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ABILITY OF THREE *PLEUROTUS* SPECIES FOR EFFECTIVE USE OF GIANT GRASS COMPOST

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ABSTRACT

Pennisetum sinese is a giant grass with a fast-growing and high rooting rate, high sugar, protein content, and high biomass yield, which causes it to be an efficient and economic energy crop of high productivity, application in phytoremediation, and fodder production. The composting system of this grass that is adapted to the simplest formulation is easy and economically feasible in small farms for cultivating oyster mushrooms. In this study, giant grass compost was employed as a substrate for cultivating three *Pleurotus* species: *P. florida*, *P. pulmonarius*, and *P. ostreatus* to assess their enzyme activities, growth, and yields. Lignin peroxidase (LiP) was the most active enzyme in each species, while other enzymes were differently expressed between species and developmental phases. The average mass of fruiting bodies formed on the giant grass compost was 173.4 g, 166.5 g, and 152.2 g. The biological effectivity was 82.6%, 78.6%, and 72.5% for *P. pulmonarius*, *P. ostreatus*, and *P. florida*, respectively. The obtained results indicate the usefulness of giant grass compost for the cultivation of the three studied *Pleurotus* species.

Key words: composted substrate, giant grass, lignocellulolytic enzyme, *Pleurotus* species

INTRODUCTION

Mushrooms are a particular class of macro fungi with a unique epigeous (superficial) fruiting body that generally grows in decomposing organic matter. The majority of cultivated mushrooms, including *Pleurotus* spp., can be grown on the raw materials of crop or agro-industrial wastes, such as, amongst others, coffee bean dregs, cereal straw, banana leaves, brewery waste, maize cobs, sawdust, and cotton wastes (Philippoussis et al. 2001; Obodai et al. 2003). Oyster mushrooms have an enzymatic complex containing the ligninolytic or oxidative enzymes (manganese-base peroxidase, lignin peroxidase, and laccase) that break the lignin polymers down (Arora et al. 2002; Knop et al. 2015), and the hydrolytic enzymes (endoglucanase, i.e., CMCase, beta-glucosidase, exoglucanase, FPase, and xylanase) that break the cellulose and hemicellulose polymers down (da Luz et al. 2012; Amore et al. 2013). The enzyme activities and nutritional composition of oyster mushrooms differ depending on their species, nature, and composition of the cultivation substrate (Çağlarırmak 2007; Bhattacharjya et al. 2015; Knop et al. 2015; Xie et al. 2016).

Pennisetum sinense is a perennial giant grass with a high cellulose and crude protein content, which can provide a biomass yield of up to 200 t FW·hm^{-2} (Zhang et al. 2015). In addition to being widely used as a raw material for livestock feed and biomass energy (Lin et al. 2012), it controls soil erosion and improves soil properties (Lin et al. 2014). Partial degradation through composting can make this grass more easily digestible by mushrooms. Compost used as a soil amendment gives numerous biological benefits and restricts the growth of harmful mushroom microbial competitors (Oh et al. 2003; Martínez-Blanco et al. 2013). Compost stimulates the formation of aggregates (mycelia thread biofilm) that improve soil structure, increase disease resistance in some cases, stimulate the indigenous soil microflora with the substances it provides, enhances resources, reduces the volume of waste products by over 40%, and reduces pollution (Dada & Fasidi 2018).

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Given that the present residues can vary greatly from one region to another, the composting system that is adapted to the simplest formulation, ideally with only one agro-industrial component is simple and economically feasible in small farms for cultivating oyster mushrooms.

It was reported that various species of *Pleurotus* can be viably cultivated using only elephant grass (*Pennisetum purpureum*) (Bernardi et al. 2007). In the study on the cultivation of oyster mushrooms using one-component composts of corn-cob hay, sawdust, and wheat straw, *P. ostreatus* readily produced fruiting bodies on composted sawdust with a yield level comparable to commercial production of *Agaricus campestris* (Block et al. 1958). *P. ostreatus* and *A. bisporus* were effectively produced on wheat straw compost (Vajna et al. 2010; Vieira & Pecchia 2018).

