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Full Length Article

Effects of Extracts of Spent Mushroom Substrates on Growth of Edible Fungi

Wei-Rui Zhang[1], Sheng-Rong Liu[1], Zhuo-Xiu Zhao[2], Yun-Bo Kuang[1], Xiao-Fei Dong[1] and Jun-Feng Ruan[1]
[1]*College of Life Science, Ningde Normal University, Ningde 352100, China*
[2]*Edible Fungus Management Stations of Pingnan Country, Ningde 352100, China*
[*]For correspondence: zhang.w.r@163.com

Abstract

Spent mushroom substrate (SMS) is produced annually in large quantities and its disposal has become a challenging problem for mushroom growers. Recycling of SMS in mushroom cultivation might be the best method for its disposal. However, currently the selection of a suitable mushroom species in cultivation for recycling a certain SMS is still a trial-and-error process. The study described herein was a preliminary investigation on SMS recycling for mushroom production. By individually supplementing the extracts of SMS of *Pleurotus eryngii*, *Flammulina velutipes* and *Hypsizigus marmoreus* into agar medium in Petri dishes, their effects on the mycelial growth of a broad range of mushroom species were investigated. Out of the 10 examined species, the extracts of SMS of *P. eryngii* had a positive effect on the mycelial growth of five species namely *P. ostreatus*, *P. geesteranus*, *Pholita nameko*, *Auricularia polytricha* and *F. velutipes*. Three strains *P. ostreatus*, *P. geesteranus* and *A. polytricha* showed vigorous mycelial growth upon supplementation of the extract of *F. velutipes* SMS, with the surface mycelial density remaining almost unchanged. In contrast, only *A. polytricha* grew well upon addition of the extract of *H. marmoreus* SMS in the growth medium. The present study provides fundamental knowledge directing the robust selection of suitable mushroom species in cultivation for recycling the SMS of *P. eryngii*, *F. velutipes* and *H. marmoreus* and the rational design of SMS-based cultivation substrates.

Keywords: Mushrooms; Mycelial growth; Recycling; Spent mushroom substrate; Surface mycelial density

Introduction

Mushrooms are good sources of proteins, vitamins, and minerals, and many of them like *Lentinula edodes*, *Ganoderma lucidum* and *Cordyceps militaris* produce various bioactive substances, such as polysaccharides, which have important pharmacological activities including antitumor, antioxidant, and immunomodulatory effects (Mizuno *et al*., 1995; Wasser and Weis, 1999; Wang *et al*., 2012). The cultivation of mushrooms is considered to be an economically viable application of biotechnology. Besides being an efficient source of food in the form of fruit bodies, the cultivation of mushrooms also reduces environmental pollution by utilizing various waste products generated from industry, agriculture, forestry, and food processing, as growth substrates (Sánchez, 2010). Artificial cultivation, mainly on lignocellulosic solid wastes, is currently the most widely used method for the production of mushrooms. Sawdust and cottonseed hull are the most commonly used materials used for artificial cultivation.

Spent mushroom substrate (SMS) is the main by-product of mushroom industry after sporocarp has been removed from the cultures. The large quantities of spent mushroom wastes produced within a concentrated area by the mushroom industries pose a serious environmental problem (Williams *et al*., 2001). Currently, some spent substrates are used as fertilizers, which serve as a source of organic nitrogen (Hackett, 2015), as well as animal feedstock (Zhang *et al*., 2005). However, most of the spent substrate is burnt to generate steam that is used for substrate sterilization and heating of mushroom farms, or is thrown away; such disposal activities are neither environment friendly nor are they economical (Zhu *et al*., 2013). Recently, SMSs have been employed for isolating several useful metabolites, like polysaccharides and various enzymes (Singh *et al*., 2003; He *et al*., 2016; Zhang *et al*., 2016). However, despite these achievements, the best and most economically viable method for disposal of SMS is considered to be its utilization in successive mushroom cultivation. Several studies have been conducted with the objective of recycling the spent substrates as components of substrates used for the cultivation of mushrooms (Royse, 1992; Jo *et al*., 2008; González Mature *et al*., 2011). However, screening of a mushroom species that can effectively utilize a particular recycled SMS for fruiting is currently a trial-and-error process.

