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Growth performance and fruiting body characterisation of the paddy straw mushroom (*Volvariella volvacea)* cultivated on different types of solid wastes

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Abstract

In this study, the effects of solid waste formulations on the developmental duration, characteristics of the fruiting body, yield, and biological efficiency (BE) of *Volvariella volvacea* were evaluated. The solid wastes were 100% paddy straw (PS) as a control, 100% palm oil empty fruit bunch (EFB) and 100% waste paper (WP). In terms of combination, 1:1 ratios were used for PS:EFB, EFB:WP and PS:WP growth substrates. The growth performance of the fruiting body was evaluated from inoculation to the egg stage for 45 days. The characterisations were evaluated based on the individual fruiting body's weight, height, and length. The yield and BE were evaluated using the percentage of the total yield harvested and dry weight of the solid wastes. According to analysis of variance (ANOVA) at $p \leq 0.05$, *V. volvacea* grown on 100% WP took by far the longest time to achieve the egg stage (33.50 ± 4.95 days), compared to 100% PS, which took the shortest time (25.67 ± 0.58 days). Meanwhile, for the combination substrates, PS:EFB was the fastest to formulate the egg stage, at 29.67 ± 1.15 days. Despite having the slowest harvesting duration, WP showed the highest size quality (24.00 ± 10.58 g) when cultivated on its own. Besides, WP showed the highest yield (124.00 ± 32.05 g) and BE (12.40 ± 3.20%) when supplemented with PS. These findings shed important light on the potential use of solid wastes as a promising alternative for the cultivation of *V. volvacea* that might increase the BE of this species.

Keywords: Biological efficiency, Solid wastes, Growth substrate, *Volvariella volvacea*

1. Introduction

Edible mushrooms are fleshy fruiting macrofungus bodies formed from a complex enzymatic and microbial reaction of mycelium with different lignocellulosic substrates such as decaying wood, organic matter and agricultural wastes [1]. The number of edible mushrooms worldwide was most recently estimated to range from 2.2 to 3.8 million, with 120,000 species having been identified and 3% having been named [2]. The collection and consumption of edible mushrooms was a traditional practice in many human cultures before they became widely cultivated for commercial purposes. The edible mushrooms commonly cultivated commercially in Malaysia include abalone (*Pleurotus cystidiosus*), the oyster mushroom (*Pleurotus spp.)*, black jelly (*Auricularia auricula*), ganoderma (*Ganoderma applanatum*), shiitake (*Lentinusedones*) and the paddy straw mushroom (*Volvariella volvacea*) [3].

Also known as the Chinese mushroom, *Volvariella volvacea* or the paddy straw mushroom is believed to have originated from China in the early 18th century as a gift or food exclusive to royalty due to its high nutritional content of protein and fibre and its low-fat content [4]. *V. volvacea* is also known as a warm mushroom since a high-temperature and humid environment is required to produce fruiting bodies. Despite its history of cultivation dating back to the 18th century, limitations in the cultivation process remain. This primarily involves low substrate conversion, which affects and hinders the commercial potential of *V. volvacea*. This poses a problem as the rising human population will lead to increased demand for varieties of agro-industrial-based products. Therefore, the biological efficiency (BE) of edible mushrooms must be improved to meet the market demand.

BE is a simple parameter used in research to calculate the effectiveness with which a mushroom converts lignocellulosic materials into a fruiting body. The BE of *V. volvacea* is among the lowest compared to other edible mushrooms. For comparison purposes, the BE of *Plerotus* spp. and *Lentinula edodes* range from 47.0% to 134.5% and from 22.7% to 85.6%, respectively. In contrast, the BE of *V. volvacea* ranges between 0.1% and 26.7% [5-8]. Its poor BE shows that *V. volvacea* cannot fully utilise the nutrients offered by a growth substrate to produce a fruiting body. The solid wastes most commonly used in *V. volvacea* cultivation are agricultural wastes such as paddy straw (PS), palm oil empty fruit bunch (EFB), cotton wastes and banana leaves. Meanwhile, research has been conducted on cultivating edible mushrooms using municipal solid waste and industrial wastes, such as waste paper, kitchen food wastes and seafood processing wastes [9,10]. Solid wastes are suitable for *V. volvacea* cultivation because they can produce a variation of cellulose enzymes to break down cellulose, thus enabling access to the required nutrients from the solid wastes. This is important as *V. volvacea* requires high-cellulose and low-lignin substrates and it utilises them to form fruiting bodies [11]. The high cellulose content of all these solid wastes makes them a potential alternative substrate to use in *V. volvacea* cultivation. In this paper, the effects of different solid waste combinations on the developmental duration, characteristics of the fruiting body, yield and BE were evaluated.

2. Materials and methods

2.1 Materials

Mature substrate seedlings were obtained from the Malaysian Nuclear Agency. The types of solid wastes used for the growth substrates were PS, EFB and WP. The other materials used included kapok, limestone, 50 × 25 cm polyethylene (PE) mushroom spawn bags, a 120 × 90 cm three-tier rack and a hygrometer.

