

Mycelia Growth of Shiitake (*Lentinula edodes*) on 4-Wood Species from Leguminaceae Family

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Abstract

Cultivation of Shiitake mushroom (*Lentinula edodes*) in Indonesia has not been developed yet, due to its low productivity. It happens because of the limited information of the good ways on cultivation Shiitake in Indonesia. One of the factors that affect the growth of mushroom is the media. In Indonesia, the information about the media composition by using various species of wood have not been found yet. Therefore, this research was conducted to determine the effect of using different wood species to the growth of Shiitake. This study used four wood species from the leguminaceae family, those were: Gamal (*Glicidia sepium*); Johar (*Samanea saman*); Leucaena (*Leucaena leucocephala*); and Sengon (*Falcataria moluccana*). Those were used as the media by adding 12.5% (w/w) of rice bran and 6% (w/w) of CaCO_3 , and adjusting the moisture content by adding the distillate water to 65, 70, and 75%. Then, the media was put in to the petridisk ($\varnothing 90$ mm) and inoculated with Shiitake mushroom. During the mycelia growth, the length of mycelia was measured every 2 days until fifty days, and then glucosamine content was analyzed. In addition, chemical analysis was also conducted to each media. The results showed that different wood species resulted different chemical content of media, except the acid soluble lignin content. The growth rate of mycelia was affected by the moisture content of media, meanwhile the glucosamin content is influenced by the moisture content and various species of wood. The best combination to cultivate the Shiitake mushroom was by using media made of gamal with 70% moisture content. There were positive correlation among the ethanol-toluene extracts content and the mycelia growth, however there was negative correlation among the hemicellulose content the mycelia growth.

Keywords: Shiitake mushroom, Leguminaceae, mycelial growth, moisture content, media's chemical content.

Introduction

Shiitake (*Lentinula edodes*) is an edible mushroom which is famous in Japan and may called also as Chinese Black Mushroom. This mushroom posses high trade value, however, unfortunately this Shiitake mushroom cultivation is not so well known in Indonesia and still needs many information on its development.

Generally mushroom's growth is affected very much by media condition such as, species which is used, moisture content, media composition, pH, and the environment condition of the media. The previous research results on ear-mushroom which was cultivated by 3 different species showed that very significant difference of micellium growth rate on each media from 3 different wood species was found (Irawati *et al.* 2012). For Shiitake it self, the optimum environment condition of growth site on temperature 5~35°C and 6~25°C for its fruiting bodies' growth, with optimum humidity among 95~100% (Widyastuti 2009). On the other hand, the proper media condition for Shiitake growth in Indonesia has not been known well. Recently, Shiitake is cultivated by people by using sengon media only, however, sometimes in the areas which are not the centre of sengon faces difficulties to get that wood. To reduce the dependency on sengon, therefore, other wood species which can be used as Shiitake media need to be found as an alternative.

In Indonesia some other wood species which are

classified into family Leguminaceae are supposed to posses good characteristics for mushroom growth. Species from family Leguminaceae is usually easy to be found in community's yard especially in country side. Community plants species from Leguminaceae very much because they use the leaves as cattle feed or to fertilize soil. Information about wood species in Indonesia which is suitable for Shiitake mushroom cultivation and also physical condition of the media suh as moisture content have not been found yet. To support the development of Shiitake mushroom cultivation in Indonesia, therefore, research about the effect of various wood species especially which are classified as family Leguminaceae and optimum media's moisture content was conducted.

Materials and Methods

Fungus and Substrate Materials

Seed of Shiitake mushroom F1 (pure culture) was obtained from kelompok tani Sedyo Lestari, Bantul, Yogyarta. Wood meal (9~80 mesh) of 4-wood species from family Leguminaceae which are Gamal (*Glicidia sepium*), Johar (*Samanea saman*), Lamtoro (*Leucaena leucocephala*), and Sengon (*Falcataria moluccana*) were used as the basal cultivation substrates. Commercial rice bran (9~80 mesh size) was used as a nutritive additive. Chemical contents of wood and rice bran whic are used are shown on Table 1.

Table 1. Chemical component content of 4-wood species and rice bran (%).

