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Original research article

# Production of Fruiting Body and Antioxidant Activity of Wild *Pleurotus*

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ABSTRACT

The HS isolate of wild *Pleurotus* is a wood-rotting fungus found in Bogor, Indonesia. This study was conducted to determine the growth and fruiting body production of HS isolate on three types of substrates, antioxidant activities, and total phenolic contents (TPCs). HS isolate was grown on *Paraserianthes falcataria* sawdust (PFS substrates), oil palm empty fruit bunch (EFB) substrates, and mixture of PFS and EFB substrates (M substrates) with proportion 1:1, respectively. Analysis of antioxidant activity of mycelial and fruiting body extracts was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, whereas TPCs were conducted using Folin–Ciocalteu method. The results showed that HS isolate could grow and produce fruiting bodies on all substrates, but based on all observation parameters, M substrates were the best ones for the growth and fruiting body production of HS isolate with biological efficiency of 88.86%. Fruiting body extract of HS isolate had a better ability to reduce DPPH free radical (IC50, 0.45 ± 0.04 mg/mL) with total phenolic compound of fruiting body extract being higher (4.62 ± 0.08 mg gallic acid equivalent/g extract) than those of mycelia extract. Based on this study, HS isolate is potential as a source of natural antioxidants.



## 1. Introduction

*Pleurotus* spp. is a wood-rotting fungi that become one of the large-scale cultivated mushrooms. The oyster mushroom strains are in the 3rd place among the world mushroom production after white button mushroom and shiitake (Gyorfi and Hajdu 2007). This mushroom is widely cultivated throughout the world because of quick mycelial growth and fruiting, short life cycle, slightly affected by diseases and high adaptability to varied agroclimatic conditions, as well as low cost of production (Bonatti 2004; Synytsya *et al.* 2009; Silveira *et al.* 2014).

*Pleurotus* spp. also has high ability to use a wide variety of lignocellulosic waste (Yildiz *et al.* 2002). One of the solid waste that is available in huge quantity in Indonesia is oil palm empty fruit bunch (EFB). EFB is a solid lignocellulosic residual that is leftover from the processing of oil palm fruit into crude oil palm at the mills. The production of EFB is approximately 20.7 million metric tons per year (Isroi *et al.* 2012). The utilization of EFB as a substrate for *Pleurotus* spp. cultivation can be one suitable solution for the management of solid waste in the oil palm plantation region. The use of EFB has been reported by Sudirman *et al.* (2011) for fruiting body production of *Pleurotus* F isolate.

*Pleurotus* spp. is increasingly popular because of possessing nutritional and medicinal values (Fernandes *et al.* 2015). Several species of *Pleurotus* with their medicinal values have been reported by many researchers, such as *P. eryngii* showed beneficial effects as antiatherosklerotic, antitumor, anti-inflammatory, enhancing immunity, hepatoprotective, and antihipolipidemic (Chen *et al.* 2012; Ma *et al.* 2014); *P. sajor caju* had potentiality as antirheumatoid, anti-inflammatory, antitumor, and inhibits tumor cell lines of human laryngeal carcinoma (Patel *et al.* 2012; Finimundy *et al.* 2013; Silveira *et al.* 2014). *P. cornucopiae* was reported by Jang *et al.* (2011) as medicinal mushroom that has antihypertensive activity, whereas *P. ostreatus* and *P. eryngii* contain glucan with prebiotic activity (Synytsya *et al.* 2009).

*Pleurotus* spp. is also known having an antioxidant activity. Recently, many research on antioxidant activity in a wide variety of *Pleurotus* spp. such as *P. abalonus*, *P. gaesteranus*, *P. tuber-regium*, *P. cornucopiae*, and *P. ferulae* had been reported (Alam *et al.* 2012; Wang *et al.* 2012; Yim *et al.* 2012; Wu *et al.* 2014; Zhang *et al.* 2014). The activity was correlated with the presence of phenolic compounds and other compounds that can scavenge free radicals.

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Zhang *et al.* (2012) had reported two novel water-soluble heteropolysaccharides of PSPO-1a and PSPO-4 from *P. ostreatus* fruiting bodies. Both compounds showed high radical scavenging activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Meanwhile, Lin *et al.* (2014) also reported high DPPH radical scavenging activity of *P. eryngii* fruiting body extracts. Those extracts contain phenolic compounds, flavonoids, carotenoids, and tocopherols.

