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Effects of Nutrient Composition on Yield and Quality of Mushroom in *Lentinula edodes* Cultivation Using Softwood Sawdust*1

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ABSTRACT

This study was performed to evaluate the efficiency of using softwood as the sawdust medium for *Lentinula edodes* cultivation, effect of nutrient on the mycelial growth, spawning, the mushroom yield, and quality. The nitrogen nutrition significantly enhanced the mycelial growth of *L. edodes*. The glutamic acid in the *L. leptolepis* and *P. koraiensis*, and asparagine in the *P. densiflora* were appeared to slight increase in the mycelial growth. The vegetable oil showed very effective on the mycelial growth in the *P. koraiensis* sawdust medium. Carbon/nitrogen ratio of all the test was reduced after mycelial growth. The mycelial growth was exclusively dependent on reduction of carbon. The mushroom yield (32.7%) of the *P. densiflora* sawdust medium (carbon source: 3% active carbon, nitrogen source: 0.4% asparagines) was the best in mushroom production of *L. edodes*, followed by the *Q. variabilis* sawdust (35.4%) of the control medium. The diameter of mushroom cap was obtained from the *P. densiflora* sawdust (carbon source: 3% sucrose, nitrogen source: 0.4% potassium nitrate) and *P. koraiensis* sawdust (carbon source: 3% sucrose, nitrogen source: 0.4% potassium nitrate), and the *P. koraiensis* sawdust (carbon source: 3% xylose, nitrogen source: 0.4% glutamic acid, supplement: 0.05% amino acid), with values 71.5 mm, 71.5 mm and 72.1 mm, respectively. In the polypropylene bag cultivation, the weight losses of the block medium gradually increased for 80 days in the dark (13.8~16.8%) and then became stable in the range of 20.7~25.8%.

Keywords : *Lentinula edodes*, mycelial, *Quercus variabilis*, *Larix leptolepis, Pinus densiflora, Pinus koraiensis*, polypropylene bag, cultivation, mushroom yield

1. INTRODUCTION

Mushrooms are known for their nutritional and medicinal value (Breene, 1990) and for containing a variety of bioactive compounds. *Lentinula edodes* (Shiitake), which is the second

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most popular edible mushrooms worldwide due-to its delicious flavour, great nutritional value and immunity-enhancing components (Jong and Nirmingham, 1993), belongs in the group of white rot basidiomycetes.

L. edodes mushroom is important in Asian countries including China, Japan, Taiwan, and Korea. This is attributed to its nutritional value and the possibility of its medical application (Jong and Nirmingham, 1993). In Korea, the *L. edodes* mushroom is mostly produced by log and sawdust cultivation. The cultivation of *L. edodes* in Korea has increased annually since the early 1970s. In 2000, the yields of dried and fresh *L. edodes* mushrooms were 2,278 tones and 17,454 tones, respectively, with the yield of dried and fresh *L. edodes* mushrooms increasing 10% and 3%, respectively in 2001.

Logs of broad-leaved trees have been used for cultivating some edible mushrooms. For example, Luo (1989) and Quimio *et al.* (1990) utilized the logs of oak trees to cultivate Auricularia in China and the Philippines. A similar work, the cultivation of *L. edodes* using oak family trees, was reported in the literature by Kuo and Kuo (1983), Singer and Harris (1987) and Miles and Chang (1997). These studies were embarked upon in order to evaluate the cultivability of *L. subnuduson* logs of selected Nigerian hardwoods and the effects of chemical treatments of the logs on fructification. The *L. edodes* mushrooms are cultivated on natural logs or sterilized sawdust. Recently, instead of log cultivation, which requires a long term to form fruiting bodies, a method of sawdust-based cultivation has been gradually extended. The cultivation of *L. edodes* on sawdust medium has become more popular in recent years than on logs in Korea. In Korea in 1995, cultivation techniques using the sawdust succeeded and these cultivation techniques were transmitted to commercial mushroom farms. Most of the cultivation process and processing of lumber resources can be mechanized extensively. Because the method of the sawdust cultivation is conducted under controlled temperature and humidity conditions inside the cultivation rooms, it has some advantages. For example, the yield of fruiting bodies is almost independent of climatic conditions throughout the year. Simply due to the mechanization of every process of cultivation, the cultivation period is shortened to one-third or one-sixth of that of the log cultivation. A shortage of the logs occurred and sawdust of the commonly used oak trees became limited, and their purchase price increased with time because of the increase of mushroom farming. Softwood species have been investigated for the cultivation of *L. edodes* but the *L. edodes* mushroom has grown poorly on the softwood such as *P. densiflora*, where both the fruit body production and quality of the mushroom decreased (Kim *et al.*, 2002; Yang *et al.*, 2003). Some components of the softwood that are inhibitory to mycelial growth must be removed in order to utilize the softwood for the cultivation of *L. edodes*, and the removal method should be duly considered.