Chemical content	Sengon	Gamal	Johar	Lamtoro	Rice bran
Ethanol-toluene extractives	2.92±0.35	5.18±0.14	3.12±0.40	1.98±0.20	6.87±0.10
Holocellulose	80.78±0.63	83.85±0.12	84.97±0.55	83.56±1.07	81.58±0.82
α-cellulose	45.34±1.11	53.72±0.55	47.15±0.44	43.69±0.97	51.4±0.10
Hemicellulose	35.45±1.62	30.12±0.21	37.82±0.06	39.87±1.02	30.18±0.91
Klason lignin	23.30±0.30	27.47±0.39	20.16±0.79	22.00±0.28	36.73±0.20
Acid soluble lignin	2.83±0.08	1.35±0.05	2.60±0.01	1.48±0.03	1.8±0.034
Hot water soluble extractives	-	-	-	-	19.5±0.20

Wood meal and commercial rice bran were mixed in a weight ratio of 8:1. Moisture content (MC) of wood meal and rice bran was determined by $103\pm2^\circ\text{C}$ and scaling them until we have constant weight. Calcium carbonate was added at a concentration of 6% (w/w) to adjust the pH of the cultivation substrate to between 6 and 7. MC of the substrate was adjusted to 65, 70, and 75% by adding tap water.

Chemical Components of Media

Chemical components of media was determined before cultivation. Extraction with organic solvents was performed to determine the quantities of holocellulose, α-cellulose, Klason lignin, and ash. Before chemical analysis, the samples were ground with a rotary speed mill and then sieved through 40~80 mesh size. Subsequently, the samples were dried in an oven at 45°C . Extraction with organic solvents was performed as follows: 5 g of sample was extracted with 120 ml of a mixture of ethanol and toluene (1:2, v:v) by a Soxhlet extractor for 6 h. The amounts of Klason lignin, holocellulose, and α-cellulose were determined by ordinary methods (Carrier *et al.* 2011; Irawati *et al.* 2013). To determine the ash content, a 1 g sample was heated in a muffle furnace at 600°C for 2 h, and then, after cooling in a desiccator, it was reheated at 600°C for 1 h. For all chemical component analyses, 3 replications were performed for each sample.

Mycelial Growth Rate

To measure the mycelial growth rate, 20 g of cultivation substrate was packed in a Petri dish (90 mm diameter) by following the method in published paper before (Irawati *et al.* 2012). Five replications were performed for each wood meal substrate. The substrates were subsequently autoclaved at 121°C for 20 min, and inoculated with mycelial plugs (5 mm diameter) of Shiitake, previously grown on potato dextrose agar medium. The culture was maintained at 25°C in the dark. Colony diameter was measured every 2 days in four directions until the mycelia reached the edge of each Petri dish.

Glucosamine Content

To determine the glucosamine content in the mycelium, 5 g of cultivation substrate was packed in a Petri dish (45 mm diameter). Chitin content in the mycelium was

assayed by the method of Braid and Line (1981) and 3 repetitions were performed for each sample. To degrade fungal chitin into *N*-acetyl glucosamine, 1 g of the dry sample after 50 days of culture was hydrolyzed with 5 ml of 5 M HCl at 80°C for 20 h. The absorbance of the characteristic bluish green color was measured at 630 nm using a spectrophotometer. The calibration curve was drawn with *N*-Acetyl-D-(+)-glucosamine (Wako Pure Chemical Co., Japan) as a standard at concentrations of 0, 10, 20, and 30 mg/ml.

Results and Discussion

Chemical Contents of Media

Originally, chemical content of media was different with chemical content of wood (Table 1), because there were rice bran and CaCO_3 addition into the media. Chemical content of media, then, was used as basic calculation and analysis. Table 2 shows that difference of wood species significantly affected to ethanol-toluene extractives content, hot water soluble extractive content, holocellulose, α-cellulose, hemicellulose, and Klason lignin, but it did not affect to acid soluble lignin. Media made of johar wood showed the highest ethanol-toluene extractives content (8.84%) and hot water soluble extractives content (16.3%) indicating that media made of johar wood is estimated to posses substrate contents such as waxes, fat, resin, tannin and eter components which are soluble into high polar and non-polar extractives and also high tannin content, gum, sugar, and colour substrates which are soluble in the water.