The HS isolate of wild *Pleurotus* is found in Bogor, Indonesia. The fruiting body of HS isolate is white, fleshy, not rubbery, not easily deteriorates, having a nice fragrance like *Pleurotus*, and good taste and aroma (Sudirman 2009). The spore prints are also white. Based on the identification by the molecular method, HS isolate is *Pleurotus ostreatus* f. florida (Sudirman 2015, personal communication). HS isolate could produce the fruiting body on rubber sawdust, *Albazia falcataria* sawdust, rice straw, and the mixtures of each sawdust and rice straw (Sudirman 2009). In addition, the fruiting body extracts, substrate extracts, and mycelial extracts of HS isolate showed activity against *Bacillus subtilis* and four strains of Enteropathogenic *Escherichia coli*. In this study, we reported the growth and production of fruiting bodies of HS isolate on other lignocellulosic wastes of *Paraserianthes falcataria* sawdust (PFS), oil palm EFB, and a mixture of PFS and EFB substrates (M substrates), the antioxidant activities, and their total phenolic contents (TPCs) of mycelia and fruiting body.

## 2. Materials and Methods

### 2.1. The isolate of *Pleurotus*

HS isolate is a collection of Lisdar I. Sudirman, Research Center for Biological Resources and Biotechnology (Pusat Penelitian Sumberdaya Hayati dan Bioteknologi (PPSHB)), Bogor Agricultural University.

### 2.2. Production of fruiting bodies

HS isolate was cultivated on PFS substrates, oil palm EFB substrates, and M substrates with a 1:1 ratio. Each substrate was added with 15% rice bran, 1.5% gypsum, and 1.5% lime ($CaCO_3$), and then tap water was added until its water content was adjusted to 70–75%. Each substrate was placed in 10 plastic bags (500 g per bag), then sterilized using autoclave for 30 minutes, inoculated with mushroom spawn, and incubated in a mushroom house at 28–30°C. Cultivation parameters were observed for each bag. These include fresh weight of fruiting bodies (FW), biological efficiency (BE), productivity rates (PRs), pileus numbers (PNs), pileus diameters (PDs), vegetative phase (VP), reproductive phase (RP), and growth and development phase (GDP). VP was determined from the time of mushroom spawn inoculation until the mycelia cover the entire surface of the substrates, RP was determined from the time of plastic bag opening until the last harvest, whereas GDP was the total phase of VP and RP. PR was calculated based on the total FW of fruiting bodies divided by GDP (Sudirman 2014, personal communication). BE was determined using the following formula: BE = (total FW/wet weight of the substrates × 100%) × 4 (Stamets 1993).

### 2.3. Production of mycelia

Mycelia were obtained from liquid culture of HS isolate. One piece of HS isolate inoculum (diameter, 6 mm) was inoculated on the surface of 100 mL of potato sucrose broth medium in an Erlenmeyer flask (volume, 250 mL). The cultures were incubated at room temperature under static state for 30 days.

### 2.4. Extraction of mycelia

The mycelia of HS isolate were separated from its culture filtrate and then rinsed three times with each of 20 mL of distilled water. Extraction was conducted according to the method of Sudirman (2009). Mycelia were mashed using a mortar and then extracted three times using 100 mL of methanol, respectively, and agitated on a rotary shaker at 115 rpm and 25°C for 24 hours. The methanol extracts were separated from the residues by filtration using a Buchner funnel supported by a vacuum pump and dried using a rotary evaporator at 40°C. The dried extracts were dissolved back into methanol and stored in a freezer at 4°C before analysis.

### 2.5. Extraction of fruiting bodies

Fruiting bodies of HS isolate were obtained from cultivation on PFS substrates. Dry fruiting bodies were mashed using a blender and then extracted according to the method of Mau *et al.* (2002). As much as 5 g of powdered mushrooms were extracted three times using 100 mL of methanol, respectively, and agitated on a rotary shaker at 115 rpm and 25°C for 24 hours. The next procedure was the same as described in the extraction of mycelia.

