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The addition of *Aspergillus flavus* on composting process of cotton waste for cultivation of paddy straw mushroom [*Volvariella volvacea* (Bull.) Singer 1951]

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Abstract

Paddy straw mushroom [*Volvariella volvacea* (Bull.) Singer 1951] is a tropical edible mushroom and commonly cultivated in Indonesia. Composting process for preparing substrate plays an important role during mushroom cultivation. The purpose of this study is to investigate the effect of the addition of *Aspergillus flavus*, the lignocellulolytic fungi in the composting process of cotton waste for cultivation of *V. volvacea*. The result showed that there was an increase in temperature of compost of 27-58°C and pH of 7-8 for seven days observation. The concentration of cellulose, hemicelluloses and lignin was decreased from the 2nd days of the composting process in both the addition and without the addition of *A. flavus*. The highest concentration of glucose was 0.089 mg/mL without addition of *A. flavus* and 0.122 mg/mL with the addition of *A. flavus*. The highest concentration of xylose was 0.070 mg/mL without addition of *A. flavus* and 0.173 mg/mL with the addition of *A. flavus*. The fresh weight of the fruiting body of *V. volvacea* was 11.153 g and 9.395 g with the addition of *A. flavus* and without the addition of *A. flavus* in the composting process, respectively.

1. Introduction

Recently, edible mushrooms have been becoming favorite food. Not only because of its delicacy but also because of high nutrition and active compounds contained in edible mushrooms. Cultivation of edible mushrooms has now become popular all over the world. This activity can help to reduce poverty as well as to strengthen livelihoods for rural farmers and peri-urban dwellers. Mushroom cultivation also provides opportunities for improving the sustainability of small farming systems through the recycling of organic matter, which can be used as a mushroom growing substrate, and then the spent mushroom substrate can be returned to the land as organic fertilizer.

There are twelve species of edible mushroom which are commonly grown for food and medicinal purposes. One of them is *Volvariella volvacea* is generally known as rice straw or paddy straw mushroom (PSM). Specifically, it is called “choku” in China, “fukurotake” in Japan, “hed-fang” in Thailand, “kauk-yo-hmo” in Myanmar, “kabuting dayami or kabuting saging” (rice straw or banana mushroom) in Philippines and “jamur merang” in Indonesia. These mushroom originally is from China and they have reportedly been cultivated for almost 300 years. They were introduced to other Southeast Asian countries more than a hundred years later by migrating overseas Chinese (Chang, 1999). PSM is well known as a tropical edible mushroom. Its growth optimum on 32-37°C and relative humidity on 85-96%. Recently, PSM was cultivated in a several lignocellulosic waste instead of rice straw. One of these is cotton waste, the byproduct of textile industry.

A mushroom is defined as “a macrofungus” with a distinctive fruiting body which can be either epigeous or hypogeous. The fruiting body is large enough to be seen with the naked eye and to be picked up by hand (Miles and Chang, 1997). Taxonomically, Mushrooms belong to the Kingdom of Fungi, a group very distinct from plants,

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animals, protista and monera (Hagen, 2012). Mushrooms are different from other heterotrophic organisms in the way of intake of food to the cell. Because they do not have a chlorophyll, they do not produce their "food" by photosynthesis process but obtain it by absorbing it into their cells. A wide variety of lignocellulose waste can be degraded efficiently by extracellular enzymes secreted by mushrooms. The extracellular lignocellulolytic enzyme is produced by fungi to assist in hydrolyzing the complex compound to be the simple one so that it can be easier absorbed by the fungal cell.

The aim of the study is the augmentation of *Aspergillus flavus*, a cellulolytic fungus during the composting process of cotton waste as a growth medium during cultivation of PSM. During the composting process, several parameters such as temperature and pH as well as biochemical aspects such as concentration of glucose, xylose, cellulose, hemicellulose and lignin were determined. Furthermore, the production of the fruiting body was also determined.

2. Materials and methods

2.1. Materials

Strain of *A. flavus* was obtained from the Laboratory of Edible Mushroom, Research Center for Applied Microbiology, National Research and Innovation Agency of Indonesia. It was cultured on PDA medium. The commercial spawn of *V. volvacea* was obtained from YK Mushroom Company, Purwakarta, Indonesia.

2.2 Preparation of Aspergillus flavus cell

The cellulolytic *A. flavus* was grown in 500 mL potato dextrose broth medium in an Erlenmeyer flask. The culture was then incubated for 5 days in room temperature on a shaker at 200 rpm. This culture was used when watering cotton on a composted process.