Holocellulose, α-cellulose, dan hemicellulose are carbohydrate polymer which are available in the wood. The highest holocellulose and α-cellulose which were found in media made of wood were 84.67% and 60.04%, respectively. Cellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide on earth, and an important renewable resource. Basidiomycetous fungi belong to its most potent degraders because many species grow in environment rich in cellulose (Baldrian and Valaskova 2008). Some edible mushroom species which are classified into Basidiomycetous. Hemicellulose content was calculated based on subtraction between holocellulose content and α-cellulose. Table 2 shows that the highest hemicellulose content was found in media made of sengon wood 28.73%. During wood degradation, generally, hemicellulose which is

polysaccharide with short sugar structures is degraded at first (Higuchi 1985).

The highest Klason lignin was found in the media made of gamal wood (28.56%) and it was statistically different with the media made of other wood species. Przybylowicz and Donoghue (1990) pointed out that lignin has special role in Shiitake mushroom growth. Shiitake mushroom also obtains the nutritions needed from lignin

degradation process result. Shiitake mushroom's hypha extracts enzymes which are able to destroy the undegradable materials such as cellulose and lignin from the wood and change them into the simple monomers then give back to the hypha of fungi as food. Meanwhile, no significant difference was found in the acid soluble lignin content among the wood species, statistically.

Table 2. Chemical contents of media from 4 wood species (%).

Chemical content	Sengon	Gamal	Johar	Lamtoro	ANOVA Analysis
Ethanol-toluene extractives	4.45±0.02 ^a	8.36±0.11 ^c	8.84±0.04 ^d	5.73±0.12 ^b	**
Hot water soluble extractives	5.06±0.16 ^b	11.42±0.13 ^c	16.3±0.19 ^d	2.95±0.06 ^a	**
Holocellulose	81.59±0.70 ^c	75.85±0.13 ^b	70.33±0.37 ^a	84.67±0.99 ^d	**
α -cellulose	52.86±0.25 ^a	58.97±0.50 ^b	51.63±0.76 ^a	60.04±0.64 ^b	**
Hemicellulose	28.73±0.78 ^c	16.89±0.48 ^a	18.7±0.84 ^a	24.63±1.61 ^b	**
Klason lignin	22.15±1.00 ^a	28.56±0.30 ^b	23.77±0.14 ^a	22.32±0.96 ^a	**
Acid soluble lignin	0.03±0.002	0.03±0.001	0.03±0.004	0.03±0.001	ns

The same superscript letter followed by the average value in the same row shows no significant difference among the four tree species by Tukey-Kramer test at the 5% level. n = 3. ± = Standard deviation.

