Effect of Physicochemical Properties of Oil-palm-waste-based Substrates on Mycelia Growth Rate of *Pleurotus ostreatus*

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ABSTRACT Malaysia produces a large quantity of empty fruit bunch (EFB) and oil palm frond (OPF) every year, these wastes are not efficiently utilized, and causing disposal problems and pollute the environment. On the other hand, it has been reported that oyster mushrooms grown on oil palm by-products produced good yield. Thus, this study was carried out to investigate the effect of physical and chemical properties of the substrate on the mycelia growth rate of *Pleurotus ostreatus* on three treatments: namely, 100% EFB, 100% OPF, and the mixture of 50% EFB and 50% OPF. All treatments had five replicates and arranged in a completely randomized design (CRD). Chemical and physical properties; concentration of C, N, K, P, Cu, Ca, Mg, Fe, Zn, moisture content, ash content, volatile solids content, pH, electrical conductivity, wet bulk density, particle density, and porosity were measured. The number of days taken for mycelia to entirely colonized the substrate bag was recorded. It was found that pH, EC, and the concentration of C, N, P, Cu, Ca, Mg, and Zn were significantly affected by substrate formulations. It took 23-25 days for mycelia to entirely colonized the substrate in all treatments. However, the mycelia growth rate of *P. ostreatus* was not significantly affected by the physicochemical properties of substrate formulations.

KEYWORDS: *Pleurotus ostreatus*; mycelia growth rate; empty fruit bunch; oil palm frond; physicochemical properties
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INTRODUCTION

Malaysia is the second-largest palm oil producer globally, the industry worth 9.7 billion USD in 2017 (Tang & Al Qahtani, 2020). Despite being the backbone of economic development in Malaysia, the palm oil industry also causes environmental pollution due to its massive waste generation on upstream and downstream activities (Abdullah & Sulaim, 2013). In 2009, oil palm frond and empty fruit bunch were mounted up to 44.8 million tonnes and 7.0 million tonnes respectively during harvesting and pruning even though there are many efforts to utilize and convert this waste into value-added products (Awalludin *et al.*, 2015), including as substrate for mushrooms cultivation. This is helpful to the mushroom industry due to the limited supply of conventional substrate material, which is rubber tree sawdust (Ali *et al.*, 2013).

Mushrooms are rich in flavor and nutritional value (Rosmiza *et al.*, 2016). Oyster mushroom is the most preferred variety consumed by Malaysians (Haimid & Rahim, 2013). Domestic production of mushrooms in Malaysia is insufficient to fulfill the local demand. However, this industry still owns the potential to grow as the local and market demand for mushrooms has increased continuously (Mat-Amin & Harun, 2015). Oil palm by-products such as empty fruit bunch, palm oil fruit mesocarp, and palm pressed fiber had shown positive results on oyster mushroom cultivation when used as growing substrate (Dzulkefli & Zainol, 2018; Zakil *et al.*, 2019; Silva *et al.*, 2020).

With the growing interest to expand the mushroom industry in Malaysia and the effort to utilize oil palm by-products as valuable resources for farmers to generate income, it is important to understand the factors affecting the growth of mushrooms cultivated on substrate formulated with oil palm by-products. Thus, this study aimed to investigate the effect of physicochemical properties of the substrate on the mycelia growth rate of *Pleurotus ostreatus*.

METHODOLOGY

Preparation of Pure Culture and Spawn

Pleurotus ostreatus was obtained from Korperasi Pembanggunan Desa, Kundasang (6˚ 1.323′ N, 116˚ 36.312′ E). The basidiocarp was cut in half, fresh tissue with a size of 5 mm x 5 mm was obtained from the center and transferred to a 39 g L^{-1} potato dextrose agar (PDA) plate using sterilized forceps and scalpel under aseptic condition, then it was incubated in dark condition with temperature 25±2 °C for 5 days. Healthy and active mycelia that were free from impurities were sub-cultured into a new PDA plate using a 5 mm cork borer to obtain purified culture.