Sawdust-based cultivation at present is conducted exclusively using heat-resistant polyethylene bags, which cannot drain excess water formed by the mycelial from the cultures. The sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing *L. edodes* in the United States (Royse, 1997; Royse, 2001), but other basal ingredients may include straw, corncobs, or both. Starch-based supplements such as wheat bran, ricebran, millet, rye and maize may be added to the sawdust medium for the production of oak mushrooms. These supplements serve as major nutrients to optimize growth conditions (Royse, 1997; Royse, 2001).

Softwood sawdust is not used for *L. edodes*

cultivation in Korean commercial mushroom farms. This study used a mix of sawdust and different nutrients as growth media for the cultivation of *L. edodes* mushroom by the mycelial growth and polyethylene bag method. This study was performed to evaluate the efficiency of using softwood as the sawdust medium for *L. edodes* cultivation, the effect of nutrient on the mycelial growth, change of carbon/nitrogen ratio in the sawdust medium and the effects of the addition of nutrients on the weight loss of the block sawdust media from spawning, the time of the first harvest, the mushroom yield, and the size of mushroom.

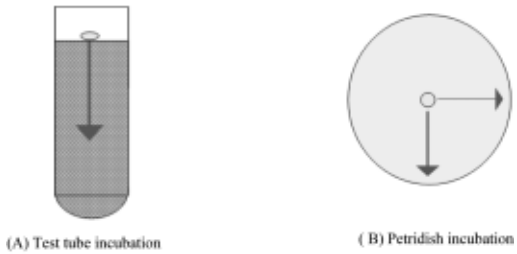

Fig. 1. Measurement method of mycelial growth in the sawdust medium. (A) Test tube : Measurement of mycelial growth length of vertical direction on the basis of inoculation surface (mm), (B) Petridish : Measurement of mycelial growth length of horizontal direction on the basis of inoculation surface (mm).

2. MATERIALS and METHODS

2.1. Substrates and Fungal Strain

The sawdusts of *Quercus variabilis, Larix leptolepis*, *Pinus densiflora* and *Pinus koraiensis*, which contained bark, were obtained from the Forest Research Institute, in Seoul, Korea. The sawdust of each species was collected in the spring of 2007 and was stored in an enclosed building until it was used. The moisture content of all air-dried sawdustwas approximately 10～13% by weight. All the sawdust was screened to a 10～60 mesh size. Three hundred gram of the *L. leptolepis*, *P. densiflora* and *P. koraiensis* sawdustswere used after hot water extraction in 3 ℓ of distilled water for 3 h by a soxhlet apparatus, washed and used as mushroom media. At this time, control sample was used as sawdust of *Q. variabilis.*

The strain of *Lentinula edodes* (Berk.) used in this study was FRI-Sanlim No. 5, a stock culture of the Forest Research Institute, Seoul, Korea. The stock culture was maintained on potato dextrose agar (PDA) medium at 4ºC. Mycelium of *L. edodes* was sub-cultured on the PDA medium for 2 weeks at 25ºC.

2.2. Determination of Nutrient on Mycelial Growth

Sawdust medium was prepared with nutrients on the hot water extracted sawdust for the mycelial growth of *L. edodes*. Mixingrate of sawdust medium contained the following on the basis of air-dried weight (%): sawdust, 76%; rice bran, 20%; carbon nutrition (glucose, active carbonand xylose, respectively), 3%; nitrogen nutrition (potassium nitrate, ammonium chloride, asparagine and glutamic acid, respectively), 0.4%; and calcium carbonate, 0.6%. Vegetable oil (Sajo Haepyo Co. Korea) was applied to rice bran oil.

