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Abstract. The study aimed to find alternative substrates for growing spawn of oyster mushroom (*Pleurotus ostreatus*). The experiment was laid out in a completely randomized design with three compositions of substrates. The substrate compositions were: (1) Substrate A (corn 100%), (2) Substrate B (corn and sawdust mix in a ratio of (1:1, v/v)), and (3) Substrate C (corn and sawdust mix in a ratio of (3:1, v/v)). All compositions were replicated 18 times. The complete colonization (days) and mycelium growth rate (cm day^{-1}) were recorded. Means were analyzed by Analysis of Variance (ANOVA) and duncan test was performed if there were significant between the substrate compositions. The results showed that substrate C was the best substrate indicated with the fastest complete colonization of 14.17±0.92 and the highest growth rate of 0.85±0.06 cm day^{-1}.

1. Introduction

Oyster mushroom (*Pleurotus ostreatus*) is one of the edible mushrooms cultivated intensively worldwide. The global mushroom production has increased in the last two decades with China as the main producer with more than 80% of total production [1]. In Indonesia, mushroom cultivation and production increase every year, which is cultivated by traditional and modern method [2]. There are several reasons for the production increase: ease of growth on various substrates (agricultural and forest wastes) and the high nutritional contents and medicinal properties of the oyster mushroom [3]. The oyster mushroom has many nutritional contents. The dry oyster mushroom contains protein (10-30,4%), fat (1,6-2,2%), carbohydrate (57,6-81,8 %), fiber (7,5-8,7 %), ash (6,1-9,8 %), 345-367 C (cal) energy value per 100 grams dry weight [4]. It also contains amino acids such as leucine (6,8 grams), isoleucine (4,2 grams), valine (5,1 grams), tryptophan (1,3 grams), lysine (4,5 grams), threonine (4,6 grams), phenylalanine (3,7 grams), methionine (1.5 grams), histidine (1.7 grams) per 100 grams of protein [4].

Oyster mushroom is cultivated on media containing lignin, carbohydrate (cellulose and glucose), protein, nitrogen, fiber, and vitamin[2]. Nutrition sources for mushroom growth can be obtained from various sources such as sawdust, paddy straw, switchgrass (*Panicum virgatum*), corn straw, water hyacinth, dried

banana leaves, and other organic materials [5-7]. Most of the substrates for oyster mushroom growth are waste products and easy to be obtained.

One of the critical steps in the cultivation of mushroom is spawn preparation. The first step of oyster mushroom preparation is inoculation of mycelium to an agar media such as potato dextrose agar (PDA). The next step is the propagation of the agar culture in grain substrate. In Indonesia, the agar culture, referred as filial 0 (F0), is propagated by farmers two to three times using grain and sawdust substrates to yield filial 1 (F1) filial 2 (F2), and filial 3 (F3). In this study, we evaluated the mycelium growth of oyster mushroom on corn and sawdust.

2. Materials and Methods

2.1 Agar culture

Mycelium from fresh fruiting bodies of oyster mushroom was used as primary inoculum in agar culture. The fruiting body of oyster mushroom was obtained from local oyster mushroom farming, Celebes Mushroom, in Desa Taeng, Gowa District, South Sulawesi Province, Indonesia. Agar culture (F0) was prepared through the tissue culture method by inoculating mycelium from the fresh fruiting body into potato dextrose agar (PDA) (Merck, Darmstadt, Germany) and incubated at room temperature (30±2°C) for seven days[8].

2.2 Experimental design

The experiment was performed using a completely randomized design (CRD). There were three treatments of F1 spawn substrates compositions, those were:

1. Substrate A (corn 100%)
2. Substrate B (combination of corn and sawdust in a ratio of (1:1) (v/v))
3. Substrate C (combination of corn sawdust in a ratio of (3:1) (v/v))

All treatments were replicated 18 times. Time for full colonization (day) and mycelium growth rate (cm day^{-1}) were measured. Means were analyzed by Analysis of Variance (ANOVA). Duncan test was performed if there were significant between the substrates tested.

2.3 Preparation of F1 spawn substrates

Substrates for making F1 spawn used in this study were corn and sawdust. The corn was soaked in tap water for 24 hours to increase the moisture content and then boiled in tap water for ±20 minutes. The sawdust was not given any treatment, such as fermentation or composting.

2.4 Preparation of F1 spawn

The F1 spawn substrate was filled into a glass bottle (substrate amount is 12 cm in height in the bottle). All spawn substrates were sterilized in an autoclave at 121^{o}C 15 psi for 15 minutes. The F1 spawn substrates were let cool down and inoculated with F0 spawn (1x1 cm in size). The F1 spawns were incubated in the dark at room temperature (27^{o}C ±2) until complete colonization.

3. Results and Discussions

3.1 Effect of different substrates on mycelium growth

The time for complete colonization and growth rate were measured. The result showed that F1 spawn with substrate C was found to have the highest growth rate of 0.85±0.06 cm day^{-1} and the fastest complete colonization of 14.17±0.92 days (Table 1). The complete colonization of Substrate A and B were 15.83±0.79 days and 14.72±0.57 days, respectively, and the growth rate of 0.76±0.04 cm day^{-1} and 0.82±0.03 cm day^{-1}, respectively (Table 1). The complete colonization time of all spawn substrates tested were significantly

different at ($P < 0.05$), and so was the growth rate. Mycelium of *P. ostreatus* was dense and uniformly white in all substrates (Fig. 1).