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It has been reported that the nutrient and energy present in the spent substrate is not fully exploited by the mycelia (Philippoussis *et al*., 2001; Mandeel *et al*., 2005). This is especially true for low-temperature fruiting-type mushrooms such as *Pleurotus eryngii, Flammulina velutipes* and *Hypsizigus marmoreus* under factory cultivation where usually only one flush of fruiting bodies is harvested because the mushroom yield of the successive flushes is not high enough to make profit. As these three species are the major commercially cultivated mushroom strains, efficient recycling of SMS derived from the cultivation of these three mushroom species is of great importance. This study was carried out to generate preliminary knowledge regarding the recycling of SMS derived from *P. eryngii, F. velutipes*, and *H. marmoreus* for mushroom cultivation by determining the effect of their extract on the growth of mycelia of a board range of mushroom species.

Materials and Methods

Spent Mushroom Substrates and Pretreatment

The SMS of *P. eryngii, F. velutipes* and *H. marmoreus* were obtained from three local mushroom factories. They were generated from three different substrate formulae as listed in Table 1. All the SMS were collected after harvesting only one flush mushroom from bags; they were fragmented by hand and were oven dried. The dried SMS were crushed in a hammer mill, and the resulting powder was used.

Strains

Ten mushroom species namely *P. ostreatus, L. edodes, P. geesteranus, F. velutipes, Auricularia polytricha, Pholita nameko, P. eryngii, Coprinus comatus, Agrocybe aegerita* and *Oudemansiella radicata* were obtained from Sanming Institute of Edible Fungi, Fujian Province, China. All the species were cultivated at 25°C for 7 days, and were stored at 4°C and sub-cultured, every four week.

Media

In our preliminary experiments, it was found that few of the mushroom species grew poorly on the commonly used PDA medium. For achieving better mycelial growth, screening of suitable media for each of the tested mushroom species was performed. Five different agar media namely potato dextrose agar (PDA) medium, mushroom complete medium (MCM), yeast malt extract (YM) medium, sawdust medium (SM) and cottonseed hull medium (CM) were used to select the most suitable medium for mycelial growth of each of the examined mushroom species.

All the media contained 20 g glucose and 18 g agar per liter; the other components were as described below: (1) PDA medium consisted of potato extract (200 g boiled potato in 500 mL distilled water); (2) MCM medium contained 2 g peptone, 0.46 g KH_2PO_4, 1.0 g K_2HPO_4, and 0.5 g $MgSO_4$; (3) YM medium contained 2 g each of yeast extract and malt extract; (4) SM contained sawdust extract (500 g boiled in 1000 mL distilled water) and wheat bran extract (100 g boiled in 300 mL distilled water); (5) CM consisted of cottonseed hull extract (250 g boiled in 1000 mL distilled water) and wheat bran extract (50 g boiled in 200 mL distilled water). All the media were autoclaved at 121°C for 20 min.

The modified media were prepared as described below. For preparing SMS-containing SM or CM (per liter), the extract obtained from 100, 200, 300, or 400 g of dried SMS powder (boiled in appropriate volume of distilled water for 30 min) was used to replace the sawdust extract in SM or cottonseed hull extract in CM. When YM was used as the control medium, the extract was added directly. These modified media were designated as CKX1, CKX2, CKX3, and CKX4, where "X" is replaced with "P" if the extract of SMS from *P. eryngii* was used, with "F" for *F. velutipes* SMS, and "H" for *H. marmoreus* SMS, and numbers 1, 2, 3, and 4 indicate that extracts from 100, 200, 300 and 400 g of dried SMS powder, were used, respectively.

Plate Culture

Each of the test mushroom strains was initially activated in a 9 cm Petri dish containing PDA medium. The incubation was performed at 25°C in complete darkness for various days, depending on the mushroom strains. Then, a mycelial agar block (1 cm diameter) was cut from the periphery of actively growing colony cultures using a sterile cork borer, and plotted on the center of a 9 cm Petri dish containing the medium. The incubation was done at 25°C under complete darkness for 7 days, and the diameter of the mycelial colony was measured along two perpendicular directions, and was averaged (mm/day). The surface mycelial density was estimated with naked eye.

