and 15.61%, respectively, the substrate was not colonized by the fungus in T_1. This indicates that adding cocoanut bark to the substrate promotes the development of *P. sajor-caju* and *P. ostreatus* were raised on rice bran (5%) and banana tree straw by Bonatti *et al*. (2003) [6]. The substrate was placed inside tightly sealed polyethylene containers and sterilized for 1.5 hours at 121 °C. Containers were then chilled so they would be ready to receive the inoculum. At 25 °C, incubation took place for 20 days. Then, a further 40 days of incubation were followed by the introduction of "frutification". Analyses of the following were performed: moisture, fat, carbohydrate, ash, protein, and raw fiber. There were no discernible variations between the nutritional facts of the two species under study. The biological efficiency of *P. sajor caju* was greater (7.51%) than that of *P. ostreatus* (6.34%). Dias *et al*. (2003) [11] used wheat bran as a supplement to grow *P. sajor-caju* on coffee husk, maize straw, bean straw, and corncob substrates. Compost was placed in bottles and then sealed. After sterilizing the material for one hour at 121 degrees Celsius, it was cooled to room temperature before the spawn was added and incubated at 24 degrees Celsius until full colonization. The process of "frutification" induction was then carried out by leaving bags open for about 90 days. Bean straw without the supplement has the greatest BE (87.5%) of the measured residues. The least effective material was coffee husk (25.1%). Soccol *et al*. (2006) [39] examined the impact of caffeine and tannin on the growth and "frutification" of *P. ostreatus* and *P. sajor-caju* in coffee husk. In order to conduct the experiment, substrates made of coffee husk were mixed with various concentrations of caffeine and tannin in the quantities of 30, 50, 100, 500, 1000, and 2500 mg l-1 and 100, 500, 1000, 5000, and 10000 mg l-1, respectively. It was discovered that mycelium development did not occur at caffeine concentrations of 500 mg/l or higher. Tannin-enriched substrates at concentrations below 100 mg l-1 induced fungal growth. Caffeine and tannin concentrations in the substrates were reduced by 39.3% and 20.8%, respectively, according to the results of the analyses. These findings indicate promising prospects for growing *Pleurotus* species on coffee husk without any prior treatment. Akyüz and Yildiz (2008) [2] grew *P. eryngii* using soy straw and wheat straw as substrates The trial was divided into three treatments: bean straw in Treatment 2, soy straw in Treatment 1, and wheat straw in Treatment 3. Rice bran was added to each of them in amounts of 5 and 10%.The experiment lasted 100 days. Treatment 1 demonstrated the highest biological effectiveness (93%), with an addition of 5% rice bran. Treatment 3 had the lowest BE (7%) with a 10% dose of rice bran. According to treatments, *P. ostreatus* cultivation was also done (Baysal *et al*., 2003) [4] using paper residues supplemented with peat from the Bolu region of Turkey, hen manure, and rice bran. The treatments were T_1 (control): paper residue (100%), T_2: paper residue (90%) + peat (10%), T_3, paper residue (80%) + peat (20%), T_4: paper residue (90%) + hen manure (10%) Rice bran was added to paper waste, which dramatically boosted yield. On the other hand, the addition of peat and hen manure significantly decreased productivity. Thus, it was determined that oyster mushrooms might potentially be grown on the study's substrata.

For the cultivation of *P. ostreatus*, weeds were also employed as substrates (Das and Mukherjee, 2007) [9]. The following plants were used: *Tephrosia purpurea* (Papilionaceae), *Lantana camara* (Verbenaceae), *Parthenium argentatum* (Asteraceae), *Ageratum conyzoides* (Asteraceae), *Sida acuta* (Malvaceae), *Leonotis* sp. (Lamiaceae), and *Sida argentatum* (Asteraceae). The plants were cut into small pieces, sectioned, and immersed in water before any extra water was drained. Each species underwent inoculation both with and without the addition of wheat straw, followed by incubation. Wheat straw added to Leonotis species greatly boosted *P. ostreatus* yield (1.30 kg/kg). The cultivation of edible mushrooms was found to be effectively accomplished by using weeds as substrates.