Prepared sawdust was packed in the test tube and petridish, and then inoculated to mycelial of *L. edodes*, respectively. A five mm diameter mycelial plug was cut from the margin of an actively growing fungal culture and placed fungus side down at the edge of the agar on the test tube medium and petridish medium.

Mycelial growth was measured to mycelial growth length in the two conditions (Fig. 1). Test tube was measured to mycelial growth length of vertical direction on the basis of inoculation surface, and petridish was measured to mycelial growth length of horizontal direc-

tion on the basis of inoculation surface. And each result was the average of five replicates.

2.3. Determination of Sawdust Medium Weight and C/N Ratio

The weight loss of the sawdust medium was used to calculate the percentage of weight loss (1 - [weight of the sawdust medium after incubation, g / fresh weight of the sawdust medium before incubation, g] × 100). The medium yield of the sawdust medium was used to calculate the percentage ([weight of the sawdust medium after incubation, g / fresh weight of the sawdust medium before incubation, g] × 100). Subsamples of sawdust medium for analysis of elemental were oven-dried at 105ºC for 24～48 h and were ground in a Wiley Mill to pass a 40 mesh screen. Total elemental composition of samples was analyzed by CHNS-932 Analyzer (Leco).

2.4. Preparation of Sawdust Block Medium for Mushroom

Three hundred grams of the resulting sawdust mixture werebagged in a polypropylene bag with a hole 4 cm in diameter covered with a 0.44 μm filter and closed with a plastic cap with an additional 0.44 μm paper filter.

The sawdusts medium were formed into blocks of 300 g in the polypropylene bag by a square mold (150 W × 60 L × 100 H mm). The apparent gravities of the sawdust medium bagged were 0.96 of *Q. variabilis*, 0.75 of *L. leptolepis*, 0.70 of *P. densiflora* and 0.67 of *P. koraiensis*.

Experiments were conducted in a completely randomized design with ten treatments and seven replicates. The sawdust medium in the polypropylene bags were sterilized at 121ºC for 90 minutes, and then cooled to 25ºC. Sawdust medium in the test tube and petridish was inoculated on with four mycelial plugs (diameter 10 mm) from the PDA medium with mycelial of *L. edodes*.

2.5. Determination of Nutrient Composition in Sawdust Block Medium Cultivation

The block medium was adjusted to about 65 ± 2% based on the fresh weight of the mixture of solid materials with nutrients (300 g). The block medium was made to a block of 300 g in the polypropylene bag by a square mold (150 W × 60 L × 100 H mm).

Carbon nutrition 3%, nitrogen nutrition 0.4% and supplement 0.05% were added on the basis of air-dried weight of sawdust 80% and rice bran 20% (Table 1).

2.6. Spawn Run, Cultivation and Harvesting Mushroom

Valuation of spawn run (mycelial growth) from the block medium was measured bytheir weight loss. The block medium were incubated at 24 ± 2ºC for 80 days in the dark, and then they were transferred to 24 ± 2ºC for 40 days in the light. At the end of dark and light incubation, the weight of the block media were used to calculate the percentage of weight loss (1 - [weight of the block medium after incubation, g/fresh weight of the block medium before incubation, g] × 100). After incubation for 120 days, all block medium were taken out of the polypropylene bags, washed with tap water to induce fruiting, and soaked in cool water (13 ± 2ºC) for 24 h. The block medium was incubated for fruiting bodies in chamber (Ilshin Lab Co., CC0150, Korea) at 15～18ºC and 90～98% relative humidity until fruiting bodies developed. The room was ventilated with fresh air moving at 2 m/s and illuminated 9～12 h/day. This experiment was performed by the method shown in Fig. 2.

Table 1. Preparation of sawdust medium for *L. edodes* based on the different nutrient content

Group	Species of sawdust	Content of block medium		
		Carbon source	Nitrogen source	Supplement
A	*Larix leptolepis*	Sucrose	Potassium nitrate	-
	Pinus densiflora	Sucrose	Potassium nitrate	-
	Pinus koraiensis	Sucrose	Potassium nitrate	-
B	*Larix leptolepis*	Glucose	Glutamic acid	-
	Pinus densiflora	Active carbon	Asparagine	-
	Pinus koraiensis	Xylose	Glutamic acid	-
C	*Larix leptolepis*	Glucose	Glutamic acid	Amino acid
	Pinus densiflora	Active carbon	Asparagine	Amino acid
	Pinus koraiensis	Xylose	Glutamic acid	Amino acid
Control	*Quercus variabilis*	Sucrose	Potassium nitrate	-