Chemical Analysis

The major nutritional composition of three SMS was determined for evaluating their suitability for use in mushroom cultivation. The content of lignin was assayed according to the method described by Goering and Soest (1970). The content of protein was determined by the Kjeldahl method, with a factor of 4.38 (Chang and Miles, 1989). Crude fiber was assayed according to the method of Nyanga *et al*. (2013). Crude fat was determined by extraction with petroleum ether for 8 h using a Soxhlet apparatus (Fernandes *et al*., 2014). The ash content was determined by incinerating the dried sample in a muffle furnace (SX-2.5-10, Shanghai Hongji Instruments Co., Ltd., Shanghai, China) at 565°C for 3 h (Khan *et al*., 2008). The assay on cottonseed hull was used for reference.

Statistical Analysis

Data are expressed as means ± standard deviation of three replicates. The differences between the means of individual groups were assessed by a one-way ANOVA with Duncan's multiple range test at 95% confidence level.

Results

Comparison of the Compositions of Three Spent Mushroom Substrates

The major nutritional components in the three SMS and in cottonseed hull (a commonly used substrate in mushroom cultivation) are listed in Table 2. The protein content in the SMS of *P. eryngii*, *F. velutipes*, *and H. marmoreus* was 3.28-, 2.41- and 2.56-fold higher, respectively, than the content in the cottonseed hull. The three spent substrates had lower contents of crude fiber (32.32–33.86%) than the content present in the cottonseed hull (40.91%). In comparison to the content of crude fat in the cottonseed hull (1.45%), the content in the SMS of *P. eryngii* (4.22%) and *H. marmoreus* (5.23%) was higher, but that in the SMS of *F. velutipes* was lower (1.27%). The lignin content ranged from 17.90% to 19.72% in the three spent substrates and was higher than that in the cottonseed hull (16.96%). The ash content in the three spent substrates was significantly higher than that in the cottonseed hull.

Screening of Media Suitable for Mycelial Growth of Examined Mushroom Species

As show in Table 3, the results demonstrate that the mycelial growth in each of the test mushroom strains varied with the media type. SM and CM were favorable media for *P. nameko, A. aegerita, P. ostreatus*, and *O. radicata*. For *L. edodes, P. geesteranus* and *A. polytricha*, PDA and SM were suitable media for mycelial growth. The suitable medium was MCM and YM for *C. comatus*. For *F. velutipes* growth, PDA, MCM and CM were excellent. In the case of *P. eryngii*, only YM supported its growth well. In the subsequent experiments, SM was selected as the control medium for cultivating *L. edodes, P. nameko, A. aegerita, P. ostreatus, P. geesteranus, A. polytricha*, and *O. radicata*, YM was used as the control medium for *C. comatus* and *P. eryngii*, and CM was chosen as the control medium for *F. velutipes*. Based on these control media, the modified media containing the extract of different SMS were prepared.

Effect of the Extract of SMS of *P. eryngii* on the Mycelial Growth of Mushrooms

The promotion of mycelial growth rate in *P. nameko, P. ostreatus, P. geesteranus, A. polytricha*, and *F. velutipes*, was obvious at all the test levels of the extract of *P. eryngii* SMS in the media, with the rates increasing by 1.26–36.90%, 0.31–6.48%, 15.61–19.75%, 28.68–39.77%, and 5.42–15.70%, respectively, in comparison to those growing on the respective control medium (Table 4). No obvious difference in the surface mycelial density among the four supplemented media and the control medium was observed for any of the five test strains.

For *L. edodes*, decreased mycelial growth rates were observed for the four supplemented media as compared with that for the control medium. Differently, there was no difference observed in the density of surface mycelia among the four supplemented media as well as on the control medium.

For *C. comatus* grown on the supplemented CKP1 medium and on the control medium, no statistical difference in the growth rate was observed; however, a further increase in the supplement level decreased the growth rate. The density of surface mycelia was similar between CKP1 and the control, whereas the mycelia became sparse with the increased level of the extract.

Regarding *A. aegerita*, its growth on CKP1 and CKP2 was faster than that on the control medium. In contrast, the growth rate was lower on the CKP3 and CKP4 media than on the control. There was no difference in the density of surface mycelia among the four supplemented media and the control medium. For *P. eryngii* and *O. radicata*, the mycelial growth rate was negatively affected by the supplementation of the extract at all the four levels, while the mycelial density was similar to that grown on the control, with the exception of *P. eryngii* on CKP1.

