Study on Terpenoid Degradation of *Pinus massoniana* Sawdust for Mycelium Germination and Cultivation of Edible Fungi

Jinping Zhang * and Yue Ying

The material basis and degradation of the terpenoids were investigated in Pinus massoniana sawdust during the composting process. In order to better utilize P. massoniana sawdust for the culturation of edible fungus, three treatments were designed according to the amount of brown sugar that was added: 4% (A1), 5% (A2), and 6% (A3). The brown sugar was added based on the dry weight of the P. massoniana sawdust. The results showed that the brown sugar addition of 5% yielded the fastest heating rate of composting and the longest temperature duration above 60 °C. The gas chromatography-mass (GC-MS) analysis showed that the amount of brown sugar did not affect the degradation of the terpenoids. The relative terpenoid content decreased from 3.89% to 2.10% and 0.31% after 30 d and 60 d of composting, respectively. Fourier transform infrared (FTIR) analysis indicated that the terpenoids decomposed a lot throughout the composting process. The mycelium cultivation demonstrated that the P. massoniana sawdust treated with 30 d of composting promoted the growth of edible fungi.

Keywords: Pinus massoniana sawdust; Material basis, composting; Terpenoids; Edible fungi

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INTRODUCTION

Pinus massoniana Lamb. is widely cultivated in 17 provinces (districts) in China (Su and Shen 2000). There are 1.2×10^7 hm^2 of *P. massoniana* forests in China, which account for 7.74% of the total forest area. As the main afforestation tree species and the main material for industrial, agricultural, construction, and civil utilization, hundreds of millions of tons of *P. massoniana* sawdust is produced in China annually. While some sawdust is used as fuel, most of it is discarded (Lin *et al.* 2004; Deng and Li 2009; Yang *et al.* 2010; Liu 2015). The intercellular substance of the *P. massoniana* wood fiber contains a large amount of extractables, such as resin, tannin, polyphenols, and terpenes, which limit the application of *P. massoniana* sawdust (Zhang and Du 2017).

Sawdust of broad-leaved species is the primary raw material for the cultivation of edible fungi. This sawdust consumes many broad-leaved forest resources every year. With the implementation of the "Natural forest protection project" (Li 2004), forest felling has been restricted, which has limited the production of various edible fungi that use broad-leaved wood as the raw material. *P. massoniana* sawdust is similar to broad-leaved wood in that it contains the essential nutrients for the growth of edible fungi such as cellulose, lignin, and polypentose. However, *P. massoniana* sawdust contains terpenoids and other substances that inhibit the growth of edible fungi. (Liu *et al.* 2005; Zhu 2008). Studies have shown that the addition of 0.1% turpentine (the main component is terpenoid) to the broad-leaf sawdust culture medium can prevent the germination of the mushroom mycelium.

Therefore, it is believed that the terpenoids contained in the sawdust of *P. massoniana* are the main factors affecting the use of *P. massoniana* sawdust as the substrate for the cultivation of edible fungi (Pan 1989; Wu *et al.* 2008). Currently, the processes for reducing the terpenoids in *P. massoniana* sawdust include the steaming method and the distillation method, among others. However, these processes are complicated, costly, and ineffective. Other processes, such as the lime water immersion method, the outdoor composting method, and the chemical method (Qiu 2003; Cai *et al.* 2015; Zhang and Du 2017) can reduce the terpenoids at a lower cost. However, these processes require long processing times and additional land resources. Therefore, it is necessary to develop a simple, feasible, efficient, and practicable method to reduce the terpenoid content in *P. massoniana* sawdust.

High-temperature fermentation is a commonly used method for the treatment of solid organic waste, as it uses microbial action to degrade and transform harmful substances such as terpenoids (Qiu 2003; Chen and Huang 2013). In this study, *P. massoniana* sawdust was used as raw material, and the carbon-to-nitrogen (C/N) ratio was adjusted by using soybean meal. The exogenous nutrients and microbial agents were used to carry out the composting of the *P. massoniana* sawdust. Moreover, the degradation of the terpenoids during the composting was studied. By comparing and analyzing the mycelial germination of shiitake mushroom and *Auricularia nigricans* cultivation with *P. massoniana* sawdust before and after composting as the substrate, the composting effect was determined. This provided a scientific basis for utilizing *P. massoniana* sawdust as the substrate for edible fungus cultivation.

EXPERIMENTAL

Research Field

The experiment was conducted in the Fuyang District, Hangzhou City, Zhejiang Province, China, from March 2018 to May 2018. The geographical coordinates were 30°3′36″ north latitude and 119°57′9″ east longitude with an elevation of 30 m. During the experiment, the highest and lowest temperatures in the area were 20.5 °C and 5 °C, respectively.