2.3 Composting of cotton waste

Two-hundred-kilogram cotton waste, as a growth medium of *V. volvacea* was soaked in water overnight to increase the water content up to 70%. The cotton waste then was divided into 10 parts. Five parts were composted with addition of inoculant of *A. flavus* and the other five parts were composted without the addition of inoculant of *A. flavus* as a control. Each treatment and control have 5 replications. Composting process was done by mixing the cotton waste with 2% of lime ($CaCO_3$) (w/w), 15% of rice brand (w/w), and 1% molasses (v/w). It was composted for 7 days.

2.4 Determination of parameters

The compost was analyzed for temperature, pH, lignocellulose, glucose and xylose, as well as N-acetylglucosamine. The analysis of lignocellulose (lignin, hemicellulose and cellulose) used the method of Chesson-Datta with minor modification (Ma'aruf *et al*., 2017). Glucose and xylose was analyzed by the method described by Ghose (1987). The sampling for determination of parameters was done on the 0, 3rd, 5th and 7th day of the composting process.

2.5 Cultivation of Volvariella volvacea

After the composting process, the media was put into a 40 × 28 × 15 cm plastic basket and pasteurized on 75°C for 2 hrs. It was then placed on a mushroom house shelf. The inoculation of the medium with the spawn of PSM was carried out when the temperature of the medium had reached 35°C. After spawning, the medium was incubated for 6-9 days for the growth of mycelium. During that period, the mushroom house was closed tightly for 6 days. On the seventh day, proper ventilation is provided and a small amount of water can be introduced onto the beds to maintain temperature and humidity in the house as well as in the substrate. The primordia usually can be formed 9-10 days after spawning.

3. Results and discussion

In mushroom production, composting a substrate is a basic process that prepares nutritional materials for the growth of mushroom mycelium and on which it produces fruiting bodies. Substrate for mushroom cultivation comes from the agricultural waste (Baghel, 2018). Microbiological activity and biomass content of the composting material changed during the production of compost used as a substrate in mushroom cultivation (Derikx *et al*., 1990). Both parameters showed a correlation with temperature. The physical characteristics such as temperature and pH of a substrate was shown in Table 1. The results show that the temperature of substrate was increased from 28.06°C at 0 days to 54.95°C at 7 days in control and from 27.33°C at 0 days to 58.57°C at 7 days in composting with the addition of *A. flavus* culture. The temperature of substrate in composting with the addition of *A. flavus* was higher than that of control. It indicates that the biochemical reactions occur more actively in the composting process with the addition of *A. flavus* than that of the natural composting of substrate. Mushroom compost develops as the chemical nature of the raw ingredients is converted by the activity of microorganisms, heat, and some heat-releasing chemical reactions. High microbiological activity causes temperatures of the composting material to rise (Satyanarayana and Grajek, 1999).

Evidence that the composting process is going well

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Table 1. Temperature and pH of substrate during composting process.

Days of composting	Temperature (°C)		pH	
	Control	Treatment	Control	Treatment
0	28.06±0.09	27.33±0.57	7.22±0.03	7.43±0.08
3	47.95±0.85	50.83±0.92	6.54±0.21	6.75±0.09
5	48.37±0.11	54.32±0.86	8.63±0.17	8.67±0.16
7	54.95±0.25	58.57±0.51	8.62±0.07	8.66±0.19

is a variation of pH during its process (Table 1.). It shows that the initial pH of the cotton waste is 7.2-7.4. The pH decreased on the 3rd day from 6.5 to 6.7 days and increased after the 5th day. It was 8.6 on the 7th day of the composting process. Hubbe *et al.* (2014) reported that initial pH values in the range of 7.0 to 7.5 have been recommended. Study by Hultman (2009) showed that the production of lactic and acetic acids during initial degradation of substrate often leads to decreasing pH until the 3rd to 5th of the composting process. Later, in the thermophilic stage of composting, the pH increases, resulting in release of ammonia, and thereafter the pH usually returns back to near-neutral conditions as the compost becomes mature. When the organic acids are decomposed by microorganisms, and the associated rise in pH is sometimes taken as evidence of successful composting. A physical appearance of cotton waste before and after the composting process was shown in Figure 1. The composted cotton wasted is darker and crumblier.