Effect of the Extract of SMS of *F. velutipes* on the Mycelial Growth of Mushrooms

As shown in Table 5, there was no statistical difference in the mycelial growth rate among the four supplemented media and the control medium for *P. ostreatus, P. geesteranus*, and *A. polytricha*. The density of surface mycelia of the three species was also unaffected by supplementation at all the tested levels. For the remaining seven mushroom species, the mycelial growth rate was negatively affected by the supplementation of the extract, with the most negative effect observed for *P. eryngii* and *C. comatus*. The density of surface mycelia of *L. edodes*, *P. nameko*, *P. eryngii* and *O. radicata* was affected by supplementation of the extract at all tested levels. The surface mycelia growth of *C. comatus* and *A. aegerita* in the modified media (CKF1 and CKF2) was similar to that in the control medium, but sparse growth was observed with the increase of the level of supplementation (CKF3 and CKF4). *F. velutipes* showed sparse mycelia in the three supplemented media (CKF1, CKF2 and CKF3), but normal growth was observed on CKF4 at a higher level of the added extract.

Table 1: The three formulae used for factory cultivation of *P. eryngii*, *F. velutipes*, and *H. marmoreus*

Materials	Formulae (% dry weight basis)		
	P. eryngii	*F. velutipes*	*H. marmoreus*
Sawdust	30	0	10
Cottonseed hull	30	38	50
Corncob	18	24	10
Sugarcane bagasse	0	0	9
Wheat bran	15	35	15
Corn powder	5	2	4
Gypsum	2	1	2

Table 2: Chemical composition of the three tested spent media substrates and cottonseed hull used in this work

Substrate	Protein (%)	Crude fat (%)	Crude fiber (%)	Lignin (%)	Ash (%)
Spent *P. eryngii* substrate	13.32±0.72	4.22±0.32	33.86±3.14	18.31±1.12	9.23±0.63
Spent *F.velutipes* substrate	9.80±0.26	1.27±0.21	32.32±2.81	17.90±0.89	7.75±0.44
Spent *H. marmoreus* substrate	10.41±0.34	5.23±0.61	32.63±1.84	19.72±2.04	15.78±0.81
Cottonseed hull	4.06±0.21	1.45±0.21	40.91±3.17	16.96±1.51	2.63±0.22

Data are presented as mean ± standard deviation of three replicates

Table 3: Mycelial growth rate and density of surface mycelia of the 10 examined mushroom species grown on different media

Media	Mycelial growth rate (mm/day)/density of surface mycelia (from 1 to 3)									
	L. edodes	*P. nameko*	*C. comatus*	*P. eryngii*	*A. aegerita*	*P. ostreatus*	*P.geesteranus*	*A. polytricha*	*F. velutipes*	*O. radicata*
PDA	4.86±0.11a/3	3.34±0.13b/2	4.86±0.23bc/3	3.27±0.03b/2	4.28±0.14c/3	4.57±0.07c/2	6.98±0.22ab/3	5.43±0.06a/3	5.33±0.08a/3	4.28±0.15b/2
MCM	3.77±0.07b/3	3.10±0.10b/2	5.37±0.07ab/3	2.45±0.03d/2	4.43±0.17cd/3	3.45±0.09d/2	6.12±0.31c/1	4.98±0.08b/3	5.25±0.16ab/3	4.87±0.05a/2
YM	3.10±0.05c/2	2.46±0.06c/2	5.58±0.14a/3	4.67±0.12a/3	4.64±0.08bc/3	5.70±0.28b/3	6.27±0.16c/2	4.92±0.16b/3	4.75±0.07c/3	4.72±0.21ab/2
SM	4.63±0.18a/3	4.80±0.16a/3	4.13±0.12d/1	3.35±0.08b/2	4.97±0.04ab/3	6.34±0.27a/3	7.19±0.33a/3	5.22±0.14ab/3	5.01±0.05bc/3	5.03±0.14a/3
CM	3.80±0.07b/3	4.53±0.07a/3	4.73±0.22c/3	2.94±0.04c/2	4.90±0.10a/3	6.17±0.11ab/3	6.35±0.12bc/3	3.64±0.11c/3	5.34±0.06a/3	5.08±0.17a/3
Preferred media	PDA, SM	SM, CM	MCM, YM	YM	SM, CM	SM, CM	PDA, SM	PDA, SM	PDA, MCM, CM	SM, CM

Note: The colony density was judged by the density of mycelial hyphae. The numbers have arbitrary unit, depicting the density of hyphal mycelia from 1 to 3. The number 3 is for the densest mycelia