Experimental Materials

The *P. massoniana* sawdust was collected from the Laoshan Forest Farm in Qiandao Lake, Zhejiang Province, China. The sawdust particles were between 1 mm and 3 mm in diameter. The soybean meal was acquired from Zhengzhou, China. The brown granulated sugar was purchased from a local supermarket. The terpene-degrading bacteria was a mixture of *Enterobacter cloacae* and *Serratia marcescens* with a volume ratio of 1:1. The bacteria was screened and deposited in the laboratory of the China General Microbiological Culture Collection Center (CGMCC), which is affiliated with the Microbial Culture Collection Management Committee. The effective microorganism (EM) bacteria were purchased from Henan Nanhua Qianmu Biotechnology Co. (Zhenzhou, China). The main components of the EM bacteria were *Bacillus*, *Lactobacillus*, *Bifidobacterium*, yeast, photosynthetic bacteria, acetic acid bacteria, *Actinobacillus*, and other original species.

Composting Process and Sample Collection

The stacking process was carried out in an insulated and highly ventilated

ecological box with dimensions of 73 cm × 115 cm × 80 cm (220 L; Biolan, Eura, Finland). A total of three experimental groups (A1, A2, and A3) were set up and the soybean meal content was adjusted to the initial C/N ratio of 30, 1.5% EM bacteria, and 1.5% terpene-degrading bacteria. Then, brown granulated sugar dosed at 4%, 5%, and 6% of the dry weight of the raw materials was added to the groups A1, A2, and A3, respectively. Subsequently, water was added to adjust the initial moisture content of each group to 55%. The samples were mixed evenly by stirring and were loaded into the stacking barrels separately to start the aerobic fermentation process. During the composting process, the composting temperature and the ambient temperature were recorded every day at 3 pm. The composting temperatures were measured at 30 cm below the surface, in the middle of the composting material, and at 30 cm from the bottom of the compost container. The compost was turned over every 5 d, and the samples were partially sealed and stored in a refrigerator at 4 °C for use. The samples were partially dried at 85 °C and pulverized. The particles that passed through an 80-mesh sieve but not a 100-mesh sieve were used for the infrared spectroscopy analysis. The remaining sample particles were used for the material basis analysis.

Parameters of the Composting Materials

The content of organic carbon (TOC) was determined according to the national standard HJ 615(2011); the contents of nitrogen and phosphorus were determined according to the national standards LY/T 1271(1999). The contents of copper, zinc, manganese, iron, sodium, and potassium were determined in accordance with the national standards, LY/T 1270(1999). The contents of arsenic, cadmium, lead, chromium, and mercury were determined according to the national standard LY/T 1296(1999). The contents of cellulose, hemicellulose, and lignin were determined in accordance with the national standards GB/T 2677.10(1995), GB/T 2677.9(1994), and GB/T 2677.8(1994). Three parallel measurements were performed for each sample, and the average mass percentage of each major component was calculated based on the data obtained. The tannin and saponin contents were determined according to the standards NY/T 1600(2008) and SN/T 1852(2006).

Determination of Terpenoids

The *P. massoniana* sawdust (1 kg in dry weight) was extracted with 95% refluxing methanol three times. The obtained extracts were extracted with petroleum ether, ethyl acetate, and n-butanol successively six times for each solvent. After passing through a 0.45 μm organic film-based filter, the extract was loaded on the gas chromatography-mass spectrometry (GC-MS) analyzer (6890N-5975B; Agilent, Santa Clara, USA) for testing, equipped with an HP-5MS column (film thickness 30 m × 0.25 mm ID × 0.25 μm).

The initial temperature for the GC-MS analysis was 60 °C, which was maintained for 2 min. The temperature was increased to 80 °C at a rate of 2 °C/min and was maintained for 5 min. Finally, the temperature was increased to 280 °C at a rate of 4 °C/min and was maintained for 5 min. The inlet temperature was 260 °C with an injection volume of 1 μL. The split injection method was used with a split ratio of 50:1. The carrier gas was high-purity helium (99.999%) with a column flow rate of 1.5 mL/min. The GC-MS interface and the ion source temperatures were 250 °C and 230 °C, respectively. The electron ionization (EI) mode was utilized with an electron energy of 70 eV and a scanning mass range of 50 m/z to 500 m/z.