Globally, oyster mushrooms take the second position of mostly cultivated mushrooms and account for 25% of total mushroom production (Vasudewa et al. 2007). However, only a few investigations were carried out, on the use of composts for growing *Pleurotus* spp. The use of giant grass compost in mushroom cultivation is advantageous for regions, which have a high production of this grass. Therefore, the goal of this study was to assess the enzyme activity, growth, yield properties, and nutritional content of three *Pleurotus* species growing on giant grass compost.

MATERIALS AND METHODS

Preparation of primary and secondary spawn

The experiment was carried out at the Academy of Juncao Science and Technology, Fujian Agricultural and Forestry University. Three *Pleurotus* strains P377, P27, and P30 were originally obtained from different geographic locations in China and collected at the Fujian Agricultural and Forestry University. They were identified further as *P. florida*, *P. pulmonarius*, and *P. ostreatus*, respectively, using DNA sequencing, according to (Menolli et al. 2010). The above strains were selected after the initial screening process. They were maintained on PDA medium (as the first) and also in polyethylene bags filled with 500 g of sterile substrate made up of raw materials of 50% *Pennisetum sinense*, 28% *Miscanthus floridulus*, 20% wheat bran, and 2% of gypsum (as the second spawn).

Preparation of cultivation substrate and mushroom growing

Fully mature plants were cut and ground to 0.5–1.5 cm particle size, sun-dried for 3 days, then piled 1 × 1 × 1 m, and composted for 21 days. The heap was turned over every 5 days, and the compost temperature was monitored daily. The highest and lowest temperatures were noted at 6 and 21 days, respectively. After 21 days the compost was sun-dried. For the experiment, the giant grass compost was mixed with 10% wheat bran and 2% gypsum powder, then moistened to a water content of 58% and loaded in polyethylene bags according to the methods of Rajapakse et al. (2007) and Okal et al. (2021). Five hundred grams of substrate mixture (pH 7.8) was loaded into the polyethylene bags. According to Rajapakse et al. (2007) and Okal et al. (2021), the polyethylene bags were sealed with perforated caps to allow for some aeration, then pasteurized for 6 hours at 80 °C and 90% relative humidity. After cooling, the experimental substrate was inoculated with 30 g of the second spawn substrate. For each of the evaluated species, 18 replicate bags were prepared. The bags were incubated in the climate box QHX-400BSH-III (Shanghai CIMO) at a temperature of 24 °C, a humidity of 50%, and in the dark. To compare growth rates between *Pleurotus* species, the time required for the substrate to fully colonize and for primordial initiation was recorded in each bag. After the bags had colonized completely, they were opened and taken to the growing room with a temperature range of 19–24 °C, and 80–85% humidity that was kept by regularly spraying with water (Ahmed et al. 2013). The mycelial samples were obtained by randomly selecting one bag from each species, and after thoroughly mixing, three replicate samples were taken at the substrate inoculation stage, mycelial colonization stage, and fruiting body stage. In harvesting, fruiting bodies were twisted and uprooted from the base. The harvest from two subsequent flushes for each bag was recorded to determine its total yield (g). Samples of fruiting bodies and colonized substrate were frozen utilizing liquid nitrogen and then kept at −80 °C until later use. To find the biological efficiency (BE) for each species, we used the formula (Zervakis & Balis 1992):

$$\mathrm{BE} = \frac{\text{Fresh weight of fruit body} \times 100}{\text{Dry weight of the substrate}}$$