Data are presented as mean ± standard deviation of three replicates; means followed with different letters within each column are statistical significant at $P < 0.05$ according to Duncan's multiple range tests

Table 4: Effect of the extract of spent substrate of *P. eryngii* on the growth rate and density of surface mycelia of various mushroom species

Media	Mycelial growth rate (mm/day)/density of surface mycelia (1 to 3)									
	L. edodes	*P. nameko*	*C. comatus*	*P. eryngii*	*A. aegerita*	*P. ostreatus*	*P. geesteranus*	*A. polytricha*	*F. velutipes*	*O. radicata*
CK	4.62±0.12a/3	4.77±0.13b/3	5.59±0.09ab/3	4.70±0.06a/3	4.95±0.05b/3	6.32±0.11a/3	7.24±0.33b/3	5.23±0.11b/3	5.35±0.05c/3	5.02±0.09a/3
CKP1	4.02±0.11c/3	4.83±0.07b/3	6.06±0.27ab/3	3.15±0.14d/2	5.54±0.26a/3	6.34±0.29a/3	8.44±0.39ab/3	7.31±0.20a/3	5.95±0.16ab/3	4.50±0.19b/3
CKP2	4.38±0.13ab/3	5.21±0.03b/3	5.26±0.32a/2	3.47±0.06cd/3	5.13±0.12b/3	6.73±0.25a/3	8.37±0.58ab/3	7.11±0.57a/3	6.19±0.37a/3	4.60±0.07b/3
CKP3	4.21±0.19bc/3	6.34±0.04a/3	5.08±0.06bc/2	3.81±0.15bc/3	4.47±0.17c/3	6.54±0.44a/3	8.50±0.50ab/3	6.76±0.12a/3	5.64±0.11bc/3	4.60±0.08b/3
CKP4	3.61±0.08d/3	6.53±0.05a/3	4.56±0.13c/2	4.03±0.21b/3	4.37±0.09c/3	6.70±0.28a/3	8.67±0.30a/3	6.73±0.08a/3	5.73±0.18abc/3	4.56±0.05b/3

Note: For *L. edodes*, *P. nameko*, *A. aegerita*, *P. ostreatus*, *P. geesteranus*, *A. polytricha*, and *O. radicata*, SM was used as the control medium, for *C. comatus* and *P. eryngii*, YM was the control medium, and for *F. velutipes*, HM served as the control medium. These control media were designated as CK. The colony density was judged by the density of mycelial hyphae. The numbers have arbitrary unit, depicting the density of hyphal mycelia from 1 to 3. The number 3 is for the densest mycelia

Data are presented as means ± standard deviation of three replicates; means followed with different letters within each column are statistically significant at $P < 0.05$ according to Duncan's multiple range tests

Effect of the Extract of SMS of *H. marmoreus* on the Mycelial Growth of Mushrooms

The data on the mycelial growth rate and density of surface hyphae of the 10 tested mushroom species in response to the extract of SMS of *H. marmoreus* are listed in Table 6. For *A. polytricha*, there was no statistical difference in the mycelial growth rate among the four supplemented media and the control medium, and furthermore the density of surface mycelia was also unaffected. For the remaining nine mushroom species, a considerable decrease in the mycelial growth rate was observed caused by the supplementation of the extract into the media, with the most negative effect observed for *L. edodes*, *P. nameko*, *A. aegerita* and *O. radicata*. In addition, the density of surface mycelia of these nine strains was also negatively affected to a great content by the supplementation.

Table 5: Effect of the extract of spent substrate of *F. velutipes* on the growth rate and density of surface mycelia of various mushroom species