Rice husk obtained from Sapi Rice Plantation (5˚ 53.673′ N, 117˚ 22.851′ E) was used as grain spawn. It was cleaned by rinsing under tap water, soaked for 30 minutes before boiling it for 15 minutes, and left on a strainer for 1 hour. Once excess moisture was removed, 300 g (wet basis) of rice husk was packed in heat resistant polyethylene bag (7.5 cm x 29 cm). The opening of heat-resistant polyethylene bags was plugged with cotton and closed with a cap. The rice husk-filled bags were sterilized using an autoclave at 121 °C for 20 minutes, and then inoculated with mycelia after it was cooled to room temperature.

Preparation of Substrates

Empty fruit bunch and oil palm frond were collected from local palm oil mill and oil palm plantations, dried in solar dryer dome, until constant weight was achieved and chopped into smaller pieces (5-7 cm) using a chipper. 2.5 kg (dry basis) of mushroom substrates were mixed uniformly with the basal substrate, rice bran, and calcium sulphate at a ratio of 100:10:1 (Rakib *et al.*, 2020) with adequate water and then packed in heat-resistant polyethylene bags (7.5 cm x 29 cm). The opening of filled bags was plugged with cotton and closed with a cap. Each filled bag weighed 500 g and was sterilized at 80 °C for 8 hours. Once cooled, it was inoculated with 2% (w/w) grain spawn under aseptic condition. Three substrate formulations were tested; namely, 100% empty fruit bunch (T1), 100% oil palm frond (T2), and a mixture of 50% empty fruit bunch and 50% oil palm frond (T3). Each treatment had 5 replicates, resulting in a total of 15 experimental units. All experimental units were arranged in a completely randomized design (CRD), kept in dark condition with 25 °C – 30 °C.

Data Collection

Moisture content (MC), wet bulk density (WBD), ash content (AC), and volatile solids content (VSC) were determined according to Mihilall *et al.* (2011). pH and electrical conductivity (EC) were measured with a benchtop meter (Eutech PC 27000) according to Owaid *et al*. (2017). Determination of particle density (PD) and porosity were done according to Almomany *et al.* (2019) with slight modification for particle density where the oven-dried sample was placed in a 100 mL cylinder until the 40 mL mark, weight of the sample was recorded. Then, 60 mL of distilled water was poured into a cylinder and stood for 5 minutes to remove trapped air in the sample. After that, the volume of water that remained in the cylinder which also known as the volume of displaced water by the sample was recorded.

Total C and total N were determined using the dry combustion method by CHN analyzer (CHN-600, LECO Corporation, St. Joseph, MI). The concentration of total K, P, Cu, Ca, Mg, Fe, and Zn were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Optima 5300 DV, Perkin Elmer), where the samples were extracted using the dry-ashing method (Isaac & Johnson, 1975) with slight modification where 1.0 g of sample was weighed into a porcelain crucible, and ashed in a muffle furnace at 300 °C for 1 hour. The temperature was then increased to 520 °C for another 4 hours until the sample turned white. Once the sample was cooled, a few drops of deionized water and 1 mL of concentrated HCl (36%) were added to the sample. The sample was evaporated to dryness using a hot plate in a fume hood. Five milliliters of 20% HNO_3 were added to the sample and placed in a water bath for 1 hour. Once the digestion process has completed, the solution was filtered with a syringe filter (0.45 μm, PTFE) and made up to 50 mL with deionized water. Days taken from substrate incubation to mycelia entirely colonize substrate bag (MFC) were recorded.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed, and Tukey HSD test was used to compare treatment means. Data were subjected to Pearson's correlation analysis to evaluate the correlation between mycelia growth and physicochemical properties. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 26.

RESULT AND DISCUSSION

Physical Properties of Substrates

The MC and WBD of substrate were significantly affected by different substrate formulations except for PD and porosity (Table 1). The lowest MC was recorded in T1 which was 58.15 %. This could be influenced by the difference in fiber composition of substrates. Empty fruit bunch contains 13% - 37% of lignin, which is higher as compared to OPF that only contains 20%- 21% (Dungani *et al.*, 2018). Lignin is water-resistant and provides mechanical support to cell walls to prevent breakage (Singh *et al.*, 2013). Thus, the lack of moisture being absorbed resulted in lower MC and WBD in T1.