The result showed that F1 spawn substrates consisting of corn and sawdust had a shorter time for complete colonization, while the highest growth rate might be due to the availability of required nutrients for *P. ostreatus* in F1 spawn corn and sawdust. The primary function of sawdust is to provide cellulose, hemicellulose, and lignin, which is utilized during the growth of mycelium[9]. On the other hand, corn provides nitrogen with that essential for mycelium growth. All fungi require nitrogen to synthesize nitrogen-containing compounds such as protein, including cell wall components such as chitin composed of N-acetylglucosamine acid, N-acetylmuramic acid, and amino acids[10].

Spawn is crucial in mushroom cultivation. The effect of mixing grain and the other substrates on mycelial growth of spawn showed by Thongklang & Luangharn (2016)[11]. Their study revealed that spawn composed of sorghum mixed with corn cobs in ratio of 1:1 (w/w) had the fastest mycelium growth indicated with highest growth rate of 16.83 mm day^{-1}, faster than spawn produced from sorghum alone as the substrate which had growth rate of 8.11 mm day^{-1}.

Many studies have been conducted to know the effect of spawn on mycelium growth and production yield of oyster mushroom. Mycelium growth on the production substrate (spawn run) is important to be considered. Shorter spawn run means time from spawning process to harvest is faster. The effect of spawn substrate on spawn run and production yield of *P. ostreatus* was demonstrated by a study previously [12]. They examined kurakkan, sorghum, corn, and paddy as the spawn substrates and found that spawn composed of kurakkan gave the fastest spawn run (mycelium fully colonized the production substrate). They also recorded significant differences in biological efficiency (BE) ratio. The BE value of *P. ostreatus* cultivated using spawns produced with kurakkan, sorghum, corn, and paddy were 30.76%, 25.38%, 16.57% and 11.99%, respectively[12]. The effect of spawn substrate on production yield was also discovered on *P. ostreatus* cultivation[13].

Table 1. Complete colonization, growth rate, mycelium density, and mycelium color of *P. ostreatus* F1 spawn on different growth substrate

No.	F1 Spawn	Complete Colonization (Day)	Growth rate (cm day^{-1})	Mycelium	
				Density	Color
1	Substrate A (Corn 100%)	15.83 ± 0.79^{a}	0.76 ± 0.04^{a}	Dense	White
2	Substrate B (Mixture of corn and sawdust (1:1))	14.72 ± 0.57^{b}	0.82 ± 0.03^{b}	Dense	White
3	Substrate C (Mixture of corn and sawdust (3:1))	14.17 ± 0.92^{c}	0.85 ± 0.06^{c}	Dense	White

Results are given as the mean±SD: a, b, c notation showed the significance between all treatments ($P<0.05$)

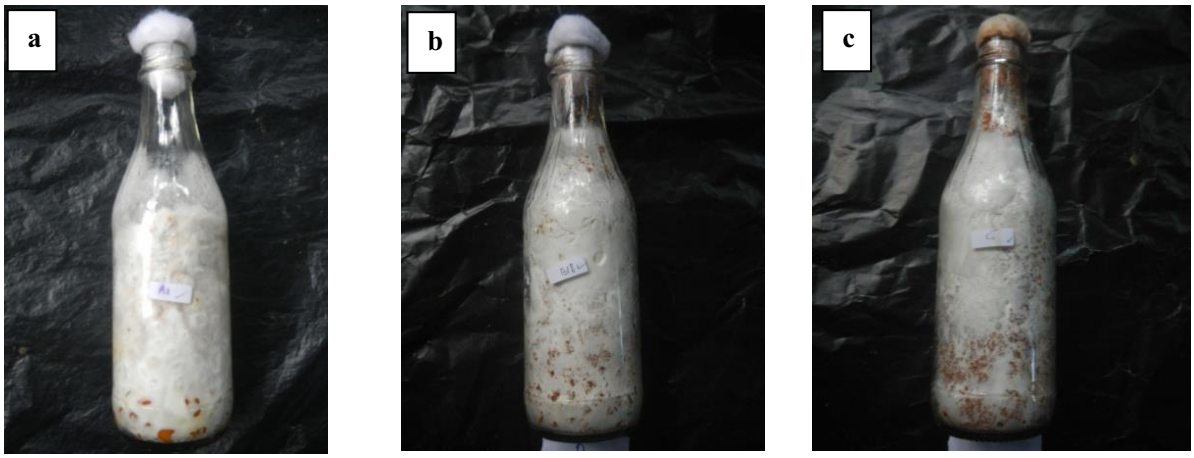

Figure 1. Mycelium growth of oyster mushroom (*P. ostreatus*) F1 spawn growth on different substrate. a) Substrate A (Corn 100%); b. Substrate B (combination of corn and sawdust (1:1)); and c). Substrate C (combination of corn and sawdust (3:1)).

The spawn substrate effect on spawn run and production yield was also recorded on the other mushroom species. Spawn produced from sorghum grains had fastest spawn run on milky mushroom (*Calocybe indica*), which took only 13.7 days to fully colonize the production substrate, followed by corn, cotton seed, horse gram, black gram, and cow pea of 19 days, 18.3 days, 18.3 days, 15.3 days, and 15.3 days, respectively[14]. The effect of spawn substrate on production yield of shiitake mushroom (*Lentinula edodes*) has also been examined [15]. Their results showed that spawn's substrates along with genetic factors were significantly influenced the BE ratio of shiitake mushroom [15].

4. Conclusion

Study suggested that spawn substrate consisting of corn and sawdust had shorter time for complete colonization. However, further study is important to investigate the effect of spawn substrates on production of *P. ostreatus.*

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