Media	Mycelial growth rate (mm/day)/density of surface mycelia (1 to 3)									
	L. edodes	*P. nameko*	*C. comatus*	*P. eryngii*	*A. aegerita*	*P. ostreatus*	*P. geesteranus*	*A. polytricha*	*F. velutipes*	*O. radicata*
CK	4.62±0.12a/3	4.77±0.13a/3	5.59±0.09a/3	4.70±0.08a/3	4.95±0.05a/3	6.32±0.11a/3	7.24±0.33a/3	5.23±0.11a/3	5.35±0.06a/3	5.02±0.09a/3
CKF1	4.01±0.15b/2	3.11±0.18c/2	3.97±0.15b/3	2.83±0.23b/2	4.28±0.05b/3	6.23±0.23a/3	8.01±0.22a/3	5.17±0.16a/3	4.83±0.10b/2	3.97±0.25b/2
CKF2	4.04±0.12b/2	3.17±0.10bc/2	3.93±0.18b/3	2.86±0.20b/2	4.29±0.18b/3	6.09±0.10a/3	8.46±0.65a/3	5.12±0.24a/3	4.84±0.12b/2	3.96±0.18b/2
CKF3	3.90±0.17b/2	3.49±0.12b/2	3.37±0.04c/2	2.75±0.07b/2	4.03±0.05c/2	6.16±0.25a/3	8.29±0.75a/3	5.24±0.04a/3	4.82±0.08b/2	3.47±0.13c/2
CKF4	3.37±0.07c/2	3.52±0.07b/2	3.59±0.09c/2	2.87±0.14b/2	4.00±0.04c/2	6.17±0.16a/3	7.99±0.16a/3	5.11±0.27a/3	4.83±0.02b/3	3.39±0.18b/2

Note: Symbols are the same as defined in Table 4

Data are presented as mean ± standard deviation of three replicates; means followed with different letters within each column are statistical significant at $P < 0.05$ according to Duncan's multiple range tests

Table 6: Effect of the extract of spent substrate of *H. marmoreus* on the growth rate and density of surface mycelia of various mushroom species

Media	Mycelial growth rate (mm/day)/density of surface mycelia (1 to 3)									
	L. edodes	*P. nameko*	*C. comatus*	*P. eryngii*	*A. aegerita*	*P. ostreatus*	*P. geesteranus*	*A. polytricha*	*F. velutipes*	*O. radicata*
CK	4.62±0.12a/3	4.77±0.13a/3	5.59±0.09a/3	4.70±0.08a/3	4.95±0.05a/3	6.32±0.11a/3	7.24±0.33a/3	5.23±0.11a/3	5.35±0.05a/3	5.02±0.09a/3
CKH1	1.13±0.01b/1	2.42±0.06b/2	3.14±0.04c/2	3.08±0.02b/3	1.10±0.01d/1	4.12±0.03b/2	5.49±0.13b/2	4.91±0.05a/3	3.88±0.05d/3	2.02±0.08c/1
CKH2	1.20±0.01b/1	2.44±0.11b/2	3.27±0.01bc/2	3.06±0.01b/3	1.62±0.03c/1	4.07±0.01c/2	5.15±0.27b/2	4.89±0.07a/3	4.20±0.09c/3	2.12±0.10c/1
CKH3	1.23±0.08b/1	2.63±0.11b/2	3.37±0.01b/2	3.09±0.02b/3	2.26±0.05b/2	4.10±0.05c/2	5.40±0.04b/2	5.05±0.03a/3	4.62±0.15b/3	3.87±0.12b/2
CKH4	1.07±0.04b/1	2.63±0.07b/2	3.34±0.04b/2	3.07±0.01b/3	1.21±0.01d/1	4.36±0.04c/2	4.97±0.09b/2	5.06±0.51a/3	3.17±0.01e/3	1.92±0.05c/1

Note: Symbols are the same as defined in Table 4

Data are presented as mean ± standard deviation of three replicates; means followed with different letters within each column are statistical significant at $P < 0.05$ according to Duncan's multiple range tests

Discussion

Previous studies have demonstrated that nutritional components play a critical role in the growth of mycelia (Kim *et al*., 2002; Malinowska *et al*., 2009). In the three investigated spent substrates, high protein contents were detected. This might be because of three reasons: (1) the original cultivation substrates contained high levels of protein-rich additives, such as wheat bran and corn powder, (2) protein that existed in the substrate was only partially utilized by the mycelia, and (3) the mycelia synthesized appreciable amounts of cellular proteins, and extracellular enzymes for degrading the substrate for cellular nutrition during spawn running and development of the fruit body. Another important component lignin in the three spent substrates still remained at a high level. This could be possibly because of the fact that some supplements like sawdust have high lignin content and only a portion of it is degraded by the mycelia during the cultivation period. Regarding crude fiber, fat, and ash in the substrates, their levels were all acceptable. Overall, these results suggest that the three examined spent mushroom substrates could support the mycelial growth and development of fruiting body in mushrooms.