### 2.6. Determination of antioxidant activity

Antioxidant activity was analyzed using DPPH method based on Salazar-Aranda *et al.* (2011) using ascorbic acid as a standard antioxidant compound. Five hundred microliters of mycelial and fruiting body extracts with the concentrations of 0.3125, 0.625, 1.25, 2.5, and 5 mg/mL, respectively, were added to 500 μL of DPPH. The mixtures were shaken vigorously and incubated for 30 minutes at a room temperature in the dark condition. Absorbances were measured using a spectrophotometer at 517 nm. Scavenging activities were calculated using the following formula:

$$\text{Scavenging activity}(\%) = \frac{A - B}{A} \times 100$$

A is the absorbance of control containing 125 μM of DPPH in ethanol, and B is the absorbance of the sample. The antioxidant activity is expressed as IC50. IC50 was calculated by interpolation from the graph plotting each concentration and its scavenging activity. Tests were conducted in triplicates.

### 2.7. Determination of TPCs

TPCs were determined by the Folin–Ciocalteu method according to Tangkanakul *et al.* (2009) using 80 ppm of gallic acid as a standard of phenolic compounds with concentrations of 1.6–19.2 μg/mL. Two milliliters of mycelial or fruiting body extracts were reacted with 10 mL of 10% Folin–Ciocalteu reagent and then incubated at room temperature. After 30 seconds and before 8 minutes, 8 mL of 7.5% $Na_2CO_3$ solution was added to that mixture, and then the total volume was adjusted to 25 mL using distilled water. The solutions were then incubated for 30 minutes at room temperature. The absorbances were measured at 765 nm using a spectrophotometer. TPC is expressed as milligrams of gallic acid equivalents (GAEs)/g extract. Tests were carried out in triplicates.

### 2.8. Data analysis

Cultivation parameter data were shown as mean ± standard error of the mean. Data were analyzed using analysis of variance and further tested with Duncan's multiple range test using Microsoft Excel 2010 (Microsoft) and SPSS Statistics 21.0 (IBM).

## 3. Results

### 3.1. Production of fruiting bodies

HS isolate could grow and produce fruiting bodies on all substrates, i.e. PFS substrates, oil palm EFB substrates, and M substrates, but based on all observation parameters, the M substrates were the best ones for the growth and fruiting body production of

HS isolate. FW of HS isolate for all substrates ranged between 80.59 and 111.07 g/bag with FW on M and PFS substrates being higher than those of EFB substrates with 111.07 and 106.74 g/bag, respectively. Similar results were shown with BE. The BE on M and PFS substrates also was higher than its EFB substrates with 88.86% and 85.30%, respectively, whereas BE for all substrates was observed between 64.47% and 88.86% (Figure 1).

VP, RP, and GDP of HS isolate in all substrates ranged between 21.3–29.3, 51.2–71.7, and 72.5–101 days, respectively, with the shortest VP, RP, and GDP on M substrates with values 21.3, 51.2, and 72.5 days, respectively (Figure 2).

PN and PD on PFS, EFB, and M substrates did not show significant differences ($p > 0.05$) in a range of 13.9–21.1 pieces and 5.95–6.68 cm, respectively (Figure 3). HS isolate had the highest PN on M substrates with values of 21.1 pieces, meanwhile the highest PD was obtained from EFB substrates with a value of 6.68 cm. HS isolate had PD on PFS, EFB, and M substrates in a range of 1–15.4, 0.7–14.5, and 0.9–18.2 cm, respectively. Although the averages of PD did not significantly differ on those three substrates, but M substrates tend to produce larger PD had reached 18.2 cm than other two substrates.

### 3.2. Antioxidant activity

DPPH radical scavenging activities of mycelial and fruiting body extracts are presented in Figure 4. The results showed that all fruiting body extract concentrations had higher DPPH radical scavenging activity than those of mycelial extracts that ranged between 49.31% and 77.82% but lower than vitamin C (data not shown). IC50 of fruiting body and mycelial extracts was of 0.45 ± 0.04 and 2.76 ± 0.36 mg/mL, respectively.

### 3.3. Total phenolic content

TPC of fruiting body and mycelial extracts was of 4.62 ± 0.08 and 2.02 ± 0.02 mg GAE/g extract, respectively. TPC of the fruiting body extracts was twice higher than its mycelial extracts (Table).