Analysis of enzyme activities

The Elisa method was used to perform the assays for enzyme activity of laccases, manganese peroxidase (MnP), lignin peroxidase, xylanases, exoglucanases (FPase), endoglucanases (CMCase), β-glucosidase, and amylase in the samples of mycelial and fruiting phases, according to Okal et al. (2021). Briefly, 50 µL of the standard solution (prepared by mixing 1 µg of supplied ELISA standard with 1 ml of ELISA diluent, then mixed 10 µL of the reconstituted standard with 990 µL of incubation buffer) was added to a micro-Elisa test strip plate (standard well), and 40 µL of the sample dilution buffer was added to the testing sample well. Then, 10 µL of the testing sample was added to the wells and gently mixed. Except for the blank well, 100 µL of HRP-conjugate reagent was then added to the wells, followed by the closure of each plate with an adhesive strip and incubated for 30 minutes at 37 °C. Then, a wash solution was prepared, diluted 30-fold with distilled water, and kept. The closed plates were then uncovered, followed by discarding the contents and drying with a swing. Then, the washing buffer was added to each well and allowed to stand for 30 seconds, and then drained. Except for the blank well, 50 µL of HRP-conjugate reagent was then added to each well and incubated for 30 min at 37 °C, followed by washing with buffer. Then in each well, 50 µL of chromogenic solution A and 50 µL of chromogenic solution B were gently mixed, protected from the light, and incubated for 15 minutes at 37 °C. To stop the reaction, 50 µL of H_2SO_4 solution was added to each well, and when the color changed from blue to yellow, the absorbance (optical density – OD) was then read at 450 nm within 15 minutes of adding the stop solution. A standard curve was drawn and the OD value was used to calculate the sample's corresponding density. The actual sample density for each enzyme was determined by multiplying the sample density on the graph by the dilution factor.

Analysis of the fruiting body composition

The analysis of contents of polysaccharides, fiber, carbohydrates, fat, proteins, amino acids, crude ash, and heavy metals (cadmium, arsenic, lead, and mercury) in fruiting bodies from each *Pleurotus* species was performed. To quantify the content of polysaccharide, the phenol-sulfuric acid method (Nielsen 2010) was applied. Polysaccharides were extracted in the water and precipitated in alcohol. Then, 0.05 g of the crushed sample was mixed with 1 ml of water into a test tube and homogenized in a water bath at 100 °C for 2 hours. The mixture was then centrifuged for 10 minutes at 10,000 rpm, and the supernatant was removed. The 0.2 ml of supernatant was mixed with 0.8 ml of anhydrous ethanol, and the mixture was then employed for quantifying polysaccharides at 490 nm. The content of carbohydrates was determined by the phenol-sulfuric acid method according to Nielsen (2017). Fiber content was examined by referring to the method used by (Bragg & Shofner 1993) and protein content using the BCA kit (Walker 2009; Yanos et al. 2013). The standard working solution was prepared in a ratio of 100 volumes of BCA reagent A to 2 volumes of BCA reagent B, where the reagent A was prepared by dissolving 1 g of sodium bicinchoninate, 2 g of sodium carbonate, 0.16 g of sodium tartrate, 0.4 g of NaOH, and 0.95 g of sodium bicarbonate in 50 ml of distilled water, then brought to 100 ml with distilled water, and the pH was then adjusted with 10 M NaOH to 11.25. The reagent B was prepared by dissolving 0.4 g of cupric sulfate (5 × hydrated) in 5 ml of distilled water, then brought to 10 ml with distilled water. The absorbance measurement of the known standard was performed at 562 nm. The fruiting body fat was evaluated according to (Randall 1974). The total amino acids were investigated using the RP-HPLC method described by Bartolomeo and Maisano (2006). The ion exchange method was utilized to analyze the fruiting body's Cd, Pb, As, and Hg (Huang et al. 2010; Liu et al. 2015).

Statistical analysis

The data from three independent biological replicates were analyzed with a one-way ANOVA test using SPSS software version 22. The means between enzymes of one strain and between strains were compared utilizing the Duncan test at a 5% significance level.

RESULTS

Enzyme activity

The enzyme assays performed immediately after inoculation, at the twentieth day of mycelia growth,