For the qualitative and quantitative analysis, each component was searched and matched with the standard in the NIST08 Mass Spectral Library and the fragments were compared. The relative retention time of each component was characterized in combination with the related literature reports. The relative content of each band area was calculated by the band area normalization method to conduct the quantitative analysis.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

An FTIR spectrometer (Nicolet iS50; Thermo Fisher Scientific, Waltham, USA) was used to identify the various functional groups of the *P. massoniana* sawdust. A 0.001g oven-dried sample of sawdust was prepared by drying at 105 °C for 1 h. The sample was mixed with 0.1 g of potassium bromide (KBr) powder and pressed into a thin and transparent disk by the KBr pallet. It was then loaded into the FTIR spectrometer for one test. For each spectrum, a 32-scan absorption interferogram was collected with a resolution of 4 cm^{-1} in the 400 cm^{-1} to 4000 cm^{-1} region at ambient temperature. The analysis of each sample was repeated three times. The position and band height of the absorption bands in the infrared absorption spectrum were measured using Origin 8.0 software (OriginLab, Northampton, MA, USA).

Edible Mycelium Germination Experiment

Three experimental groups were designed for this experiment, namely group A (80% composted *P. massoniana* sawdust + 15% rice bran + 2% soy flour + 2% corn flour + 1% gypsum), group B (80% untreated *P. massoniana* sawdust + 15% rice bran + 2% soy flour + 2% corn flour + 1% gypsum), and group C (80% mixed broad-leaved tree sawdust + 15% rice bran + 2% soy flour + 2% corn flour + 1% gypsum). The homogeneously mixed substrate was placed in a test tube (Fig. 1) with a diameter of 3 cm and a length of 20.5 cm. The height of the substrate in the tube was 10 cm and each substrate group consisted of 10 tubes. Under aseptic conditions, 3 g of *A. nigricans*/shiitake mushroom cultivation spawns were added to the test tube, covered with a sterile cotton plug, and cultured in a sterile environment under constant temperature and humidity. The growth of the hyphae (cm) was recorded every 3 d, and the average values were calculated (Zhang *et al.* 2018).

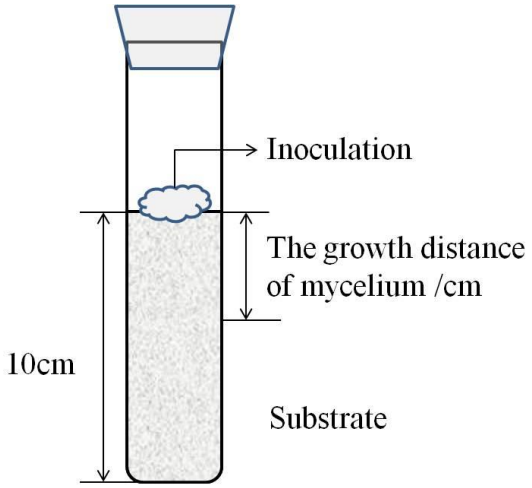

Fig. 1. Schematic diagram of the test-tube cultivation apparatus for the edible fungi

RESULTS AND DISCUSSION

Material Analysis of the *P. massoniana* Sawdust

The chemical composition and contents of the *P. massoniana* sawdust are important factors that affect its application as a cultivation substrate for edible fungi. As shown in Tables 1 and 2, the *P. massoniana* sawdust was composed of cellulose, hemicellulose, and lignin. Lignin is an optimal raw material for the cultivation of edible fungi. The tannin content in the cultivation substrate for the edible fungi affects the taste and quality of the edible mushroom, while the saponin inhibits the growth of the edible fungi hyphae (Fang and Huang 1994). Since the amount of tannin and saponin in the *P. massoniana* sawdust is relatively low and most of it degrades after being composted, they do not affect the growth of the edible fungus hyphae. The mineral elements are essential nutrients for the life activities of edible fungi, and their main function is to constitute the components of the fungi. The *P. massoniana* sawdust contains the mineral elements P, S, K, Mg, Ca, Fe, Mo, Mn, Zn, Co, *etc.*, which are required for the growth and development of edible fungi (Suzuki 1994; Xu *et al.* 2010). On the other hand, according to the Chinese standard DB13/T-2277 (2015), other heavy metal elements such as As, Hg, Cd, Cu, and Pb are below the heavy metal limits of the cultivation substrates for the edible fungi. Although the components of the *P. massoniana* sawdust (cellulose, hemicellulose, lignin, TOC, and TN) are very similar to those of other broad-leaved species for the cultivation of edible fungi (Xiao and Wang 2002; Zhu 2008), the terpene content in the *P. massoniana* sawdust is relatively high. This has an inhibitory effect on the mycelial growth and needs to be pretreated before it can be used as a cultivation substrate for the edible fungi matrix (Zhang and Du 2017).