Table 1. Physical properties of substrates

Treatment	MC (%)	PD (g cm^{-3})	WBD (g cm^{-3})	Porosity (%)
T1	58.15 ± 0.58a	0.803 ± 0.06a	0.10 ± 0.004a	87.11 ± 0. 69a
T2	69.80 ± 1.27b	1.323 ± 0.60a	0.13 ± 0.002b	85.17 ± 3.48a
T3	66.23 ± 1.87b	1.053 ± 0.15a	0.13 ± 0. 007b	86.24 ± 1.91a
P value	0.001	0.603	0.002	0.843

Note: T1 = 100% empty fruit bunch, T2 = 100% oil palm frond T3 = 50% empty fruit bunch and 50% oil palm frond, MC = moisture content, PD = particle density, WBD = wet bulk density.
Data are means ± standard error (n=4). Values in each column followed by a different lower-case letter indicate significant difference by Tukey's multiple range test ($p \leq 0.05$).

Chemical Properties of Substrates

The pH, EC, and the concentrations of C, N, P, Cu, Ca, Mg, and Zn were significantly affected by substrate formulations except for AC, VSC, and the concentrations of K and Fe (Table 2). It was found that T1 had the highest pH, and concentrations of Cu and Zn, which were 7.66, 0.11 mg kg^{-1}, and 0.51 mg kg^{-1}, respectively. T2 showed the highest in N, P, Ca, and Mg concentrations, which were 5.98%, 77.70 mg kg^{-1}, 235.34 mg kg^{-1} and 56.93 mg kg^{-1}, respectively. As for the combination of EFB and OPF (T3), EC, C, and the Cu concentration were the highest among all treatments with 5.49 ms m^{-1}, 44.34%, and 0.11 mg kg^{-1}, respectively.

Correlation Between Mycelia Growth Rate and Physicochemical Properties of Substrate

Although substrate formulations exhibited significant differences in certain physical and chemical properties, there was no significant effect on the mycelia growth of *P. ostreatus* between treatments. Days taken for mycelia entirely colonized for T1, T2, and T3 were 25, 24, and 23, respectively. There was no correlation between physicochemical properties of substrates on mycelia growth of *P. ostreatus*.

The growth of mycelia is important to provide an ideal internal condition for the growth of fruiting bodies (Tesfay *et al.*, 2019). Porosity is an important physical property in a substrate, as it is related to the bulk density as well as particle density. Higher porosity increases oxygen concentration which leads to an increase in mycelia growth (Mbogoh *et al.*, 2011). Apart from that, porosity also affects the aeration and compaction of substrate, larger particle size has smaller surface, hence creating less compaction in substrate. In the present study, there was not much difference in particle density, wet bulk density and porosity between treatments, which explains the similar mycelia growth rate. This can be concluded that EFB and OPF chopped into 5-7 cm creates an ideal porosity for mycelia growth.

pH is one of the chemical factors that can influence mycelia growth. It was reported that oyster mycelia grow best at pH 6.4, and lower pH could be toxic to the hyphae, resulting in slower mycelia colonization (Ibekwe *et al.*, 2008). Mycelia of *P. ostreatus* has the ability to tolerate a wide pH range, 5.0 – 8.0 (Yadav, 2001). This statement is supported by the present study, in which pH of substrates ranges 6.02 – 7.66 resulted in mycelia growth. Therefore, the ideal pH for mycelia growth is close to neutral, acidic condition (< 5.0 pH) will deteriorate mycelia growth.

Nitrogen is an essential nutritional source for mycelia growth, higher nitrogen resulted in shorter time to complete mycelia growth (Dzulkefli & Zainol, 2018). However, Yang *et al*. (2013) reported that excess amount of nitrogen slows down spawn run and inhibits mushroom growth, it was stated that high carbon and nitrogen ratio is preferred for mycelia growth while low carbon and nitrogen ratio is preferred for fruiting bodies. Despite the difference substrate materials used in this study, the percentage of carbon and nitrogen did not vary much. Carbon ranges 43.02% - 44.34% while nitrogen ranges 5.57% – 5.98%, resulted in 23 – 25 days for mycelia entirely colonized substrate bags.