and at fruiting stages showed various activities of lignocellulolytic enzymes depending on *Pleurotus* species grown on the same substrate. The results presented the highest activity of LiP of each *Pleurotus* species (Fig. 1) at the inoculation stage but different at the mycelia colonization and fruiting stages (Table 1). Apart from LiP, every *Pleurotus* species showed the participation of all enzymes in the degradation of lignocellulosic substances, but their activity differed between developmental stages (Figs. 1–3). Amylase, laccase, and CMCase exhibited significantly higher activities in *P. florida*, while glucosidase and MnP exhibited higher activity in *P. pulmonarius* and *P. ostreatus*. Xylanase and FPase showed significantly higher activities in *P. pulmonarius* and CMCase in *P. ostreatus*. During the stage of mycelial colonization, the complex of amylase, CMCase, and FPase had a higher activity in *P. florida*, xylanase had a higher activity in *P. pulmonrius*, and the complex of glucosidase, MnP, and laccase in *P. ostreatus* (Table 2). At the fruiting phase (Table 3 & Fig. 3), LiP activity remained to be the highest for each *Pleurotus* species. LiP and CMCase peaked in *P. pulmonarius*. Besides LiP, MnP and laccase exhibited higher significant activity in *P. florida*, whereas in *P. pulmonarius*, a complex of amylase, xylanase, LiP, and CMCase activities were significantly higher. In *P. ostreatus*, a complex of glucosidase, laccase, and FPase activities were the highest. In brief, during all growth stages, the LiP enzyme (Figs. 1–3) was the most active, displaying the highest significant activity at the fruiting phase, especially in *P. pulmonarius*.

Mycelium growth and fruiting body yield

The results showed that the mycelial growth of *P. florida* was faster (23.9 days) than that of *P. pulmonarius* and *P. ostreatus* (26.5 and 25.3 days, respectively). However, *P. pulmonarius* was the first in producing the primordia (38.9 days), followed by *P. ostreatus* (42 days), and *P. florida* (46.4 days). *P. plumonarius* produced the highest number and highest mass of fruiting bodies with the highest biological efficiency of 82.6%. The lower number of fruiting bodies in the two other species was accompanied with lower BE. Differences in stipe lengths and pileus diameters were not correlated with their number and mass (Table 4).

Content of compounds and heavy metals in fruiting bodies

The highest content of polysaccharides, proteins, carbohydrates, and fiber was detected in *P. ostreatus*, whereas the highest contents of total amino acids and fat were detected in the *P. florida*, and crude ash in *P. pulmonarius* (Table 5). Differences in the content of heavy metals were not high although statistically significant. The highest content of Hg was detected in fruiting bodies of *P. florida* and the highest contents of As, Pb, and Cd in *P. pulmonarius* (Table 6).

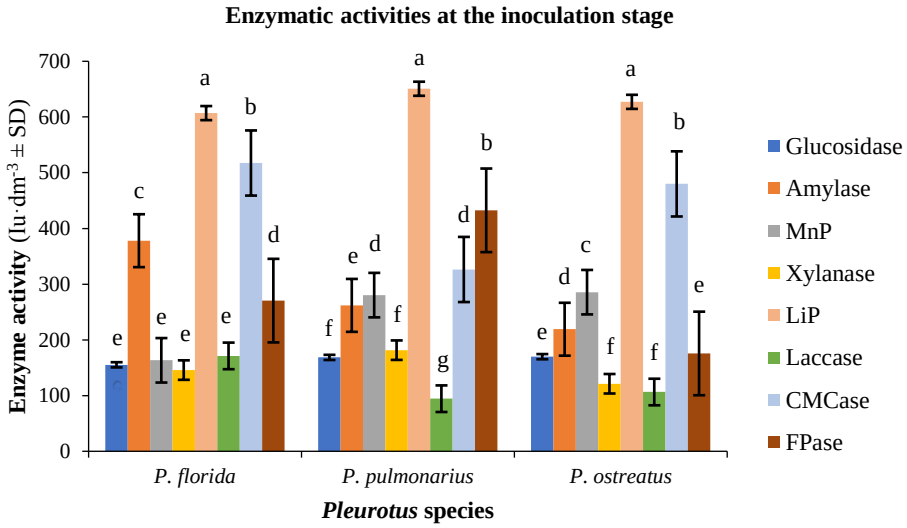

Figure 1. Comparison of enzymatic activities of three *Pleurotus* species cultured in composted giant grass at the inoculation phase (comparisons were made within species)