Table 1. Composition of the *P. massoniana* Sawdust

Component	Cellulose	Hemicellulose	Lignin	Terpenoids	Tannin	Saponin	C	N
Content (%)	48.69	22.99	30.25	3.91	0.64	1.42	47.5	0.11

Table 2. Elemental Composition of the *P. massoniana* Sawdust

Component	Content (mg/kg)
P	86.1
S	0.018
K	646
Mg	0.145
Ca	0.945
Fe	91.3
Mo	0.003
Mn	83.6
Co	0.139
As	0.012
Hg	0.004
Cd	0.188
Cr	1.6
Cu	1.42
Na	9.74
Pb	0.204
Zn	7.05

Composting Temperature

To some extent, the temperature reflects the composting process and the time required to complete the composting (Luo *et al.* 2011). As shown in Fig. 1, the temperatures of the three composting groups varied with time, which can be divided into four stages, namely the initial mesophilic phase, the thermophilic phase, the cooling phase, and the maturing phase (Bernal *et al.* 2009). The temperature of the composting increased rapidly in the initial mesophilic phase. On the 6th day, the temperature reached more than 60 °C and entered the thermophilic phase. Groups A1, A2, and A3 maintained a temperature above 50 °C for 10 d, 10 d, and 9 d, respectively. On the 16th day, the composting temperature dropped below 50 °C and the temperature gradually cooled down the ambient temperature. The composting temperature reflects the dynamic change of the microbial community to a certain extent. The organic matter in the compost was decomposed by the aerobic microorganisms, so a lot of heat was released and the process entered the thermophilic phase. This phenomenon is attributed to the decomposition of the small molecular organic compounds such as sugars and proteins by the thermophilic bacteria (Bernal *et al.* 2008). The thermophilic phase is the key stage of treating the *P. massoniana* sawdust by high temperature aerobic composting, in which the high composting temperature and long duration indicate that the microorganisms produce more heat and rapidly reproduce (Zhang 2004). The duration and temperature of the thermophilic phase for the A2 group were higher than those of the A1 and A3 groups.

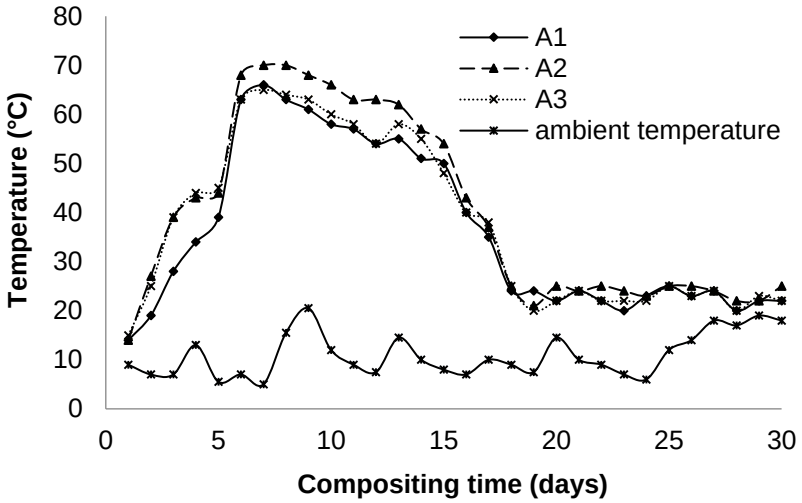

Fig. 2. The temperature profile of the composting materials over time

Changes of the Terpenoids in the Composting Process of the *P. massoniana* Sawdust

Each experimental group was sampled at 0 d, 30 d, and 60 d. The composition and contents of the terpenoids were detected by GC-MS. The results showed that the variation of the added brown granulated sugar had little effect on the degradation of the terpene compounds. The composition and contents of the terpene compounds in the samples with different composting time are shown in Table 3. Before and after the composting, the identified terpene compounds were mainly sesquiterpenes. Although there were 14 identical components between the samples before and after composting, their overall difference is obvious. Specifically, 44 kinds of terpene compounds were isolated from the

sample at the beginning of the composting process, including terpene, terpene oxide, taraxerol, and taraxerone. In contrast, 23 kinds of terpene compounds were isolated from the samples after 30 d of composting. Terpene was the main type of compound that was isolated. The content and kinds of the terpene compounds decreased after 30 d of composting compared to the system before composting. The *P. massoniana* sawdust was continuously composted at ambient temperature for 30 d. After 60 d of composting, cubebene, which had a relative content of 0.31%, was the only type of terpenoid that was detected. It can be concluded that the high temperature during the composting process rapidly degraded the terpene compounds in the *P. massoniana* sawdust. The terpene compounds were almost completely degraded after an additional short period of ambient temperature composting.