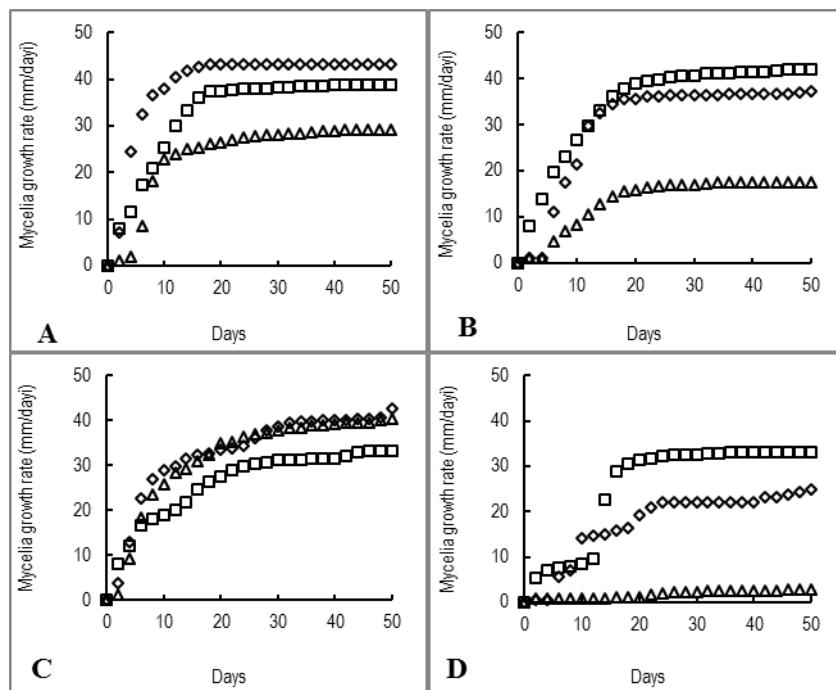


Figure 1. Graphic of Shiitake mushroom's mycelia growth. Remarks: (A) Gamal; (B) Johar; (C) Lamtoro; (D) Sengon. Diamond (65%); Square (70%); Triangle (75%).

Mycelia Growth Rate

Mycelia growth rates per 2 days on each types of substrate with different moisture content were shown in Fig. 1. Those 4 graphics showed that the average length of mycelia growth rate increased rapidly on the beginning of growth period and then it remained constant relatively. The average value of mycelia growth rate on this research was ranged from 0.61 to 3.32 mm/day. This value is included in

the range of another mushroom growth rate which is *Auricularia polytricha*, which has growth rate among 1.80–3.21 mm/day (Irawati *et al.* 2012). Statistic analysis results of mycelia growth rate on factor wood species and moisture content showed no significant effect on factor wood species and the interaction of those 2 factors, however, factor moisture content alone very significantly affected to mycelia growth rate.

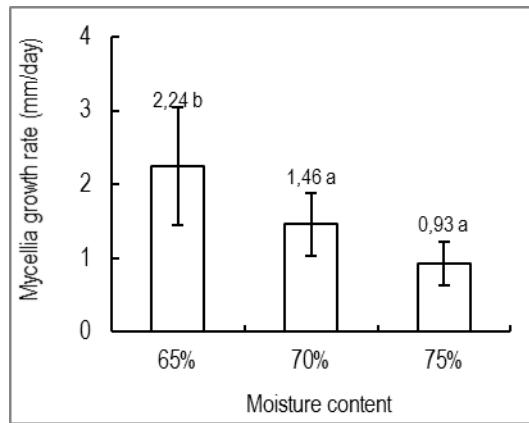


Figure 2. Graphic of mycelia growth in 3 different moisture contents.

Fig. 2 shows the values of mycelia growth rate on 65% moisture content is significantly different with mycelia growth rate on 70% and 75% moisture content. Whereas no significant difference was found on the values of mycelia growth rate on 70% moisture content and 75% moisture content, statistically. The highest mycelia growth rate value was found media with 65% moisture content which is 2.24 mm/day. Actually, according to the previous research results, Shiitake is classified in mushrooms with high water activity, means Shiitake mushroom will grow well on media which has high moisture content (Hu *et al.* 2004), however it also pointed out that water activity of Shiitake mushroom is

also affected wood physical properties as media. Wood with high water activity (easy to absorb water) needs high water addition in order to be good as Shiitake mushroom cultivation media, and vice versa. In this research, the woods used were supposed as species with low water activity, so the optimum water addition was 65%. Widyastuti (2009) pointed out that moisture content of sawdust media for Shiitake mushroom vegetative growth is ranged from 60 to 75%. Mycelia growth rate of Shiitake on media made of sugi sawdust increases along with the increment of moisture content of media from 65 to 75%, it was also happened on media made of konara wood (Hu *et al.* 2004).

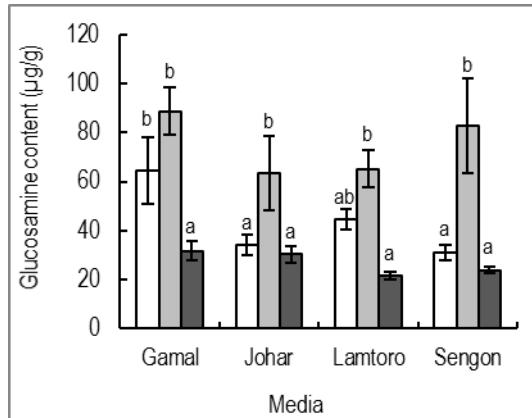


Figure 3. Graphic of glucosamine content of Shiitake mushroom's mycelia on 4 types of media. White (65%). Light gray (70%). Dark gray (75%).