The extract of *P. eryngii* SMS had a positive effect on the mycelial growth of *P. nameko*, *P. ostreatus*, *P. geesteranus*, *A. polytricha*, and *F. velutipes*. The reason for this enhancement could mainly be the presence of organic nitrogen source, growth factors, and some easily digestible carbohydrates in the extract. Hence, we believe that SMS of *P. eryngii* could be a good substrate component for the cultivation of these five mushroom species. For *L. edodes*, its growth was partially inhibited by the extract. Thus, *P. eryngii* SMS might be not an appropriate material in substrate for the cultivation of *L. edodes*. As to *C. comatus*, the mycelial growth rate began to decline at a supplement level with CKP2 medium. This is also true for surface mycelial density. These findings suggested that *C. comatus* was sensitive to the extract of *P. eryngii* SMS, and it is reasonable to deduce that the SMS of *P. eryngii* was not a suitable substrate for *C. comatus* cultivation. For *A. aegerita*, as a decreased mycelial growth rate was observed only at relatively high supplement levels (CKP3 and CKP4), while the mycelial density was unaffected. Thus, the SMS of *P. eryngii* could be a good substrate for *A. aegerita* cultivation. The use of the spent *P. eryngii* substrate has been reported for the cultivation of *A. aegerita* (Zeng *et al*., 2017); the yield of fruiting body obtained in this study was high. In the case of *P. eryngii* and *O. radicata*, as the mycelial growth rate and hyphal density were negatively affected by the supplementation of the extract, the SMS of *P. eryngii* might not be a suitable substrate for their cultivation. Herein, it should be pointed out that the decreased mycelial growth rate or sparse mycelial growth was observed for several mushrooms in some cases by the supplementation, possibly because of the existence of appreciable amounts of inhibitory substances in the extract, than could be tolerated by the strains.

As the growth of *P. ostreatus*, *P. geesteranus*, and *A. polytricha* was not found to be negatively affected by the supplementation of the extract of *F. velutipe* SMS into the media, the SMS of *F. velutipes* might be a good substrate component for the cultivation of all three strains, and their optimal supplement levels in a certain substrate should be

determined for each strain. Li (2013) successfully used spent *F. velutipes* substrate for the cultivation of *P. ostreatus*, and got the highest mushroom yield at 60% (w/w) supplementation level (based on dry weight). In the case of the remaining seven strains including *C. comatus*, the mycelial growth and density was negatively affected, but showed varying effects, hence the suitability of *F. velutipe*s SMS for cultivation of the seven mushroom species is ambiguous, and warrants further investigation. In literature, successful cultivation of *C. comatus* on *F. velutipes* SMS has been reported (Rinker, 2002).

In the case of using the extract of *H. marmoreus* SMS in the media at tested levels, among ten examined edible fungi, only *A. polytricha* growth was unaffected. This result suggested that the SMS of *H. marmoreus* might be a good substrate component for the growth of this mushroom. As of date, there is no report on *A. polytricha* cultivation using *H. marmoreus* SMS in the substrate mixture. For the remaining nine strains, both the mycelial growth rate and density were negatively affected at a great content by the supplementation of the extract; we therefore believe that the SMS of *H. marmoreus* is not a suitable substrate for the cultivation of these mushroom strains, including *P. ostreatus*. In contrast, the supplementation of the SMS of *H. marmoreus* at 12–25% (w/w) in *P. ostreatus* cultivation was reported to give promising biological efficiency, higher than that of the control (Wang *et al*., 2015). This could be related to a considerable variation in the composition of SMS of *H. marmoreus*, because the substrate formula for *H. marmoreus* cultivation might be different.

Conclusion

This study showed that the mycelial growth of mushroom species varied considerably with media. Among the ten examined mushrooms, the extract of the SMS of *P. eryngii* promoted the mycelial growth of *P. ostreatus*, *P. geesteranus*, *P. nameko*, *A. polytricha* and *F. velutipes*. For the supplementation of the extract of *F. velutipes* SMS into the media, *P. ostreatus*, *P. geesteranus* and *A. polytricha* showed vigorous growth. In the case of addition of the extract of SMS of *H. marmoreus*, only *A. polytricha* showed promising growth. Our results provide fundamental knowledge directing the robust selection of suitable mushroom species in cultivation for recycling SMS of *P. eryngii*, *F. velutipes*, and *H. marmoreus* and the rational design of SMS-based mushroom cultivation substrates.

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