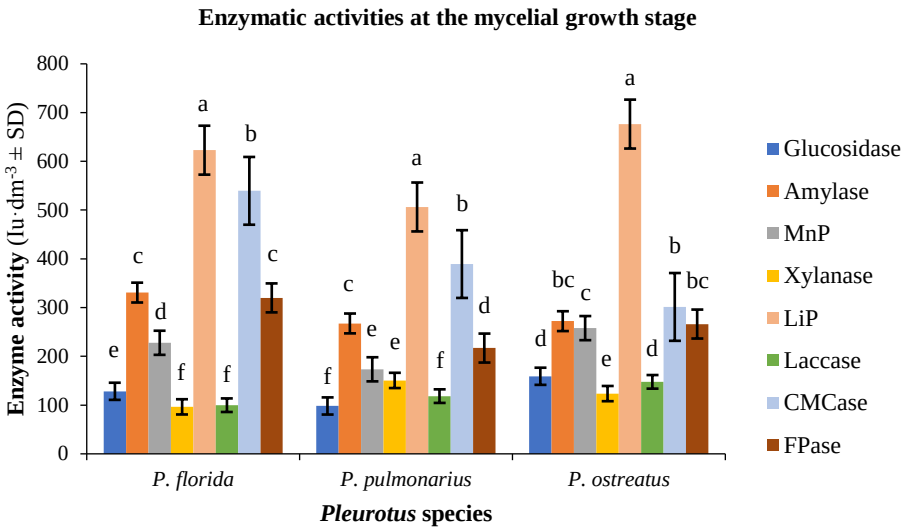

Figure 2. Comparison of enzymatic activities of three *Pleurotus* species cultured in composted giant grass at the mycelial colonization phase

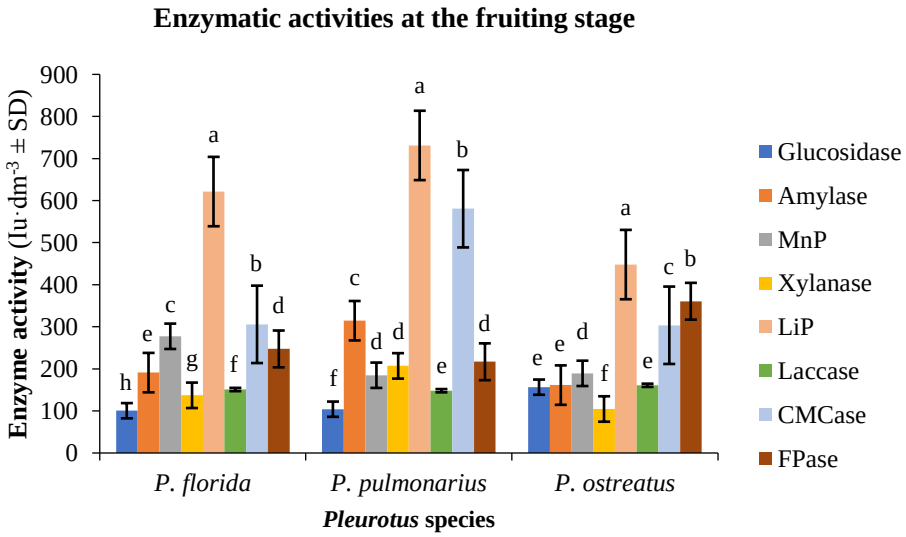

Figure 3. Comparison of enzymatic activities of three *Pleurotus* species cultured in composted giant grasses at the fruiting stage

Table 1. Enzyme activities (Iu·dm^{-3} ± SD) at the inoculation stage of three different *Pleurotus* species cultured in composted giant grass

Pleurotus species	Glucosidase	Amylase	MnP	Xylanase	LiP	Laccase	CMCase	FPase
P. florida	153.7±1.5b	372.7±5.3a	148.9±14.4b	143.5±2.3b	601.4±5.6a	163.2±8.0a	495.8±21.6a	257.3±13.1b
P. pulmonarius	164.8±3.7a	249.0±12.9b	273.4±6.9a	173.4±8.1a	632.6±18.1a	90.4±4.0b	311.4±14.9b	418.7±13.8a
P. ostreatus	165.1±4.7a	202.7±16.4c	271.7±13.9a	118.0±3.2c	603.6±23.6a	98.7±7.7b	478.6±1.3a	154.5±21.1c