Using wheat straw as the substrate, wheat bran supplemented at a 9:1 ratio, moistened with the resulting olive oil processing solution, diluted in numerous concentrations in accordance with the following five treatments, Kalmis and Sargin (2004) [21] analyzed *P. cornucopiae* and *P. sajor-caju* production. Wheat straw + 0% residual for Test 1 (Control), 25% residue for Test 2, 50% residue for Test 3, 75% residue for Test 4, and 100% residue for Test 5. Composts were sterilized and put in containers for storage. The inoculation and incubation processes were carried out after cooling to room temperature. It was determined that concentrations of 25 and 50%, with yields of 33.7 and 30.6%, respectively, were suitable for growing *Pleurotus* species. In a study with *P. ostreatus*, Kalmis *et al*. (2008) [20] employed wheat straw as the substrate, supplemented with wheat bran, and moistened with the olive oil production effluent in the same ratios as in the previous experiment. According to the authors, a concentration of 25% oil residue with a BE of 45% is the only one that may be employed. Bad mushroom formation was a result of higher concentrations. Given the severe environmental harm that olive oil processing waste causes, using it as a substrate for the production of edible fungi offers a commercially and environmentally sound solution to the issue. Zhang *et al*. (2002) [42] examined the culture of *P. ostreatus* in wheat straw and rice straw using two different processing techniques—cut into pieces and ground. Mushrooms grown on cut straw showed faster rates of growth. When yields between the two types of residue were compared, rice straw produced a 10% higher yield than wheat straw. According to Obodai *et al*. (2003) [31], rice straw substrates also have greater BE (50.64%) when compared to elephant grass (0%), banana tree straw (37.15%), maize forage (16.50%), and wheat straw (29.26%). By replacing rice straw in P. florida culture with cotton seed, Shashirekha *et al*. (2005) [37] found that protein, amino acid, and lipid concentrations increased while fiber, free sugar, and carbs significantly decreased.

In the cultivation of *P. ostreatus*, Sainos *et al*. (2006) [35] used wheat straw supplemented with wheat grains in the ratios T_1: 100/0, T_2: 75/25, T_3: 50/50, T_4: 25/75, and T_5: 0/100. The experiment lasted 10 days, or until the fungus had completely colonized the substratum. Only T_1 had a yield difference that was statistically significant (31.7% yield difference). The average for the others was 39.77%. It was determined that using wheat straw as a substrate is a great alternative for growing *P. ostreatus*. Motato *et al*. (2006) [29] grew *P. djamor* using leaves, stalk and fruit of the banana tree (*Musa paradisiaca*) and jequitiba (*Cariniana pyriformis*) sawdust, according to seven treatments, T_1: sawdust (100%), T_2: leaves (100%), T_3: stalk (100%), T_4: sawdust + stalk (50/50), T_5: sawdust + leaves (50/50), T_6: sawdust + fruit (50/50), and T_7: sawdust + leaves + stalk + fruit (25% each), with three repetitions each. According to the data, T_2 had a BE of 24.1% and saw more successful microbial development. Laccase and peroxidase activities, which indicate the presence of lignocellulitic enzymes, were higher in T_2 and T_5. In order to demonstrate the function of lignocellulitic enzymes during substrate fermentation, *P. ostreatus* and *P. sajor-caju* were also grown on the leaves and stem of the banana tree. Reddy *et al*. (2003) [34] confirmed that over the 40 days of cultivation, both organisms displayed the same activity in producing the enzymes laccase, peroxidase, lignin, xylanase, endo-1-4-b-D-glucanase (CMCase), and exo-1-4-b-D-glucanase. The goal of Alexandrino *et al*. (2007) [3] was to confirm that *P. ostreatus* produces lignocellu-lolytic enzymes. The cultivation was done using a substrate made of dry, pulverized orange leftovers. The enzymes with the highest activity were laccase and manganese peroxidase. Additionally, the utilization of this kind of material offered the fungus the nutritional circumstances it needed to develop. *P. ostreatus* was also grown using coffee dregs, wheat straw, and sawdust from *Picea abies*, a coniferous tree found in the chilly mountains of Northern Europe. Six different combinations of sawdust, wheat straw, and coffee grounds were used in the experiment: the control (60 g/125g wheat straw), CB1: 36/60/106, CB2: 74/60/106, CB3: 110/60/93, CB4: 146/60/74, and CB5: 186/60/88. The outcomes demonstrated that the use of coffee dregs as substrate did not reduce the "frutification" capacity or the biological effectiveness of the fungus's production. . Studies to confirm the presence of caffeine in the basidiomata of *Pleurotus* spp. revealed that 59% of the caffeine was not ingested by the mushroom, suggesting its capacity for degradation (Job, 2004) [19]. Using coconut shell, sawdust, crushed sugar cane, and *Typha angustifolia* (a typical tree from North America) leaves as substrates, Vetayasuporn (2007) [41] grew *P. ostreatus* with the intention of eradicating harmful microorganisms present in the substrate in a 100/15 (residue/EM) ratio. Crushed sugar cane (103.56%) had the highest BE value of any substrate, making it an effective alternative for making oyster mushrooms. Naraian *et al*. (2008) [30] used corncobs (CC) as substrate for P. florida cultivation, supplemented with urea (U), ammonium sulfate (AMS), grass (G), bran (B), cotton seed (CS), mustard seed (MS), nuts seed (NS) and molasses (M), in three different combinations each: CC + U (0.5, 1 and 1.5%), CC+AMS (0.5, 1 and 1.5%), CC + G (2, 3 and 5%), CC + B(2, 3 and 5%), CC + CS (2, 3 and 5%), CC + MS (2, 3 and 5%), CC + NS (2, 3 and 5%), and CC + M (2, 3 and 5%). Although all treatments outperformed the control in terms of productivity, substrates with greater supplement doses showed notable yield reductions. The best supplement was 2% cotton seed (93.5%), followed by 2% bran (93) in terms of effectiveness. Sales-Campos (2008) [36] cultivated *P. ostreatus* in the Amazon region's wood sawdust (*Simarouba amara* and *Ochroma piramidale*), agroindustrially crushed sugar cane (*Saccharum officinarum*), and pupunheira palm tree stem (*Bactris gasipaes* Kunth). In a ratio of 80:10:8:2, respectively, rice bran, wheat, and $CaCO_3$ were added to all residues. The growing of axenic took place for 100 days. S. amara sawdust (94.0%), *O. piramidale* sawdust (64.6%), S. officinarum (125.6%), and *B. gasipaes* strips (99.8%) all had great biological efficiency. The mushrooms were produced with high levels of protein (14.67-21.16%), fiber (18.89-31-30%), and low levels of lipids (1.27-2.14%). The use of residues as a fantastic alternative for *P. ostreatus* cultivation was concluded as a result of the good outcomes, with the Amazonian region serving as a very favorable environment for that technique.