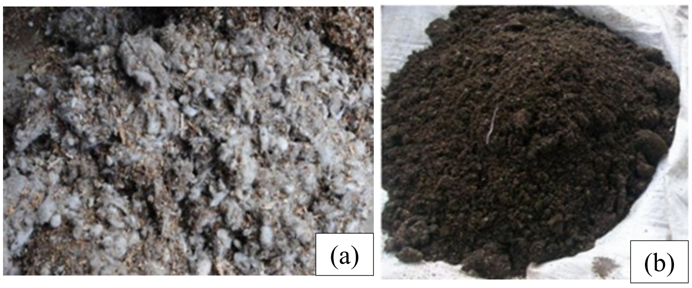

Figure 1. Cotton waste (a) before composted and (b) after composted.

Substrates for mushroom cultivation mainly contain lignocellulose compounds and act as structural components of plants. Major components of the lignocellulosic materials are cellulose, hemicellulose and lignin with varying composition in different materials (Pothiraj *et al*., 2006). During the composting process, degradation of lignocellulosic compounds happens. It provides the ideal condition for mushroom growing. The composition of lignin, cellulose and hemicellulose of substrate during the composting process was shown in Table 2. Degradation of lignocellulose was occurring during the composting process. At the end of composting, the concentration of lignin decreases from 7.12% to 2.94%, cellulose from 15.68% to 12.35% and hemicellulose from 8.09% to 6.86% in the substrate without addition of *A. flavus* cell. The addition of *A. flavus* cell increase the degradation of lignocellulosic compound as the concentration of lignin decrease from 7.05% to 2.25%, cellulose from 15.62% to 12.35%, and hemicellulose from 8.08% to 5.88%. Baghel (2018) reported that cellulolytic fungi have the lignocellulose degrading ability that can be attributed to their highly well-organized enzymatic system. There are two types of extracellular enzyme system, one which produces hydrolases for the degradation of polysaccharides such cellulose and hemicellulose as well as a unique extracellular and oxidative ligninolytic system, which cleaves open phenyl rings and thus degrades lignin.

The degradation of cellulose is conducted by a set of glycoside hydrolase (GH) enzymes with complementary catalytic activities. Cellulases have different specificities to hydrolyze the β-1,4-glycosidic linkages bonds that connect glucose units in the cellulose fiber. On the other hand, hemicellulose is a heterogeneous polymer composed of many sugars, such as xylose, arabinose, mannose, and galactose, that are C5 and C6 sugars. The concentration of glucose and xylose as the enzymatically hydrolyzed product of cellulose and hemicellulose was shown in Table 3. At the end of the composting process, the concentration of glucose was 0.089 (mg/mL) in the process without addition of *A. flavus* cell and 0.122 (mg/mL) with addition of *A. flavus* cell. The concentration of xylose was 0.070 (mg/mL) in the process without addition of *A. flavus* cell and 0.173 (mg/mL) with addition of *A. flavus* cell. Jurak *et al*. (2015) reported that during composting of wheat straw for cultivation of *Agaricus bisporus*, 50% of both xylan and cellulose were metabolized by microbial activity. It is coincidence to

Table 2. Composition of lignin, cellulose, hemicellulose, from substrate during composting process.

Days of composting	Lignin (%)		Cellulose (%)		Hemicellulose (%)	
	Control	Treatment	Control	Treatment	Control	Treatment
0	7.12±0.07	7.05±0.26	15.68±0.23	15.62±0.32	8.09±0.10	8.08±0.60
3	6.13±0.11	4.73±0.53	14.15±0.28	13.37±0.83	7.56±0.28	6.86±0.34
5	3.75±0.11	3.45±0.33	13.35±0.45	12.53±0.31	7.09±0.18	6.39±0.34
7	2.94±0.47	2.25±0.06	12.35±0.96	11.77±0.51	6.86±0.08	5.88±0.31

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Table 3. The concentration of monosaccharide glucose and xylose of substrate during composting of process.

Days of composting	Glucose (mg/mL)		Xylose (mg/mL)	
	Control	Treatment	Control	Treatment
0	0.073±0.004	0.073±0.005	0.045±0.006	0.045±0.007
3	0.086±0.002	0.115±0.009	0.067±0.004	0.111±0.015
5	0.086±0.004	0.143±0.006	0.066±0.006	0.157±0.009
7	0.089±0.002	0.122±0.001	0.070±0.003	0.173±0.002

the results in this study that the addition of *A. flavus* cell increases the concentration of glucose and xylose.