Glucosamine Content

Glucosamine content was measured to determine the number of mycelia on media substrate before fruiting bodies phase. No branches polymer from N-acetyl-D-glucosamine (chitin) is component which is available on mushroom's cell wall, generally. Acid hydrolysis to kitin will disintegrate glucosamine and comparison between glucosamine and mycelia dry-weight can be determined and used to calculate mushroom biomass in the media or substrate (Jones and Worrall 1995). Ohga (2000) pointed

out that the bigger glucosamine content makes the number of mycelia in the media substrate more abundant.

The average value of glucosamine content in this research was ranged among 21.56~88.81 µg. Fig. 3 showed that media glucosamine content made of 4 wood species with 70% moisture content posses highest value compared to media with different moisture content indicating that 70% moisture content is the most suitable moisture content for Shiitake mushroom's mycelia growth on 4 media. Media moisture content affects to mushroom growth, because ideal moisture content in the baglog will yield which

is not too wet or dry. Too high moisture content in the baglog may cause that baglog will be decomposed quickly, in contrary, less moisture content in the baglog also causes not optimum mushroom growth (Meinanda 2013).

Correlation between Chemical Content to Growth Rate and Glucosamine Content

Wood mushrooms, in their growth period, generally obtain the nutritions from the wood decomposition of their media. The main components of wood composer which are cellulose, hemicellulose and lignin are carbon resources which are needed for wood mushroom's growth (Przybylowicz and Donoghue 1990). However, Przybylowicz and Donoghue (1990) also added that resins and phenolic compounds may inhibit the mushroom growth on wood media both hardwood or softwood because those kind of extractives are very difficult to be degraded by wood mushroom, generally.

Correlation analysis results between chemical content of sawdust media and mycellia growth rate and glucosamine content are presented in Table 3. The average of Shiitake mycellia growth rate in this research positively correlated with ethanol-toluene extractives content and negatively correlated with hemicellulose content of the media. This

results indicated that media with high ethanol-toluene extractives content can be grown by mushroom mycellia faster. Whereas on the media have high hemicellulose content mushroom mycellia grew slower. Generally, extractives content (especially ethanol-toluene extractives content) may cause negative impact to mycellia growth (Przybylowicz and Donoghue 1990), however, Shiitake mushroom in this research used substrates in ethanol-toluene extractives as carbon resources for its growth. Shiitake mushroom is classified as white-rot fungi therefore Shiitake mushroom secretes ligninase enzyme to degrade lignin or other phenolic compounds in the media. At first, Shiitake mushroom is supposed to use the simple compounds in the extractives and then degrade lignin which is more complex. This matter also explains about the high correations between lignin content and growth rate or glucosamine content, however, statistically those correlation coefficients are not significant. Negative correlation between mycellia growth and hemicellulose content pointed out that Shiitake mushroom does not use hemicellulose of the media for its growth. The same tendency was also happened on holocellulose although the correlation coefficient was not significant. Shiitake mushroom does not secrete hemicellulose enzyme to degrade cellulose, allegedly.

Table 3. Correlation of chemical content to growth rate and glucosamine content.

Chemical components	Growth rate	Glucosamine content
Ethanol – toluene extractives	0.973 *	0.350 ns
Hot water soluble extractives	0.853 ns	0.169 ns
Holocellulose	-0.821 ns	-0.141 ns
α -cellulose	0.042 ns	0.476 ns
Hemicellulose	-0.988 *	-0.534 ns
Klason lignin	0.825 ns	0.927 ns

** = Significant at 1% level. ns = Not significant.

Conclusions

The growth rate of mycelia was affected by the moisture content of media, meanwhile the glucosamin content is influenced by the moisture content and various species of wood. The highest glucosamine content was found in 70% moisture content for all species, although the highest mycelia growth rate was found in 65% moisture content. The best combination to cultivate the Shiitake mushroom was by using media made of gamal with 70% moisture content. There were positive correlation among the ethanol-toluene extracts content and the mycelia growth, however there was negative correlation among the hemicelulose content the mycelia growth.

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