Table 3. GC-MS Analysis of the Terpene Components Before and After the Composting of the *P. massoniana* sawdust

Ordinal	Compound Name	Chemical Formula	Relative Contents (%)		
			Day 0	Day 30	Day 60
1	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-	$C_{10}H_{16}O$	0.003	-	-
2	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	$C_{10}H_{14}O$	0.002	-	-
3	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-	$C_{15}H_{24}$	0.009	0.012	-
4	(+)-Longicyclene	$C_{15}H_{24}$	0.007	-	-
5	(+)-Sativene	$C_{15}H_{24}$	0.003	-	-
6	Longifolene	$C_{15}H_{24}$	0.935	0.066	-
7	Caryophyllene	$C_{15}H_{24}$	0.056	0.022	-
8	alpha.-Caryophyllene	$C_{15}H_{24}$	0.008	-	-
9	(E)-.beta.-Farnesene	$C_{15}H_{24}$	0.006	-	-
10	Isocaryophillene	$C_{15}H_{24}$	0.002	-	-
11	gamma.-Muurolene	$C_{15}H_{24}$	0.003	-	-
12	Germacrene D	$C_{15}H_{24}$	0.003	-	-
13	(-)-Alloaromadendrene	$C_{15}H_{24}$	0.002	-	-
14	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	$C_{15}H_{24}$	0.003	0.028	-
15	alpha.-Muurolene	$C_{15}H_{24}$	0.004	-	-
16	delta.-Cadinene	$C_{15}H_{24}$	0.010	0.067	-
17	(-)-.alpha.-Gurjunene	$C_{15}H_{24}$	0.004	-	-
18	beta.-Guaiene	$C_{15}H_{24}$	0.002	-	-
19	alpha.-Calacorene	-	0.007	0.013	-
20	cis-Z-.alpha.-Bisabolene epoxide	-	0.002	0.058	-
21	(-).alpha.-Neoclovene	$C_{15}H_{24}$	0.010	-	-
22	(-)-Spathulenol	$C_{15}H_{24}O$	0.011	0.064	-
23	Caryophyllene oxide	$C_{15}H_{24}O$	0.074	-	-
24	Widdrol	$C_{15}H_{26}O$	0.031	-	-
25	(+)-Longiborneol	$C_{15}H_{26}O$	0.071	-	-
26	Isolongifolene, 7,8-dehydro-8a-hydroxy-	-	0.005	-	-
27	(+)-Valencene	$C_{15}H_{24}$	0.012	-	-
28	15-Copaenol	$C_{15}H_{24}O$	0.004	-	-
29	Thujopsene	$C_{15}H_{24}$	0.002	0.061	-
30	Epiglobulol	$C_{15}H_{26}O$	0.004	-	-
31	alpha.-Cadinol	$C_{15}H_{26}O$	0.020	-	-
32	Di-epi-.alpha.-cedrene-(I)	$C_{15}H_{24}$	0.024	-	-

33	Isoaromadendrene epoxide	$C_{15}H_{24}O$	0.007	-	-
34	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	0.034	0.014	-
35	Eremophilene	$C_{15}H_{24}$	0.002	0.043	-
36	(+)-.alpha.-Bisabolol	-	0.007	-	-
37	(-)-Isolongifolol	$C_{15}H_{26}O$	0.098	-	-
38	Diepicedrene-1-oxide	-	0.015	-	-
39	(+)-Calarene	$C_{15}H_{24}$	0.006	0.062	-
40	Longifolenaldehyde	$C_{15}H_{24}O$	0.005	-	-
41	Kaur-16-ene	$C_{20}H_{32}$	0.076	-	-
42	Isoaromadendrene epoxide	-	0.020	-	-
43	(+)-Pimaral	-	2.263	0.263	-
44	Styrene	C_8H_8	0.017	0.589	-
45	Cembrane	-	-	0.083	-
46	Cycloisolongifolene, 8,9-dehydro-9-formyl-	-	-	0.302	-
47	beta.-Humulene	-	-	0.014	-
48	trans-Nerolidol	$C_{15}H_{26}O$	-	0.257	-
49	Calamenene	$C_{15}H_{24}$	-	0.024	-
50	Himachala-2,4-diene	$C_{15}H_{24}$	-	0.009	-
51	alpha.-Selinene	$C_{15}H_{26}$	-	0.013	-
52	beta.-Cubebene	$C_{15}H_{24}$	-	0.026	0.31
53	Borneol	-	-	0.008	-
	Total relative content（%）		3.891	2.098	0.31

Note: "-" means that the substance was below the level of detection.