## 4. Discussion

Based on all observation parameters, the growth and fruiting body production of HS isolate on M substrates were better than those of PFS and EFB substrates. In the present study, BE of HS isolate in M substrates was higher than BE of HS isolate cultivated on different proportions of rubber tree sawdust and rice straw

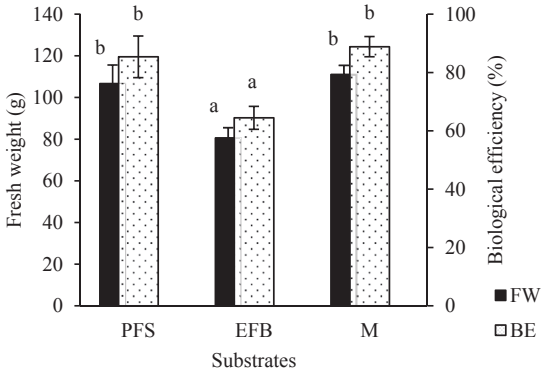


**Figure 1.** FW and BE of *Pleurotus* HS isolate grown on three kinds of substrates: PFS, oil palm EFB, and M substrates (1:1). Different letters on the same figures are significantly different ($p < 0.05$). PFS, *Paraserianthes falcataria* sawdust; EFB, empty fruit bunch; M, mixture of PFS and EFB substrates; FW, fresh weight of fruiting body; BE, biological efficiency.

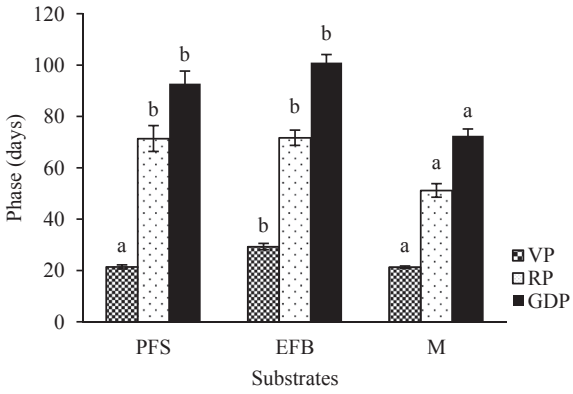


**Figure 2.** Growth phases of *Pleurotus* HS isolate on three kinds of substrates: PFS, EFB, and M substrates (1:1). Different letters on the same figures are significantly different ($p < 0.05$). PFS, *Paraserianthes falcataria* sawdust; EFB, empty fruit bunch; M, mixture of PFS and EFB substrates; VP, vegetative phase; RP, reproductive phase; GDP, growth and development phase.

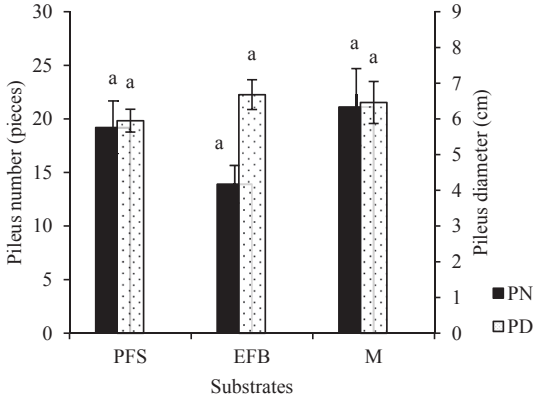


**Figure 3.** PN and PD of *Pleurotus* HS isolate on three kinds of substrates: PFS, EFB, and M substrates (1:1). Same letters on the same figures are not significantly different ($p > 0.05$). PFS, *Paraserianthes falcataria* sawdust; EFB, empty fruit bunch; M, mixture of PFS and EFB substrates; PN, pileus number; PD, pileus diameter.