Different letters in a column represent a significant difference in enzyme activity between various species of *Pleurotus* at $p \leq 0.05$ by Duncan's multiple range tests (n = 3)

Table 2. Enzyme activities (Iu·dm^{-3} ± SD) at the mycelia colonization stage of three *Pleurotus* species cultured in composted giant grass

Pleurotus species	Glucosidase	Amylase	MnP	Xylanase	LiP	Laccase	CMCase	FPase
P. florida	125.5±2.8b	320.3±10.5a	223.2±4.6b	93.6±2.8c	611.8±11.1a	97.2±2.6c	524.5±15.0a	311.1±8.8a
P. pulmonarius	93.8±4.4c	256.9±10.6b	158.1±15.3c	145.1±5.5a	481.9±24.5b	113.7±4.7b	373.1±16.2b	214.2±2.8c
P. ostreatus	156.8±2.3a	266.5±5.7b	248.3±9.6a	116.4±7.2b	649.6±26.8a	141.7±6.0a	278.4±23.0c	257.3±8.9b

See explanation in Table 1

Table 3. Enzyme activities (Iu·dm^{-3} ± SD) at the fruiting stage of three *Pleurotus* species cultured in composted giant grass

Pleurotus species	Glucosidase	Amylase	MnP	Xylanase	LiP	Laccase	CMCase	FPase
P. florida	98.7±1.8b	181.2±9.9b	272.7±4.7a	132.3±4.8b	610.3±11.2b	147.5±3.4ab	301.3±4.5b	237.6±9.8b
P. pulmonarius	99.6±4.5b	300.1±14.4a	177.6±7.2b	198.9±8.2a	715.0±16.1a	145.8±2.3b	550.3±30.4a	204.1±12.8c
P. ostreatus	151.3±5.2a	144.9±16.5c	184.6±4.7b	98.4±6.2c	441.1±6.8c	154.9±6.0a	288.4±15.2b	349.5±11.2a

See explanation in Table 1

Table 4. Mycelia growth and productive capacity of three *Pleurotus* species cultured in composted giant grass

Pleurotus species	Mycelia growth (days)	Primordia initiation (days)	No. of fruiting bodies	Fresh fruiting body weight (g)	Stipe length (cm)	Pileus diameter (cm)	BE* (%)
P. florida	23.9±1.2a	46.4±1.5c	15.8±3.2c	152.2±11.0b	5.9±0.4a	8.2±0.7b	72.5±5.2c
P. pulmonarius	26.5±3.0b	38.9±3.0a	23.2±3.3a	173.4±13.0a	5.4±0.4b	7.5±0.8c	82.6±6.2a
P. ostreatus	25.3±1.5b	42.0±1.9b	18.5 ± 2.6b	166.5 ±12.7a	5.6±0.5b	8.7±0.4a	78.6±5.8b

Means ± SD followed by letters indicating a significant difference at 5% probability; in a column, different letters represent a significant mean difference between *Pleurotus* spp ± SD at 5% probability; the total number of replicate bags per species was 18 bags (500 g per bag)

*BE – biological efficiency

Table 5. The content of nutrients in fruiting bodies (mg·g^{-1} ± SD) of three *Pleurotus* species cultured in composted giant grass

Pleurotus species	Polysaccharides	Protein	Carbohydrate	Amino acids total	Fat	Crude ash	Fiber
P. florida	3.47±0.01c	114.25±0.98b	96.29±1.10c	2.89±0.01a	51.18±0.11a	12.63±0.23b	19.89±0.48a
P. pulmonarius	3.88±0.01b	108.55±1.03c	105.54±1.55b	2.68±0.01b	43.56±0.77c	15.26±0.22a	17.30±0.62b
P. ostreatus	4.11±0.02a	121.54±1.03a	110.75±1.03a	2.35±0.07c	48.77±0.07b	11.69±0.27c	20.42±0.72a

In a column, different letters represent a significant nutrient content difference between *Pleurotus* species, at $p \leq 0.05$ according to Duncan's multiple range tests; the total number of replicate bags per species was 18 bags (500 g per bag)