FTIR Spectroscopy Analysis before and after Composting of the *P. massoniana* Sawdust

FTIR spectroscopy can be employed to study the changes in the functional groups before and after the composting of the *P. massoniana* sawdust (Xu *et al.* 2014). The FTIR spectra and the assignment of the absorption bands at the three composting stages are shown in Fig. 3 and Table 4. The FTIR spectra in the different stages exhibited similar spectral characteristics but varied in the relative intensity of the band.

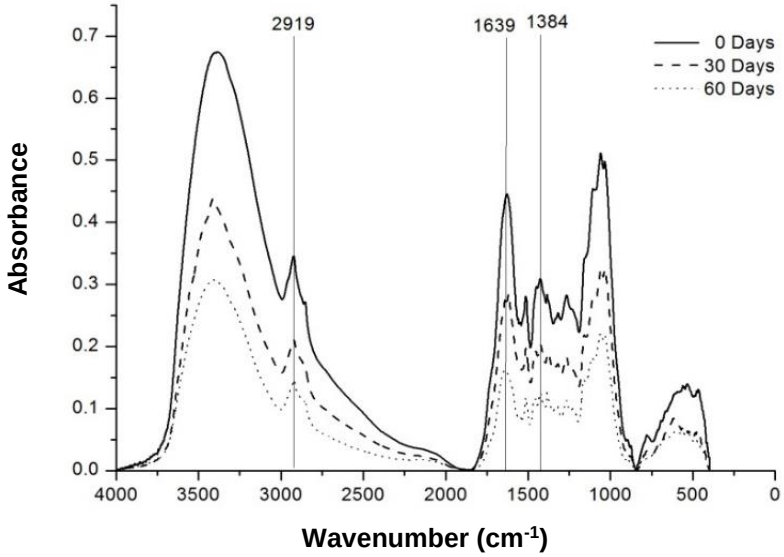

Fig. 3. The FTIR spectra of the composting samples on day 0, 30, and 60

Table 4. Assignment of the FTIR spectra

Wavelength (cm^{-1})			Assignment	Representative Compounds
Day 0	Day 30	Day 90		
3424.95	3423.64	3416.39	-OH, $-NH_3$ stretching	carbohydrates, amino acids
2925.39	2919.18	2918.52	$-CH_2-$, $-CH_3$ symmetric stretching	esters
		2850.93		
	2135.99			
1639.79	1639.84	1635.90	ring vibration of substituted aromatics C=O, N-H stretching COO- asymmetric stretching	aromatics, amides, esters, carboxylates, amines
1514.11	1509.01	1510.33	Aromatic backbone	lignin, cellulose
1454.77	1455.47	1455.32	$-CH_2$, $-CH_3$ stretching	carbohydrates, aliphatic compounds, amino acid salts
1423.08	1424.78	1424.03	C=C variant vibration $-CH_2$ connecting to carbonyl group Inorganic NH_4^+, NO_3 COO-	lignin aliphatic compounds organic carboxylate
1383.27	1384.23	1383.99	Germinal dimethyl variant Isopropyl, tert-butyl	terpenes
1313.22	1318.96	1319.51	C-H, C1-O stretching	cellulose syringyl derivative
1264.40	1265.09	1266.55	C-O-C, C-O stretching	phenols, aryl ethers,
		1225.98		
	1207.15			
1161.38	1161.94	1161.20	C-O-C, C-O, C-N stretching	carbohydrates, aliphatic compounds, amino acid salts
1111.18		1111.04	C-O-C, C-H stretching	carbohydrate, hemicellulose
1059.42	1058.18	1058.27	C-O stretching	Polysaccharides, polysaccharide analogues
1034.76	1034.58	1034.13	Si-O-SiO asymmetric stretching C-O stretching, -OH bending	siliceous substance
896.83	900.69	898.22	Ring vibration of sugar	glycoside
	878.40		C-C(O) out-of-plane bending, -C-C- stretching	carbonate carbohydrate
560.29	559.31	558.73	C-CO-C in-plane bending	ketones