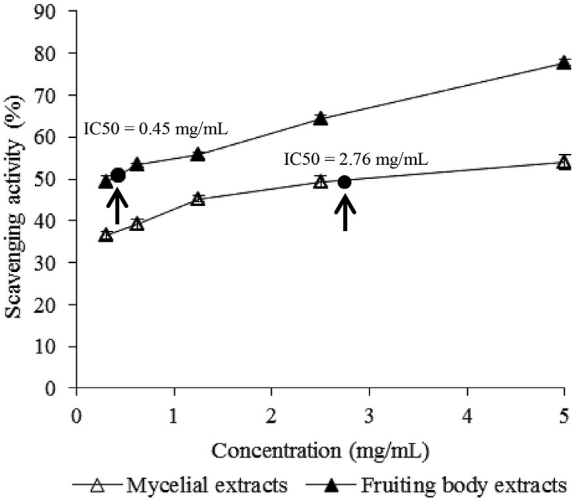


**Figure 4.** Scavenging activity of mycelial and fruiting body extracts of *Pleurotus* HS isolate at various concentrations of extracts.

Table. Total phenolic contents of mycelial and fruiting body extracts of *Pleurotus* HS isolate

| Sample | Total phenolic contents (mg GAE/g extract) |
|---|---|
| Mycelial extracts | 2.02 ± 0.02 |
| Fruiting body extracts | 4.62 ± 0.08 |

GAE, gallic acid equivalent

(69–72%) or on *Albazia falcataria* sawdust and rice straw (57–70%) (Sudirman 2009).

In the previous study, *Pleurotus* F isolate was ever grown on PFS, EFB, and M substrates (Sudirman *et al.* 2011). The BE of *Pleurotus* F isolate in the three substrates (152–167%) was higher than the BE of HS isolate that cultivated on the same substrates with the highest BE on PFS substrates. *Pleurotus* F isolate also showed greater PN (31–34 pieces) than that of HS isolate, but HS isolate showed shorter GDP and larger PD than those of *Pleurotus* F isolate.

The BE of HS isolate on M substrates was higher than the BE of various species of *Pleurotus* that is grown on variety of substrates, such as *P. ostreatus* cultivated on mango sawdust (11.99–30.76%) (Pathmashini *et al.* 2008), *P. pulmonarius* cultivated on rubber wood sawdust (65.48%) (Abdullah *et al.* 2013), *P. ostreatus* cultivated on spent beer grain substrates with the addition of 45% wheat bran (19.1%) (Wang *et al.* 2001), and *P. abalones* and *P. geesteranus* cultivated on asparagus straw (20.6–40% and 56.9–66.3%, respectively) (Wang *et al.* 2012). But, the BE of HS isolate on M substrates was lower than the BE of *P. ostreatus* f. sp. florida (P-184) grown on *Coffea arabica* pulp (168.5–179.4%) (Bermudez *et al.* 2001) compared with that of *P. ostreatus* grown on rice straw (95.46%) (Sharma *et al.* 2013) and those of *P. ostreatus* grown on mixture substrates of wheat straw and paper waste (1:1) (121.2%) (Yildiz *et al.* 2002).

The IC50 values of mycelial and fruiting body extracts of HS isolate were 2.76 ± 0.36 and 0.45 ± 0.04 mg/mL, respectively. It seems that the DPPH scavenging activity of fruiting body extracts was more higher than those of mycelial extracts. Similar result was reported by Reis *et al.* (2012) who found that fruiting body extracts of *P. ostreatus* gave much higher DPPH scavenging activity than its mycelial extracts with IC50 of 6.54 ± 0.16 and 58.13 ± 3.02 mg/mL, respectively.

The DPPH scavenging activity obtained from methanol extracts of HS fruiting body (IC50, 0.45 ± 0.04 mg/mL) was higher than that of the aqueous extracts of *P. ostreatus* and *P. sajor-caju* fruiting bodies from Thailand (IC50, 11.56 and 13.38 mg/mL, respectively) (Chirinang and Intarapichet 2009) and those of the ethanol extracts of *P. pulmonarius*, *P. djamor* var *roseus*, and *P. ostreatus* fruiting bodies from Malaysia (IC50, 4.20, 5.50, and 7.50 mg/mL, respectively) (Arbaayah and Umi 2013) but lower than the results described by Neelam and Singh (2013) for ethanol extracts of *P. florida* and *P. ostreatus* fruiting bodies from India (IC50, 0.17 and 0.19 mg/mL, respectively).

