INTI JOURNAL | eISSN:2600-7320
Vol.2022:40

Converting agro-ecological waste into potential substrates for cultivation of *P. ostreatus*

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Abstract

Pleurotus ostreatus is an edible mushroom which is rich in proteins, carbohydrates, vitamins, minerals and lipids, making it a highly beneficial food source. Commercial cultivation of *P. ostreatus* is mostly done on sawdust, however, there is a potential shortage of sawdust due to a shift of rubber plantations to more profitable oil palm plantations in Malaysia. Therefore, alternative substrates should be sourced, preferably from agricultural waste. Cultivation of *P. ostreatus* on agricultural waste that are abundant in Malaysia, namely *Imperata cylindrica*, was studied in terms of cultivation parameters and nutritional value. The sterilized substrates were treated and inoculated with spawn of *P. ostreatus* before incubation for colonization and fruiting of the mushrooms. The experiment was conducted in triplicates for each substrate. *I. cylindrica* had a faster mycelial growth compared to sawdust which was used as a control. After complete colonization, two flushes of matured fruiting bodies were harvested from both sawdust and *I. cylindrica*, where *P. ostreatus* cultivated on *I. cylindrica* took a shorter duration (58 days). Although *I. cylindrica* had better mycelial growth, however a lower yield performance and biological efficiency were observed with this substrate compared to sawdust. However, the two different substrates had similar effects on the nutritional values of *P. ostreatus* in terms of the moisture, total carbohydrates, protein, lipids and metabolised energy. Based on the results obtained, *I. cylindrica* was found to be a potential alternative substrate for *P. ostreatus* cultivation as no significant differences were observed on the total yield and nutritional values between substrates. Nevertheless, further studies on the optimization of commercial *P. ostreatus* production using *I. cylindrica* are required.

Keywords

Pleurotus ostreatus, *Imperata cylindrica*, sawdust, macronutrient content

Submission: 28 June 2022; **Acceptance:** 5 July 2022

Introduction

Pleurotus spp. is the third most cultivated and commercially produced mushroom worldwide (Ho et al., 2020). *Pleurotus* spp. is highly nutritious where it is rich in proteins (25-50%) and relatively low in fats (2-5%), in addition to the presence of vitamins and minerals (Ng'etich et al., 2013). Thus, *Pleurotus* spp. is regarded to be a powerful food source to reduce malnutrition in developing countries as it is easy and economical to cultivate (Agarwal et al., 2017). In Malaysia, commercial cultivation of *Pleurotus* spp., especially *P. ostreatus*, is mostly done using rubber tree sawdust (Zakil et al., 2020). However, there is potential shortage and occasionally, unavailability of sawdust due to limited access to rubber trees and an increase in consciousness of protecting the forests (Zakil et al., 2020). Thus, this poses a severe problem to mushroom growers and emphasizes the importance of identifying alternative substrates such as lignocellulosic substrates for *P. ostreatus* cultivation.

In Malaysia, there are large volumes of unused *Imperata cylindrica*, commonly known as Cogon grass, which is usually left to rot in fields, controlled using chemical herbicides or disposed of through open burning, leading to environmental pollution. In fact, this lignocellulosic substrate could be utilized to cultivate *P. ostreatus* to reduce the amount of wastes generated in order to maintain a sustainable environment in addition to overcoming the issue of the lack of sawdust. Furthermore, *I. cylindrica* propagates rapidly and unlike other lignocellulose, it is not used as forage, causing it to be more difficult to contain, thus a new destiny as a mushroom cultivation substrate can be given to reduce the environmental burden caused by *I. cylindrica*.

There is limited published data in Malaysia about the cultivation of *P. ostreatus* on *I. cylindrica*, where most studies focused on the use of rubber tree sawdust and oil palm fronds. This work is aimed at studying the difference in yield and nutritional content (namely carbohydrates, proteins and lipids) of *P. ostreatus* cultivated on lignocellulosic substrate, namely *I. cylindrica* compared to rubber tree sawdust.

Methodology

Cultivation of *P. ostreatus*

The spawn of *P. ostreatus*, sawdust, corn bran and calcium carbonate ($CaCO_3$), were obtained from NAS Agro Farm. Meanwhile, *I. cylindrica* were collected from grassy areas in Kluang, Johor. *I. cylindrica* was dried before being coarsely ground into 1.0-2.0 cm length pieces. Distilled water 1.5%), rice bran (10%) and $CaCO_3$ (1%) were added to the dried, ground *I. cylindrica* and sawdust separately (Tesema & Keneni, 2019). Each mixed substrate, prepared in 3 replicates, was filled into polyethylene bags and were capped individually before being autoclaved at 121°C for 30 min. After cooling to room temperature, about 7 g of spawn were aseptically inoculated into each of the sterilised bags which were then capped and stored at room temperature under dark conditions to induce colonization. After complete colonization of the substrates by the mycelia, the bags were opened and stored at room temperature in the presence of light to enable the formation of fruiting bodies. Each inoculated bag was sprayed with distilled water twice daily to keep the mycelia moist

(Tesema & Keneni, 2019). The fruiting bodies were manually harvested at maturity, indicated by the curving of the cap edges.