The main characteristic absorption bands that can be used to determine the presence of terpenoids are 2928 cm^{-1} to 2917 cm^{-1}, 1625 cm^{-1} to 1680 cm^{-1}, and 1370 cm^{-1} to 1380 cm^{-1}. The characteristic absorption band of 1370 cm^{-1} to 1380 cm^{-1} are related to the stretching vibration of germinal dimethyl on the tetracyclic aliphatic ring of the terpene compounds, which can be used to determine the presence of tetracyclic rings in the terpene compounds. However, since isopropyl and tert-butyl also have C-H variant vibration

absorption bands at 1375 cm^{-1}, the absorption bands in this range cannot be used as the sole evidence for the presence of terpene compounds. The absorption bands at 1625 cm^{-1} to 1680 cm^{-1} and 2928 cm^{-1} to 2917 cm^{-1} are the absorption bands of the C=C double bond stretching vibration of terpene compounds and characteristic of the six-membered ring in terpene compounds, respectively (Liu and Huang 2003; Gu *et al.* 2015). As shown in Fig. 3 and Table 3, the samples in the three different composting stages exhibited these three characteristic bands, which confirms that terpene compounds were present in the composting samples within 60 d of composting, and the intensity of these three bands decreased gradually with the extension of the composting time. This indicates a gradual decrease in the quantity of terpene compounds in the compost with the increased composting time.

Mycelium Germination of the Edible Fungi Cultivated by *P. massoniana* Sawdust

As shown in Figs. 4 and 5, the mycelium in group A tubes grew the fastest compared to groups B and C. The mycelia of the shiitake mushrooms and the *A. nigricans* outgrew the test-tube on the 18th and 15th days, respectively. Under the same edible fungi and environmental conditions, group B needed 24 d for both mycelia to grow, while the mycelia of the shiitake mushrooms and the *A. nigricans* in group C needed 22 and 21 days, respectively, to outgrow the test tubes. Therefore, the sawdust without treatment can inhibit the growth of the mycelium. In contrast, the germination rate of the mycelium cultivated by the *P. massoniana* sawdust treated by composting was faster than the mycelium that was cultivated by the mixed broad-leaved tree sawdust. The composted *P. massoniana* sawdust can shorten the mycelium germination cycle of the edible fungi and the production cycle of the edible fungi in the actual production process, compared with other innocuous processes for the treated sawdust of *P. massoniana* (Weng *et al.* 2013). The sawdust of the *P. massoniana* that was composted for 30 d can be used for the normal growth of the shiitake mushrooms and the *A. nigricans* mycelia.