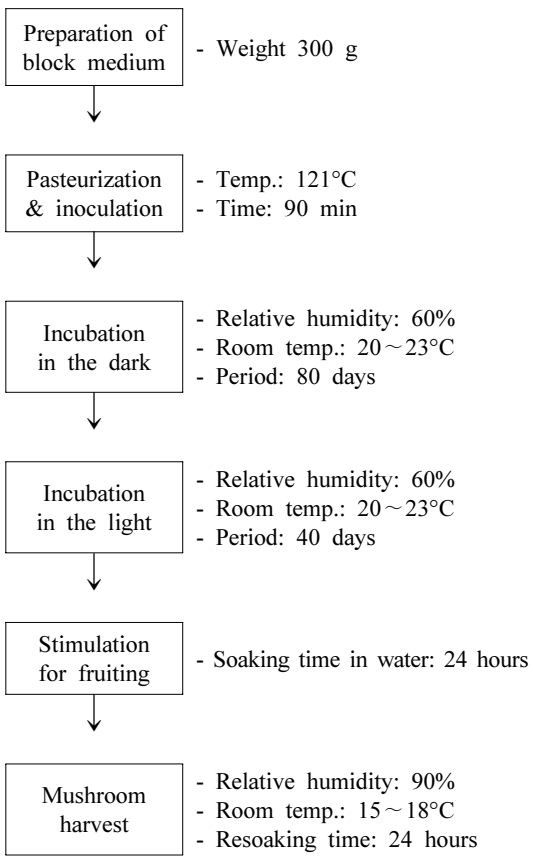

Fig. 2. Scheme of mushroom cultivation on sawdust medium in polypropylene bags for *L. edodes*.

The *L. edodes* mushrooms were harvested from the block medium at same time each day when in-rolled 80～90% of fruiting body gills began to flatten. The block medium clinging to the stipe werecut away and then the fresh mushrooms harvested at maturity were weighed, and the size of the cap and stipe of the mushrooms were measured. The diameter and thickness of the cap, and the length and diameter of the stipe from produced fresh fruiting bodies of the mushrooms were measured for the estimation of the mushroom quality. Seven replicateswere used for the mushroom product from each block medium. The data concerning spawn running were recorded after complete colonization of the block medium. The fresh mushroom yield was g fresh mushrooms harvested at maturity per g fresh block medium before incubation and expressed as a percentage.

2.7. Statistics of Data

Data was transformed to arcsin of square root of the value and analyzed using Proc Glm of SAS (Statistical Analysis System, ver.6.12). Means were separated using Duncan's new multiple range test at the $P < 0.05$.

Table 2. Influence of nitrogen nutrition in the weight loss of sawdust medium

Species	Weight loss of sawdust medium (%)			
	Potassium nitrate	Ammonium chloride	Asparagine	Glutamic acid
L. leptolepis	6.68 a *	6.26 a	6.67 a	6.77 a
P. densiflora	6.23 a	5.52 b	6.74 a	6.39 a
P. koraiensis	5.42 a	4.78 b	5.41 a	5.71 a

* Means followed by the same letters within each columnare not significantly different by Ducan's multiple range test ($P > 0.01$ in the *L. leptolepis*, $P = 0.01$ in the *P. densiflora*, $P < 0.01$ in the *P. koraiensis*)

3. RESULTS and DISCUSSION

3.1. Effect of Nutrient on the Mycelial Growth

All of the tested nitrogen nutritions significantly enhanced the mycelial growth of *L. edodes* ($P > 0.01$ in the *L. leptolepis*, $P = 0.01$ in the *P. densiflora*, $P < 0.01$ in the *P. koraiensis*) and glutamic acid in the *L. leptolepis* and *P. koraiensis*, asparagine in the *P. densiflora* were appeared to slight increase in the mycelial growth (Table 2).

Fig. 3 showedthe mycelial growth following the addition of vegetable oil in the *L. leptolepis*, *P. densiflora* and *P. koraiensis* sawdust medium. Mycelial growth in the *L. leptolepis* and *P. densiflora* sawdust medium was decreased in the mycelial growth of addition after than before. However mycelial growth in the *P. koraiensis* sawdust medium was somewhat increased. Mycelial growth length was calculated after 11 days incubation at the 25ºC.