Concentrations of Zn, Mg, Ca, Cu and P exhibited significant difference among treatments, but it did not contribute to the mycelia growth rate of *P. ostreatus* in this study. This is in contrast with studies done by Kaur & Atri (2016), and Atri & Guleria (2013), *P. sadipus* and *Lentinus cladopus* showed different mycelia growth rate when substrates were supplemented with various trace elements at different concentrations. It can be concluded that concentration of 0.34 mg kg^{-1} – 0.51 mg kg^{-1} Zn, 25.68 mg kg^{-1} – 56.94 mg kg^{-1} Mg, 74.45 mg kg^{-1} – 235.35 mg kg^{-1} Ca, 0.04 mg kg^{-1} – 0.11 mg kg^{-1} Cu and 38.32 mg kg^{-1} – 77.70 mg kg^{-1} P are suitable for *P. ostreatus* during mycelia growth stage.

Although there was no correlation between concentrations of trace elements and mycelia growth of *P. ostreatus*, it is an important factor in later stage of mushroom cultivation. Substrate material will affect the nutritional properties of fruiting bodies. Mycelia secrete hydrolytic enzyme that degrades the substrates and bio-accumulates them as nutrients, which will then be absorbed by the mycelia and accumulated in the fruiting bodies (Aaron *et al.*, 2017). This statement is supported by Sithole *et al.* (2017), mushrooms harvested from trace metal polluted soil were reported to contain higher concentrations of trace metals in their stalks and caps. Therefore, the materials used for substrate must not contain excess trace elements that may cause health risk to consumers.

Table 2. Chemical properties of substrates

Treatment	AC (%)	VSC (%)	pH	EC (ms m^{-1})	C (%)	N (%)	K (mg kg^{-1})	P (mg kg^{-1})	Cu (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
T1	13.94 ± 0.68a	85.11 ± 0.68a	7.66 ± 0.13a	3.82 ± 0.11b	44.16 ± 0.09a	5.87 ± 0.17ab	222.37 ± 10.68a	38.32 ± 2.22b	0.11 ± 0.01a	74.45 ± 3.93b	25.68 ± 1.39b	6.64 ± 0.34a	0.51 ± 0.03a
T2	14.89 ± 1.32a	86.06 ± 1.32a	6.99 ± 0.01b	3.94 ± 0.17a	43.02 ± 0.05b	5.98 ± 0.01a	206.61 ± 4.03a	77.70 ± 1.25a	0.04 ± 0.001c	235.35 ± 4.84a	56.94 ± 1.04a	5.81 ± 0.18a	0.34 ± 0.01b
T3	13.12 ± 1.35a	86.88 ± 1.35a	6.02 ± 0.03c	5.49 ± 0.08b	44.34 ± 0.03a	5.57 ± 0.01b	195.36 ± 9.13a	40.14 ± 2.20b	0.11 ± 0.003b	88.05 ± 4.89b	27.81 ± 1.28b	5.62 ± 0.31a	0.38 ± 0.04b
P value	0.577	0.577	0.000	0.000	0.000	0.043	0.130	0.000	0.000	0.000	0.000	0.069	0.007

T1 = 100% empty fruit bunch, T2 = 100% oil palm frond, T3 = 50% empty fruit bunch and 50% oil palm frond, AC = ash content, VSC = volatile solids content, EC = electrical conductivity

Data are means ± SE (n=4). Values in each column followed by a different lower-case letter indicate significant difference by Tukey's multiple range test ($p \leq 0.05$).

CONCLUSION

Mycelia of *Pleurotus ostreatus* were able to grow on substrates formulated with EFB and OPF, either singly or in combination. The physicochemical properties of substrates showed significant differences in terms of moisture content, wet bulk density, pH, electrical conductivity, and the concentrations of C, N, P, Cu, Ca, Mg, and Zn. However, there is no significant effect on the mycelia growth rate of *P. ostreatus* between treatments. A study on nutritional properties of *P. ostreatus* fruiting bodies grown on substrates formulated with EFB and OPF is recommended.

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