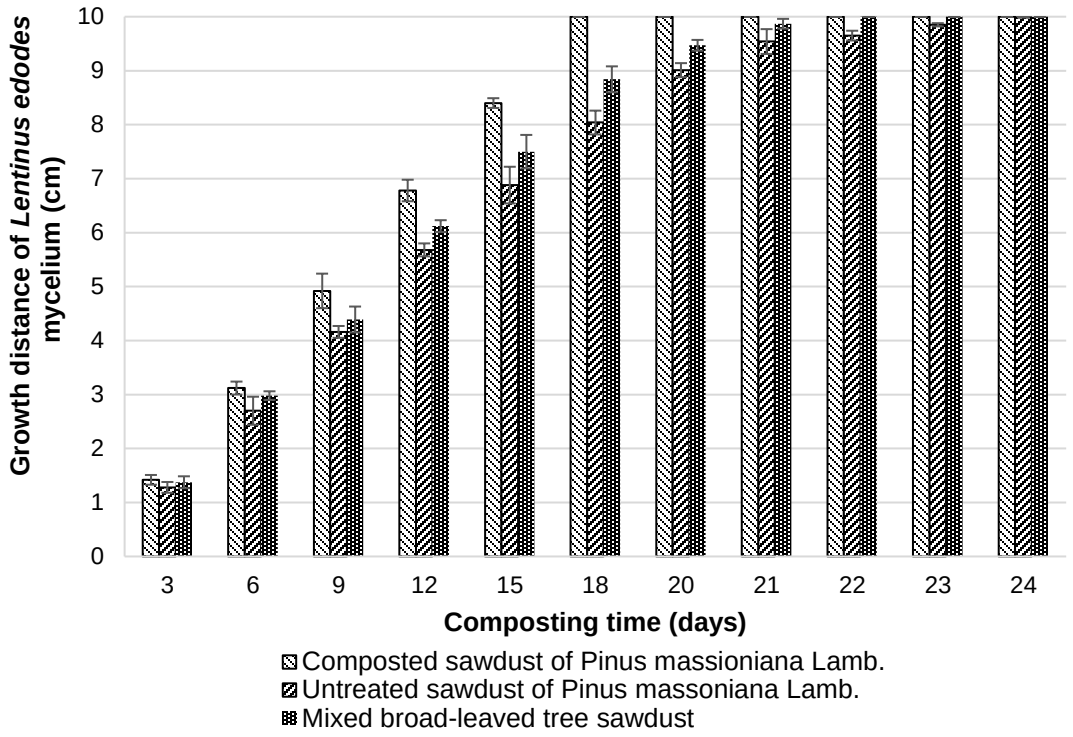

Fig. 4. The mycelial growth of *Lentinus edodes* cultivated with sawdust

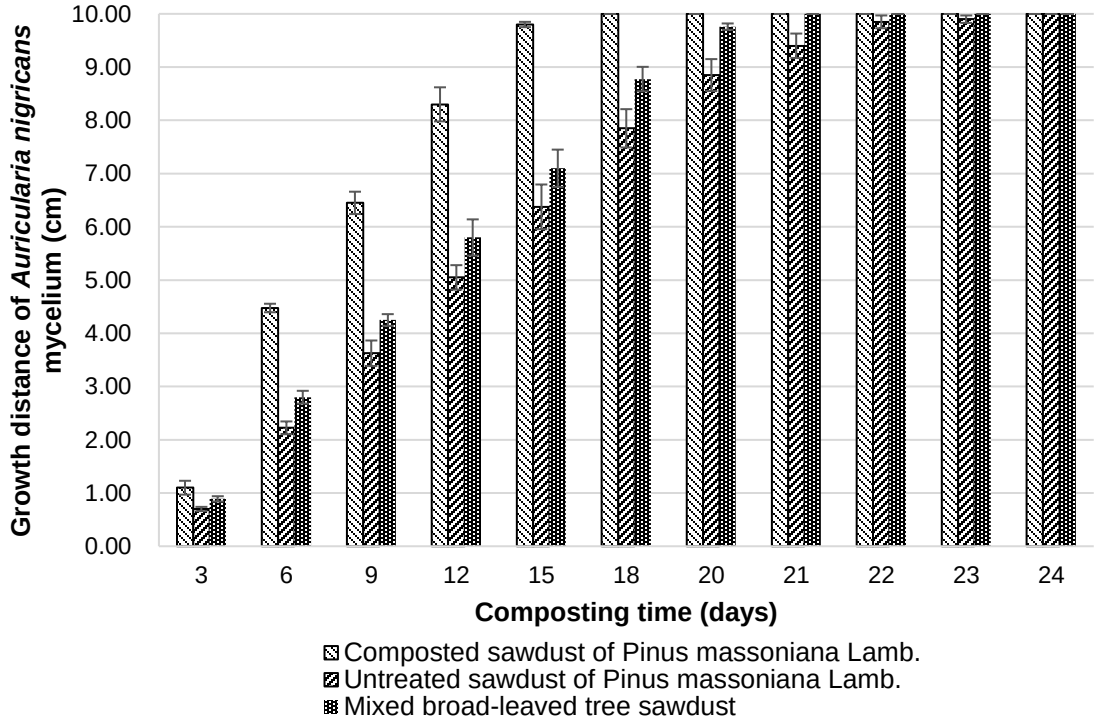

Fig. 5. The mycelial growth of the *Auricularua nigricans* cultivated with sawdust

CONCLUSIONS

1. *P. massoniana* sawdust contains terpene substances that are detrimental to the germination of edible fungi and need to be treated before they can be used as the cultivation substrate for edible fungi. The addition of brown sugar and soybean meal in the *P. massoniana* sawdust facilitated the composting process at high temperatures. Moreover, the amount of brown granulated sugar that was added affected the increased heating rates of the composting process and the temperature in the thermophilic phase. At a 5% dose of brown granulated sugar, the composting heating rate was the fastest and a temperature above 60 °C was maintained the longest. However, the duration of the whole thermophilic phase (> 50 °C) was not prolonged compared to the other two groups.
2. The GC-MS analysis showed that the relative content of the terpenoids in the *P. massoniana* sawdust was reduced from 3.89% to 2.10% after 30 d of composting. Cubebene was the only terpene substance that was detected after 60 d of composting, with a relative content of 0.31%. The FTIR analysis indicated that the terpene compounds in the composting process were gradually reduced as the composting time increased.
3. The *P. massoniana* sawdust that was composted for 30 d yielded faster cultivation of edible fungus when it was used as a major ingredient compared to the untreated *P. massoniana* and mixed broad-leaved tree sawdust. Additionally, the time required for the innocuous treatment of *P. massoniana* sawdust was greatly reduced.
4. For the terpene-degrading bacteria, a mixture of *E. cloacae* and *S. marcescens*, terpenes in the *P. massoniana* sawdust were rapidly treated at room temperature for a more cost-effective process. This made it possible for the *P. massoniana* sawdust to replace the broad-leaved tree sawdust as the cultivation substrate of edible fungi. Other effects, such as the additives used, the microbial quantities used, and other factors regarding the turpentine degradation and product quality were not studied. Therefore, further research on these factors has important theoretical and practical significance for improving the value and efficiency for the cultivation of edible fungi.

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