Cultivation Parameters Assessment

During the colonization phase, the mycelia length of each experimental bag after spawn inoculation were recorded. The yield was determined as the average fresh weight of harvested mushrooms from each substrate. The average biological efficiency of the *P. ostreatus* cultivation were calculated based on the following equation (Tesema & Keneni, 2019):

$$\text{Percentage of biological efficiency} = \frac{\text{weight of fresh mushroom per bag}}{\text{weight of dry substrate per bag}} \times 100$$

Nutrition Analysis

Moisture content was determined by measuring the weight of 2 grams of freshly harvested *P. ostreatus* before and after drying the samples in an oven for 2 hours at 135^{0}C (AOAC,1990). The average moisture content of the samples from each substrate was obtained using the formula below:

$$\text{Percentage of moisture} = \frac{initial\ weight - dried\ weight}{initial\ weight} \times 100$$

Total carbohydrate content was evaluated using the phenol-sulphuric acid method described by Serna-Saldiva (2012) and Yogachitra (2019), while total protein was determined by the Bradford assay with modifications (Yogachitra, 2019). Total lipid content was calculated using Bligh and Dyer Extraction, modified by Sündermann et al. (2016).

Data analysis

The data was presented as mean weight and percentages. One-way analysis of variance (ANOVA) was used to analyse all the data groups between the different substrates where a significance value of 0.05 was considered (Tesema & Keneni, 2019).

Results & Discussion

Colonization of *P. ostreatus* on Sawdust and *I. cylindrica*

The highest mean length of the mycelial growth was recorded on *I. cylindrica*, (Figure 1). The mycelial growth on sawdust and *I. cylindrica* were significantly different during the first 30 days of cultivation whereby *I. cylindrica* was found to be better as it had the highest mean length of mycelial growth which could be attributed to the relatively lower carbon to nitrogen (C/N) ratio. These results correspond to the previous findings by Zakil et al. (2019) who reported that nitrogen rich substrates were found to speed up the mycelium growth. In addition to the rapid colonization rate of the substrate, *P. ostreatus* cultivated on *I cylindrica* produced two harvests of mushrooms in a shorter duration (< 60 days) compared to sawdust (< 70 days).

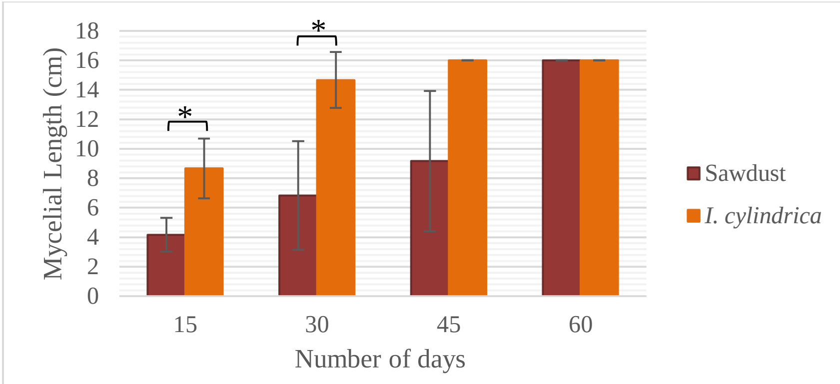

Figure 1. Average mycelial length of *P. ostreatus* cultivated on different substrates in the experimental bags over 60 days. The total length of the experimental bags were 16 cm. Significance levels were obtained from one-way ANOVA analysis with $p \leq .05$.Between-group significance differences are indicated by asterisks above the bars.

Yield and Biological Efficiency of *P. ostreatus* Cultivated on Different Substrates

In general, *P. ostreatus* cultivated on sawdust had slightly better yield (23.7 g on average) than those cultivated on *I. cylindrica* in terms of the average fresh weight of harvested mushrooms (Figure 2).

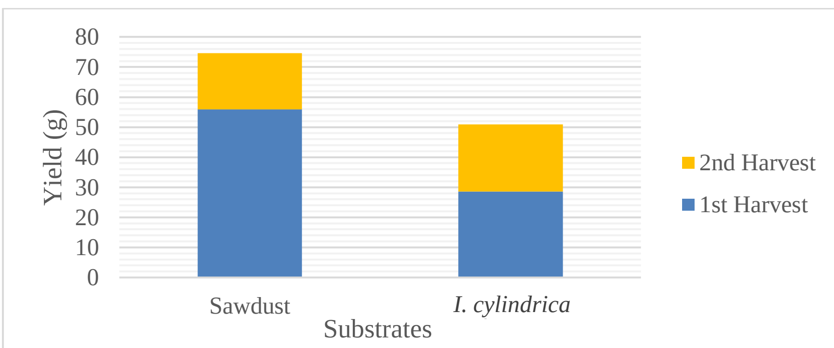

Figure 2. Total yield of *P. ostreatus* cultivated on *I. cylindrica* and sawdust over the 2 harvests. Significant differences ($p \leq .05$) were not observed on the total yield between the two substrates.