In Indonesia, cultivation of PDM usually is done in rice straw. However, because of the high demand of this mushroom, an alternative substrate is needed. Procedure of PDM cultivation using cotton waste as an alternative substrate is similar to that of rice straw as a substrate. Figure 2 shows the growth of PDM in various stages on cotton waste substrate. PSM is predominantly harvested in the egg stage, since taste, shelf life, nutritious and thus commodity value is considerably better in this than in later developmental stages. Therefore, the rapid transition from the egg to the elongation stage decreased commodity value and shelf life (Tao *et al*., 2014). The fruit body formation of PDM in this study was shown in Figure 3. The pattern of fruit body formation in a control substrate and treatment substrate is similar. The first peak was obtained on the 2nd day of fruit formation; it was 3005 g and 2320 g in the treatment and control, respectively. The 2nd peak was obtained on the 6th day with 1565 g and 1200 g in the treatment and control, respectively. The last peak for treatment and control was obtained on the 10th day showing 1080 g and 645 g, respectively. After the 12th day, the formation of the fruit body is decreased until the 17th day. The fresh weight of the fruiting body of *V. volvacea* was 11.153 g and 9.395 g with the addition of *A. flavus* and without the addition of A. flavus in the composting process, respectively. Peng (2008) reported that in Taiwan, cotton waste is used as basic raw material for growing *V. volvacea* instead of rice straw. Mushroom growers used the cotton waste as the casing of spent mushroom substrate of *A. bisporus* or *A. bitorquis*. The casing soil on the surface of used mushroom compost is removed and then covered with wetted cotton waste with a thickness of about 5 cm to grow *V. volvacea*. Cotton waste showed the best results in production of the fruit body of *V. volvacea* in comparison with paddy straw and banana leaves as substrate (Zikriyani *et al*., 2018).

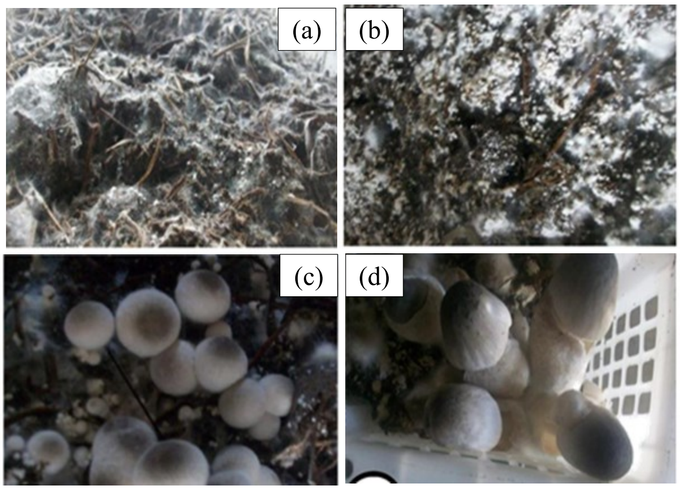

Figure 2. The growth of paddy straw mushroom (PSM) in various stage in cotton waste substrate; (a) Mycelium, (b) primordia stage, (c) egg stage, and (d) mature stage.

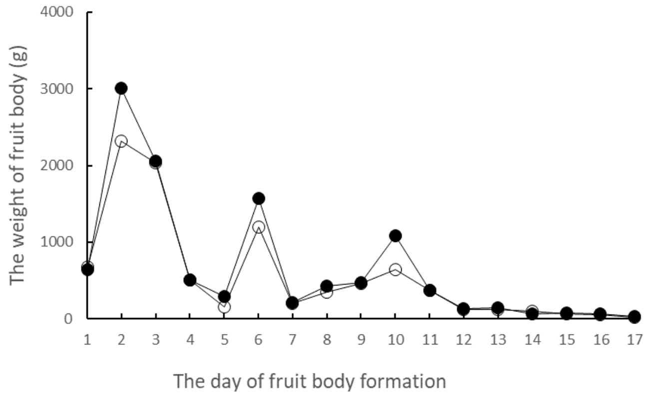

Figure 3. The fruiting body formation of PDM in composting substrate with and without the addition of *A. flavus* cell (○ = without the addition of *A. flavus* cell; ● = with the addition of *A. flavus* cell).

4. Conclusion

The augmentation of *A. flavus* cell during the composting process of cotton waste in the preparation of substrate for PSM cultivation, affect the biochemical aspect. The degradation of lignocellulose compound was increase. On the other hands, the availability of glucose and xylose, a monosaccharide as results of hydrolysis of cellulose and hemicellulose was also enhance. Furthermore, the augmentation of *A. flavus* enhance the production of fruitbody of *V. volvacea*.

Conflict of interest

The authors declare no conflict of interest.

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