Methanol extracts of HS fruiting body showed higher DPPH scavenging activity than hot water extracts of fruiting body from various medicinal mushrooms that was reported by Abdullah *et al.* (2012), such as *Ganoderma lucidum* (IC50, 5.280 ± 0.263 mg/mL), *Lentinula edodes* (IC50, 19.093 ± 0.296 mg/mL), *Volvariella volvaceae* (IC50, 17.832 ± 0.020 mg/mL), and *Auricularia auricular-judae* (IC50, 23.916 ± 0.106 mg/mL).

The DPPH scavenging activity of methanol extracts of HS mycelial (IC50, 2.76 ± 0.36 mg/mL) was higher than that of methanol extracts of *P. sapidus* mycelial (IC50, about 3 mg/mL) (Jeena *et al.* 2014) compared with those of methanol extracts of *P. ostreatus* and *P. eryngii* mycelia (IC50, 58.13 ± 3.02 and 25.40 ± 0.33 mg/mL) but lower than those of ethanol extracts of young and old mycelial *Agaricus brasiliensis* (1.43 ± 52 and 0.59 ± 35 mg/mL, respectively) (Carvajal *et al.* 2012).

TPCs of the fruiting body extracts of HS isolate were much higher than its mycelial extracts with the value of 4.62 ± 0.08 and 2.02 ± 0.02 mg GAE/g extract, respectively. TPC of HS fruiting body extracts was higher than that of *P. sajor caju*, *P. ostreatus*, and *P. sapidus* fruiting body extracts (1.53 ± 0.09, 1.32 ± 0.10, 1.10 ± 0.05 mg GAE/g extract, respectively) (Jeena *et al.* 2014) and those of *P. ostreatus* fruiting body extracts (3.20 ± 0.05 mg GAE/g extract) (Chowdhury *et al.* 2015) but much lower than that of *P. djamor* var *djamor*, *P. pulmonarius*, *P. djamor* var *roseus*, and *P. ostreatus* ranging between 43.07 ± 0.27 and 50.19 ± 0.98 mg GAE/g extract (Arbaayah and Umi 2013). TPC of HS mycelial extracts also was higher than that of *P. sajor caju*, *P. ostreatus*, and *P. sapidus* mycelial extracts (0.69 ± 0.10, 0.68 ± 0.10, 0.50 ± 0.05 mg GAE/g extract, respectively) (Jeena *et al.* 2014) but lower than those of *P. ostreatus* and *P. eryngii* with values of 5.19 ± 0.14 and 9.11 ± 0.23 mg GAE/g extract, respectively (Reis *et al.* 2012).

TPC of the mycelial and fruiting body extracts of HS isolate was lower than other mushrooms that have been proved to be efficacious as a medicine and edible mushrooms such as *G. lucidum* (47.25 ± 0.20 mg GAE/g extract) (Mau *et al.* 2002), *Grifola frondosa* (19.61 ± 1.69 mg GAE/g extract) (Yeh *et al.* 2011), and *Lentinula edodes* (70.83 mg GAE/g extract) (Sasidharan *et al.* 2010).

A higher TPC in the fruiting body extracts of HS isolate was possibly causing a high antioxidant activity of the fruiting body extracts compared with its mycelial extracts. The key role of phenolic compounds as free radical scavengers has been widely studied. According to Barros *et al.* (2007), phenolic compounds become the main component of antioxidant compounds found in mushrooms, whereas lycopene, ascorbic acid, and beta-carotene are only found in very small amounts. HS isolate had high antioxidant activity, but its TPC was lower than other mushrooms. For example, fruiting body extracts of *G. lucidum* had antioxidant activity with IC50 value of 5.280 ± 0.263 mg/mL, but its TPC was higher than that of HS isolate with value of 63.51 ± 3.11 mg GAE/g extract (Abdullah *et al.* 2012). It might be because of the presence of other compounds that contribute to antioxidant properties of fruiting body and mycelial extracts of HS isolates, such as lycopene, ascorbic acid, beta-carotene, and tocopherol. Thus, further analyses are required to evaluate those nonphenolic compounds using high-performance liquid chromatography or colorimetric assays.

Overall, HS isolate has potential to be cultivated on a variety of lignocellulosic waste. Further research is required to determine the antioxidant activity of the fruiting body obtained on different substrates. On the other hand, the provision of additional nutrients with mineral on substrates of mushroom production is needed to increase the antioxidant activity of selenium, zinc, iron, and others.

## Conflict of interest

The authors declare no conflict of interest.

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