3.2. Change of Carbon/Nitrogen Ratio in the Sawdust Medium

Earlier work showed the mycelial growth effects on the carbon/nitrogen ratios (Jonathan and Fasidi, 2001; Fasidi and Olorunmaiye, 1994; Chandra and Purkayastha, 1997). Jonathan *et al.* reported the fungus grew best on a medium with ratio 2:3 followed by 3:4 while the least growth was obtained with the ratio 5:1 (Jonathan and Fasidi, 2001).

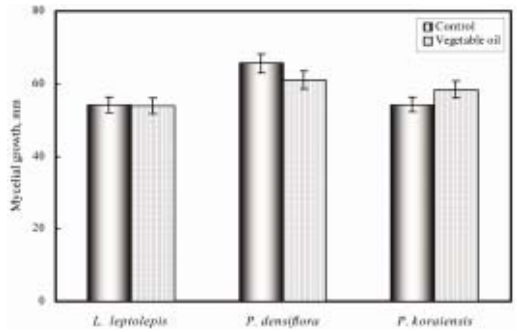

Fig. 3. Effect of vegetable oil for mycelial growth of *L. edodesin* the softwood sawdust medium of *L. leptolepis*, *P. densiflora* and *P. koraiensis* (11 days incubation at the 25ºC).

Table 3 showed the change of element content and carbon/nitrogen ratio. Analysis of element content in the softwood sawdust medium was compared before and after of incubation in the mycelial growth of *L. edodes*. Carbon/nitrogen ratio of all the tests were reduced after mycerial growth. This result was found that the mycelial growth was exclusively dependent on reduction of carbon. These results suggested that the mycelial nutrient of *L. edodes* utilize carbohydrates in the sawdust at the early growth stage.

3.3. Weight Loss of Sawdust Block Medium

The weight losses of the block medium in the polypropylene bags were measured to estimate spawn growth. The weight loss of the block media incubated in the polypropylene bags at the 80^{th} day in the dark and 120^{th} day in the light after inoculations wereshown in Table 4.

Table 3. Changes of element composition and carbon/nitrogen ratio in the sawdust medium

Species	Carbon nutrition	Fungi growth	Medium yield[a], %	Element, %				C/N ratio
				C	H	N	O	
L. leptolepis	Glucose	Before	100.0	44.5	4.5	0.7	49.3	65.0
		After	84.9	37.8	3.6	0.7	42.8	54.0
P. densiflora	Active carbon	Before	100.0	47.6	4.5	0.6	47.3	79.3
		After	85.8	38.1	3.7	0.6	43.4	63.5
P. koraiensis	Xylose	Before	100.0	45.4	4.7	0.7	49.2	64.9
		After	84.3	38.1	3.6	0.7	41.9	54.4

a) Yield, based on the dry weight of sawdust medium

Table 4. Weight loss of block medium during incubation of the dark and light

Group[a]	Species	Weight loss of sawdust block medium[b], %	
		Incubation in the dark[c]	Incubation in the light[d]
A	*L. leptolepis*	15.0 ± 2.94 a *	23.2 ± 2.99 abc
	P. densiflora	14.8 ± 2.37 a	21.7 ± 2.18 abc
	P. koraiensis	13.9 ± 1.63 a	20.7 ± 1.72 c
B	*L. leptolepis*	15.1 ± 2.67 a	23.4 ± 2.64 abc
	P. densiflora	16.6 ± 2.89 a	25.8 ± 3.12 a
	P. koraiensis	13.8 ± 2.28 a	21.2 ± 2.51 bc
C	*L. leptolepis*	15.5 ± 2.83 a	24.1 ± 1.93 abc
	P. densiflora	16.8 ± 3.01 a	25.3 ± 2.05 ab
	P. koraiensis	13.8 ± 2.82 a	22.4 ± 2.38 abc
Control	*Q. variabilis*	16.8 ± 1.66[e] a	25.7 ± 1.96 a

Note: See Table 1 (Nutrients of control, A, B and C groups: sawdust 80% + rice bran 20% + carbon source 3% + nitrogen source 0.4% + calcium carbonate 0.6% + amino acid 0.05%)

a) Group divided according to nutrient content of block media.

b) Moisture content of the block medium was adjusted to about 65 ± 2% based on the fresh weight of the mixture of solid materials with nutrients (300 g). The block medium was made from a block of 300 g in the polypropylene bag by a square mold (150 W × 60 L × 100 H mm).

c) Media were incubated for 80 days in the dark.

d) Media were incubated for 40 days in the light after in the dark incubation.

e) Values are mean ± S.D.(g) of seven separate experiments.