Table 6. The content of heavy metals (mg·kg^{-1} ± SD) in fruiting bodies of three *Pleurotus* species cultured in composted giant grass

Pleurotus species	Hg	As	Pb	Cd
P. florida	0.015a	0.043b	0.017b	0.130b
P. pulmonarius	0.012c	0.047a	0.018a	0.136a
P. ostreatus	0.014b	0.042c	0.015c	0.123c

In a column, different letters represent a significant metal content difference between various *Pleurotus* species at $p \leq 0.05$ according to Duncan's multiple range tests; the total number of replicate bags per species was 18 bags (500 g per bag)

DISCUSSION

The significant differences in enzyme activity between *Pleurotus* species found in this study were also reported by Cavallazzi (2004), Ohga (1992), and Mata and Savoie (1998). Knop et al. (2015) found that the lignocelluloses' conversion into soluble sugars by *P. ostreatus* hinges mainly on the secretion of different nonspecific oxidative enzymatic systems, which are mainly consisted of laccases, versatile peroxidases, and short manganese peroxidases. The observation of the high activity of LiP in the three *Pleurotus* species studied here is similar to the earlier findings (Johjima et al. 1999; Hammel & Cullen 2008) that it is the most efficient and best known ligninolytic enzyme oxidizing lignin in white-rot fungi. In addition, this high activity of LiP is connected with a relative capability of colonizing and degrading the recalcitrant lignin polymer by the *Pleurotus* species (Ruiz-Dueñas & Martínez 2009). The study showed that the production and activity of LiP are influenced by the pH of culture media (stable from acidic to basic pH) and fermentation time (Morais et al. 2001; Sánchez-Ruiz et al. 2021), and it is also connected with substrate composition, temperature, and the growth phase of the fungus (Kaal et al. 1995; Bellettini et al. 2019; Abon et al. 2020). The observation of a high yield of *P. pulmonarius* compared to other *Pleurotus* species in this study is similar to the findings reported by de Siqueira et al. (2012), which showed that *P. pulmonarius* yielded the highest productivity of 24.32% and BE 68.8% than *P. ostreatus* (productivity of 21.08%, BE 61.75%) and *P. eryngii* (the weakest growth) when cultivated on composted bean straw. The advantage of our study is that all three species of *Pleurotus* grown on giant grass compost gave higher yields than these reported above. The *P. ostreatus* yielded at the level of 21.08% of productivity per 10 kg of fresh bean straw compost (de Siqueira et al. 2012), 10.97 g per 50 g of dried sawdust compost (Dada & Fasidi 2018), and 183.1 g per 1 kg of fresh *Triplochiton scleroxylon* compost (Obodai et al. 2003), which is significantly less than we obtained on fresh giant grass compost in this study (166.5 g per 500 g). The protein contents (111.25, 108.55, and 121.54 mg per g of *P. florida*, *P. pulmonarius*, and *P. ostreatus*, respectively) obtained in this study, are much higher than the protein content range of 25.35–27.3% of *P. ostreatus* grown separately on six varied sawdust substrates (Bhattacharjya et al. 2015), 0.92% protein content of *P. ostreatus*, and 1.76% of *P. sajor-caju* (Çağlarırmak 2007), as well as 20.82% protein content of *P. ostreatus* and 21.3% of *P. sajor-caju* (Chirinang & Intarapichet 2009). The contents of carbohydrates, amino acids, fat, and crude ash in this research are also higher than the results reported by Çağlarırmak (2007), Chirinang and Intarapichet (2009), and Bhattacharjya et al. (2015). The observation of different contents of heavy metals between diverse *Pleurotus* species is alike to the findings reported by Wang et al. (2017). Furthermore, the heavy metal amounts obtained in the fruiting bodies of each of the three species used in this study were below minimum allowable concentrations in vegetable food (Zarcinas et al. 2004; Pan et al. 2016).

CONCLUSION

Giant grass compost can be effectively used for the production of fruiting bodies of three *Pleurotus* species, which have high enzymatic activity, allowing for further decomposition of organic matter in the compost, which results in high yield and biological efficiency.

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Conflicts of interest

No conflicts of interest.

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