Conclusion

Due to a variety of qualities (medical, nutritional, bioremediation of contaminated environments, enzyme synthesis, etc.), *Pleurotus* spp. mushrooms are among the most widely cultivated mushrooms in the world. Due to their ease of manipulation and quick development, they also offer a wide range of alternatives for use in cultivation, including the use of agro-industrial residues, leaves, sawdust, fruit peels, and industrial effluents depending on availability, which varies between locations. Due to their ability to produce hemicellulose and lignocellulolytic enzymes that break down the lignin that gives vegetables their rigidity and structure, these fungi may grow on these substrates. Due to their structure, these leftovers decompose very slowly and can be left in the environment for a very long time. Additionally, leftovers utilized in the feeding of ruminant animals are pre-treated with edible fungi to make them suitable for human consumption. The cultivation technique used and the supplements to be added rely on the species and nutritional characteristics of the used residue.

The analyzed studies demonstrated the viability of using a variety of byproducts as substrates for *Pleurotus genus* mushrooms, including sawdust, elephant grass, coast-cross, crushed sugar cane, processing waste (cotton, paper, olive oil, tannage), processing residues (cotton, paper, banana tree, corn, bean), and straws (wheat, soy, banana tree, corn, bean). A variety of materials were utilized as supplements, and wheat bran and rice bran produced the highest levels of biological effectiveness. It was determined that mushrooms grown in substrates with supplements had higher biological efficiency than mushrooms produced in substrates without nutritional sources, necessitating the addition in order to produce mushrooms of a higher quality. Bean straw was the sole residue employed as a cultivation substrate to demonstrate efficiency in production without the requirement for supplementing. It was discovered that edible fungi also break down the majority of the organic material used as substrate, making them a highly helpful approach to get rid of harmful environmental contaminants by converting them into a nutritional source for their growth. Because labor and raw materials are inexpensive and leftovers sometimes have little or no aggregate value, growing edible mushrooms offers small businesses a possible alternative.

Conflict of Interest

The authors should declare that they do not have any conflict of interest.

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