* Mean values followed by the same letter within the same column are not significantly different at the 5% level according to Duncan's New Multiple-Range Test.

In the polypropylene bag cultivation, the weight losses of the block mediumgradually increased for the 80 days in the dark (13.8～16.8%) and then became stable in the range of 20.7～25.8%. On the 80th day, the percentages of the weight losses for spawn growth of *L. edodes* were not significantly different in the dark incubation, nor was there a significant differ-

Table 5. First harvest day and yield of fruiting body according to sawdust block medium

Group[a)]	Species	First harvest, days[b)]	Yield[c)], %
A	*L. leptolepis*	143.6 ± 2.8 d *	22.8 ± 2.3 de
	P. densiflora	143.2 ± 3.9 d	20.5 ± 1.3 ef
	P. koraiensis	151.4 ± 6.0 c	19.0 ± 1.3 f
B	*L. leptolepis*	139.5 ± 1.2 d	24.7 ± 1.4 cd
	P. densiflora	169.4 ± 1.7 a	32.7 ± 2.2 a
	P. koraiensis	138.6 ± 1.0 d	17.2 ± 1.2 f
C	*L. leptolepis*	140.7 ± 5.6 d	25.9 ± 2.2 cd
	P. densiflora	169.9 ± 3.1 a	27.7 ± 2.9 c
	P. koraiensis	141.8 ± 4.6 d	31.5 ± 2.5 b
Control	*Q. variabilis*	162.9 ± 2.73[d)] b	35.4 ± 3.6 a

Note: See Table 1 (Nutrients of control, A, B and C groups: sawdust 80% + rice bran 20% + carbon source 3% + nitrogen source 0.4% + calcium carbonate 0.6% + amino acid 0.05%)

a) Group divided according to nutrient content of blockmedia.

b) Mushrooms were harvested from the block medium at the same time each day when in-rolled 80～90% offruiting body gills began to flatten.

c) Yield, % = ratio of g fresh mushrooms harvested at maturity per g fresh block media before incubation. Media were incubated for 80 days in the dark.

d) Values are mean ± S.D.(g) of seven separate experiments.

* Mean values followed by the same letter with in the same column are not significantly different at the 5% level according to Duncan's New Multiple-Range Test.

ence between medium with different species of sawdust.

The *P. densiflora* sawdust medium in the light incubation of the B group had the highest weight loss (25.8%), followed by the *Q. variabilis* sawdust medium (25.7%) and the *P. densiflora* sawdust medium of the C group (25.3%). These results suggested that the nutrients of the B group and C group added to the *P. densiflora* sawdust medium were effective for *L. edodes* spawn growth. After 120 day the incubation in the dark and light, the weight loss of block medium in the A, B and C groups was greater than 81% of the *Q. variabilis* sawdust that was the control.

3.4. Effect of Nutrient Composition in Sawdust Block Medium Cultivation

After having inoculated the mycelium of *L. edodes* on the block medium, the *P. densiflora* sawdust of the B and C groups were the slowest of their respective groups on day 169.4 and day 169.9 harvesting times. The first harvest of the fruiting body from the *Q. variabilis* sawdust of the control medium was obtained on day 162.9 (Table 5).

The mushroom yield of the *P. densiflora* sawdust medium (32.7%) in the B group was the best in mushroom production of *L. edodes*, followed by the *Q. variabilis* sawdust (35.4%) of the control medium. The mushroom yields of the *L. leptolepis* (22.8%) and *P. densiflora* (20.5%) media each increased about 2% in the A group and 12% in the B group, respectively.