Based on the total yield, higher biological efficiency was observed on sawdust, where it was 6.78% higher than on *I. cylindrica*. However, the total yield and biological efficiency of this study had no significant differences between the *P. ostreatus* cultivated on *I. cylindrica* and sawdust. Conversely, Muswati et al. (2021) and Tesfay et al. (2020), reported significant differences on the yields and biological efficiencies of *P. ostreatus* cultivated on different substrates due to the difference in chemical composition of substrates. In this study, the statistically similar of yields and biological efficiencies might due to the relatively similar amount of cellulose, hemicellulose and lignin found in both these substrates.

Nutrition Analysis of *P. ostreatus* Cultivated on Different Substrates

Moisture content of all the fruiting bodies was above 80% in both harvests on both sawdust and *I. cylindrica* (Figure 3), which is in agreement with the findings by Ashraf et al. (2013), Tesema and Keneni (2019) and Zakil et al. (2019). Generally, moisture content was found to be unaffected by the substrates but has been shown to be associated more with the type of mushroom species (Patil et al., 2010).

In general, the total carbohydrate content was observed to be higher in *P. ostreatus* cultivated on sawdust (23.05%) than on *I. cylindrica* (Figure 3). These findings were comparable to several previous studies by Bhattacharjya et al. (2015) (73%-79%), Hoa et al. (2015) (30%-51%) and Tesema and Keneni (2019) (42-53%), who cultivated *P. ostreatus* on different substrates including *Pennisetum purpureum*, *Oryza sativa* and *Triticum* spp.

The total protein content was higher in *P. ostreatus* cultivated on sawdust than on *I. cylindrica* for both harvests with a difference of 1.07% (Figure 3).

In contrast, the lipid content of *P. ostreatus* cultivated on *I. cylindrica* was higher than those cultivated on sawdust where *I. cylindrica* was 0.13% higher (Figure 3). The lipid contents of *P. ostreatus* were between 1.0% to 2.5%. These values were similar to the reported values in previous studies by Fernandes et al. (2015), Hoa et al. (2015) and Sales-Campos et al. (2011). Besides, the results of this study were found to be in accordance with the findings by Belletini et al. (2016), who reported increasing protein content could result in low lipid content in the mushrooms.

There was no statistical significance in the differences between sawdust and *I. cylindrica* in terms of the total carbohydrates, total protein and lipid contents of *P. ostreatus* in both harvests. This indicates that both substrates had similar effects in terms of the macronutrient content of *P. ostreatus*.

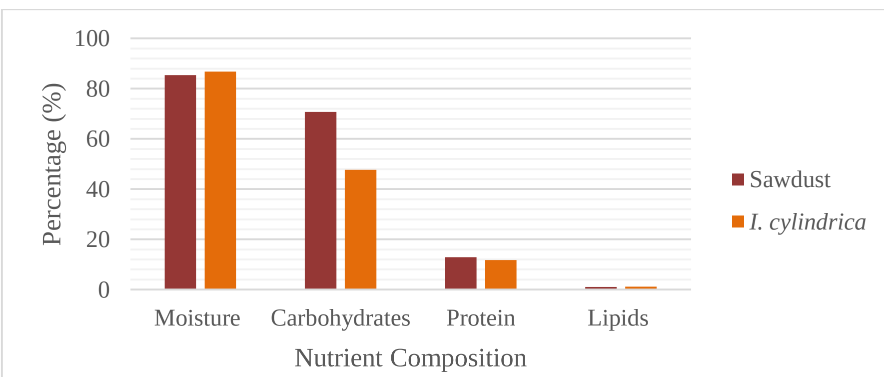

Figure 3. Nutrient compositions of *P. ostreatus* cultivated on sawdust and *I. cylindrica*. Significant differences were not observed among the substrates in each category ($p \leq .05$).

Conclusions

P. ostreatus was cultivated on *I. cylindrica* had better effects on the mycelial growth and colonization as well as duration to yield mushroom fruiting bodies compared to sawdust. This characteristic of faster mushroom production also makes *I. cylindrica* a beneficial alternative substrate as faster production leads to shorter cultivation period to obtain the maximum flushes of fruiting bodies. At the same time, the nutritional attributes of *P. ostreatus* cultivated on *I. cylindrica* was almost of the same quality as those cultivated on rubberwood sawdust. Thus, when cultivated on *I. cylindrica*, *P. ostreatus* can still serve its purpose as rich source of carbohydrates, proteins and lipids. All in all, *I. cylindrica* can be considered to be a potential alternative substrate for the commercial cultivation of *P. ostreatus* due to its availability throughout the year in large amounts. This would help in green conservation by utilizing agro-ecological waste instead allowing it to rot or be burnt, thereby polluting the environment. However, further research can be conducted on other agricultural wastes that are readily available in Malaysia including *P. purpureum*, and *O. sativa* husks to determine which of these waste that potentially contaminate the environment can be used to cultivate mushrooms instead.

Acknowledgements

The study is supported by an industry grant (AgroMas/INTI/2022-01).

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