Table 6. Measurement of mushroom size for the quality of *L. edodes*

Group[a)]	Species	Mushroom size[b)]			
		Cap		Stipe	
		Diameter, mm	Thickness, mm	Diameter, mm	Length, mm
A	*L. leptolepis*	67.3 ± 1.5 bc*	16.6 ± 0.5 ab	10.9 ± 1.7 b	27.9 ± 2.5 de
	P. densiflora	71.5 ± 2.6 a	15.4 ± 1.3 ab	9.0 ± 1.2 bc	28.8 ± 2.7 cd
	P. koraiensis	71.5 ± 1.7 a	16.9 ± 0.7 a	15.3 ± 6.5 a	27.3 ± 3.3 de
B	*L. leptolepis*	60.0 ± 1.8 e	15.1 ± 1.3 b	7.9 ± 0.8 bc	25.2 ± 0.7 ef
	P. densiflora	60.3 ± 0.8 e	16.3 ± 1.0 ab	9.5 ± 0.3 bc	28.6 ± 1.2 de
	P. koraiensis	48.3 ± 3.5 f	12.2 ± 1.1 c	7.5 ± 1.1 b	22.3 ± 1.4 f
C	*L. leptolepis*	61.8 ± 3.4 de	15.3 ± 1.1 ab	8.8 ± 1.2 bc	28.3 ± 4.8 de
	P. densiflora	68.9 ± 4.8 ab	16.9 ± 2.1 a	8.8 ± 0.8 bc	31.7 ± 1.4 bc
	P. koraiensis	72.1 ± 1.76 a	15.7 ± 1.1 ab	10.6 ± 0.5 bc	36.3 ± 2.9 a
Control	*Q. variabilis*	64.4 ± 2.3[c)]cd	15.8 ± 0.3 ab	8.6 ± 0.2 bc	32.6 ± 0.5 b

Note: See Table 1 (Nutrients of control, A, B and C groups: sawdust 80% + rice bran 20% + carbon source 3% + nitrogen source 0.4% + calcium carbonate 0.6% + amino acid 0.05%)

a) Group divided according to nutrient content of block media.

b) Diameter and thickness of the cap and length and diameter of the stipe from produced fresh fruiting bodies were measured by digimatic caliper (mm).

c) Values are mean ± S.D.(g) of seven separate experiments.

* Mean values followed by the same letter within the same column are not significantly different at the 5% level according to Duncan's New Multiple-Range Test.

However, the yield of the *P. koraiensis* medium in the A group decreased about 2% in the B group, but increased about 12% in the C group. The first harvesting time of mushrooms from the *P. densiflora* sawdust of the B group happened on day 169.4, but from the *P. densiflora* sawdust of the B group was obtained the cumulative yield of 32.7%. The mushroom yield of the *P. densiflora* sawdust medium in the C group (27.7%) with 0.05% amino acid was decreased.

For the cumulative yields during 150 days of spawning, there was a statistically significant difference among *L. leptolepis*, *P. densiflora* and *P. koraiensis* sawdust medium. However, there was not a significantly difference between the yields of the *L. leptolepis* sawdust in the B group (24.7%) and the *L. leptolepis* sawdust in the C group (25.9%), and also, there was not a significant difference between the yields of the *P. koraiensis* sawdust in the A (19.0%) and B (17.2%) groups. There was a significant difference between the yields of the *P. koraiensis* sawdust in the A, B groups and the *P. koraiensis* sawdust in the C group (31.5%). The yield of the *P. densiflora* sawdust in the C group (27.7%) with 0.05% amino acid was decreased contrary to the expectation.

The mushroom yields in all block medium of the C group were higher than that of the A group. Significant sources of variation in the analysis of variance for the yields for groups A, B and C included the sawdust species, nutrients and supplement. The best results for the diameter of mushroom cap were obtained from the *P. densiflora* sawdust and *P. koraiensis* sawdust of the A group, and the *P. koraiensis* sawdust of the C group. The values obtained were 71.5 mm,

71.5 mm and 72.1 mm, respectively. These were significant difference between any of the other groups and the control medium (Table 6).

The thickness of the mushroom cap of the *P. koraiensis* sawdust of the B group was the lowest at 12.2 mm, but that of the *P. koraiensis* sawdust in the A (16.9 mm) and C (15.7 mm) groups were thicker, than the *P. koraiensis* sawdust of the B group. There was no significant difference between the thickness of the mushroom cap of the other block medium and that of the control medium. *P. koraiensis* (15.3 mm) of the A group gave the widest diameter of stipe but the diameters of the other stipes were not significantly different from that of the control medium (8.6 mm). The stipe length of all block mediumin the A, B and C group obtained various values. Regarding the mushroom quality from *P. koraiensis* of the B group, the diameter and thickness of the cap and the diameter and length of the stipe were 48.3 mm, 12.2 mm, 7.5 mm and 22.3 mm, respectively, while the corresponding values of the C group were 72.1 mm 15.7 mm, 10.6 and 36.3 mm, respectively. In all block medium, *P. koraiensis* of the B group grew the shortest length of stipe at 22.3 mm, and *P. koraiensis* of the C group grew the longest length of stipe at 36.3 mm. Comparing the B and C groups, the mushroom quality from the block medium of the C group with 0.05% amino acid improved. Especially, the mushroom quality of *P. koraiensis* medium in the B group improved by adding 0.05% amino acid.

4. CONCLUSION

In this study, softwood sawdusts of *L. leptolepis*, *P. densiflora* and *P. koraiensis* which the inhibitory compound for *L. edodes* were removed studied for the possible utilization in the cultivation of *L. edodes* mushroom and investigated the effect that nutrients and supplements had on the yield and quality of mushroom grown on block medium in polypropylene bags.

Nitrogen nutrition significantly enhanced the mycelial growth of *L. edodes* and glutamic acid in the *L. leptolepis* and *P. koraiensis*, asparagine in the *P. densiflora* were appeared to slight increase in the mycelial growth.

Mycelial growth was exclusively dependent on reduction of carbon. These results suggested that the mycelial nutrient of *L. edodes* utilize carbohydrates in the sawdust at the early growth stage.

The first harvest period of mushrooms grown on the *P. densiflora* medium (carbon source: 3% active carbon, nitrogen source: 0.4% asparagines) and *P. densiflora* medium (carbon source: 3% xylose, nitrogen source: 0.4% glutamic acid) were the slowest at about 169 days, the same day that the mushroom grown on the *Q. variabilis* control medium (carbon source: 3% sucrose, nitrogen source: 0.4% potassium nitrate) was obtained. There was no observable relationship between the first harvest period and the yield of mushroom. However, except the *P. koraiensis* medium (carbon source: 3% xylose, nitrogen source: 0.4% glutamic acid), the mushroom yields produced on the nutrients of the B (glucose, active carbon, xylose, glutamic acid, and asparagines) and C (glucose, active carbon, xylose, glutamic acid, asparagines, and amino acid) groups were higher than that cultivated on the nutrients of the A (sucrose, potassium nitrate) group.

The diameter and thickness of the mushroom cap produced from the A (sucrose, and potassium nitrate) group were better than that of the B (glucose, active carbon, xylose, glutamic acid, and asparagines) and C (glucose, active carbon, xylose, glutamic acid, asparagines, and amino acid) groups. However, in comparison of the B (glucose, active carbon, xylose, glutamic acid, and asparagines) and the C (glucose, active carbon, xylose, glutamic acid, asparagines, and

amino acid) groups, the mushroom quality produced on the block medium with 0.05% amino acid of the C (glucose, active carbon, xylose, glutamic acid, asparagines, and amino acid) group was higher than that of the block medium of the B (glucose, active carbon, xylose, glutamic acid, and asparagines) group.

Q. variabilis medium (carbon source: 3% sucrose, nitrogen source: 0.4% potassium nitrate) and *P. densiflora* medium (carbon source: 3% active carbon, nitrogen source: 0.4% asparagines) and *P. densiflora* medium (carbon source: 3% xylose, nitrogen source: 0.4% glutamic acid) in dark incubation showed the highest weight loss at 16.8%, 16.6% and 16.8% and also, these medium showed the highest weight loss at 25.7%, 25.8% and 25.3% in light incubation, respectively. The results of the weight loss on the block medium in the polypropylene bags were not significantly different between the different nutrients of A, B and C groups.

Softwood sawdust was practicable as a block medium for production of the *L. edodes* mushroom. This study demonstrated that the substrates containing carbon sources (glucose, active carbon, and xylose) and nitrogen sources (glutamic acid, and asparagine) were more productive than the substrates containing sucrose as a carbon source and potassium nitrate as a nitrogen source and that the substrates with 0.05% amino acid to the sawdust medium of *L. leptolepis* and *P. koraiensis* were more productive than the substrates without additional amino acid.

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