2.2 Preparing growth substrate

Substrate seedlings of *V. volvacea* were obtained from the Malaysian Nuclear Agency. The solid wastes were prepared as follows: the PS and waste paper (WP) were shredded into lengths of 3 to 5 cm and the EFB was obtained in pellet form. Before inoculating the solid wastes with the healthy *V. volvacea* spawn substrates essential for producing healthy fruiting bodies, pre-treatments of the wastes were essential; these included soaking, tossing to remove the excess water, mixing with limestone and kapok, as well as sterilising. The methods used to prepare the growth substrates were based on Azhar et al. [12]. The solid wastes were weighed at 3 kg and soaked in water for at least eight hours. The wastes were then tossed overnight to remove the excess water. The solid wastes were then assessed to determine whether a good amount of moisture had been achieved. If too much excess water was noticed, an additional one to two hours of tossing was added. After tossing the excess water, the solid wastes were then mixed with 10% limestone and 10% kapok, based on the dry weight of the wastes. For this experiment, the control was 100% PS, while the experimental cultivation substrate mixtures were 100% EFB, 100% WP, and 1:1 ratios of PS: EFB, EF: WP and PS: WP. Three replications of each cultivation substrate mixture were prepared. Limestone was used to increase the growth substrate pH, while kapok was used to maintain its moisture. After mixing, the mixtures were then left to compost for a week. After composting, the mixtures were packed into 50 × 25 cm PE spawn bags.

2.3 Sterilisation process

The growth substrates were autoclaved at 121°C and 15 atm for 30 min. The sterilised growth substrates were then placed in a well-ventilated room (Room Temperature (RT) 25°C) and left overnight to cool.

2.4 Inoculation of substrate seedlings and spawning

The inoculation process and spawning methods were based on Azhar et al. and Reyes [12,13]. The amount of substrate seedlings used for inoculation was based on 10% of the weight of solid waste. Thus, approximately 300 g of substrate seedlings was used to inoculate 3 kg of each growth substrate. The growth substrates were wrapped tightly and left for two weeks to spawn in a well-ventilated room (RT 25°C). The purpose of spawning was to allow the mycelium to colonise at least 50% of the growth substrate. The inoculation and spawning method are shown in Figure 1.

Figure 1 The spawn substrate was inoculated by (A) spreading the spawn on the growth substrate. The inoculated growth substrate was then (B) wrapped and left for two weeks to spawn in a well-ventilated area.

2.5 Fruiting and harvesting

After two weeks of spawning, the growth substrates were exposed and placed in a covered rack for the formation of fruiting bodies, as shown in Figure 2. The method of harvesting the *V. volvacea* fruiting bodies was based on Azhar et al. and Reyes [12,13]. The mushroom was harvested at the egg stage by holding the egg and twisting, followed by an upward pull. The mushroom base will be sliced off the block if the pulling motion is not strong enough to pull off the mushroom. Precautions were taken to not leave any remaining mushroom during the slicing as this could have attracted bugs and caused contamination to the growing substrate. The parameters used to evaluate the performance of the solid wastes as cultivation substrates were the duration of each development phase, the earliness, the average weight, as well as the length and height of each individual fruiting body.

Figure 2 After spawning, (A) the growth substrates were exposed and (B) placed in a covered growing rack to initiate fruiting.

2.6 Experimental design

In this study, three replicates were utilised for each formulation cultivation. The durations taken to produce pinheads, small buttons, buttons, the egg stage and the first harvest were observed for 45 days. The characteristics of the fruiting bodies cultivated from different types of solid wastes were evaluated by measuring the weight, height and length of each body. The yield and BE of the solid wastes were also evaluated. The mean of the data was analysed with the statistical package for the social sciences (IBM SPSS) Statistic Data Editor software and using the ANOVA test. Any significant differences between the performance of *V. volvacea* cultivated on different solid wastes were determined using Tukey's honestly significant difference (HSD) test, with $p \leq 0.05$.

3. Results and discussion

3.1 Developmental duration

The developmental durations taken to produce the pinhead, small button, button and egg stage formations are shown in Table 1. For all the formulations, pinheads were shown to form within a range of 17 to 23 days, while the time taken until the egg stage formation and the first harvest ranged from 25 to 33 days. The pinhead formation on EFB (16.33 ± 2.30 days) and PS (17.33 ± 0.58 days) was significantly faster. These developments conform to

the findings from Apetorgbor et al. and Sakib et al. [14,15], who observed that the formation of pinheads on EFB and PS took 16 and 15 days, respectively. The differences in the durations of pinhead and harvesting formation for these substrates may have been due to the efficiency with which *V. volvacea* produces solubilising enzymes such as laccase, lignin peroxidases (LiP) and manganese peroxidase (MnP), which differs depending on the types of solid wastes used. These enzymes play major roles in breaking down lignin and cellulose during the reproductive and vegetative states [16]. Furthermore, the different combinations of solid wastes possibly produced improved amounts of macro elements like potassium, calcium, phosphorus and magnesium, as well as helping to stimulate the developmental duration and faster fruiting body formation of V. volvacea [17]. Both of these could be seen in the developmental duration of *V. volvacea* cultivated on PS:WP and EFB:WP, with both combinations showing significant improvements compared to when V. volvacea was cultivated on WP alone. These results can be supported by the reports by Apetorgbor et al., [7] who found that supplemented substrates improved the developmental duration of *V. volvacea*.

Table 1 Developmental and harvesting duration of *V. volvacea* cultivated on different formulations of solid wastes for 45 days.

Formulation	Developmental duration (days)			
	Pinhead	Small button	Button	Egg
100PS	17.33 ± 0.58^{a}	20.33 ± 0.58^{a}	23.00 ± 0.00^{a}	25.67 ± 0.58^{a}
100EFB	16.33 ± 2.30^{a}	20.33 ± 2.31^{a}	25.00 ± 1.73^{abc}	29.00 ± 1.00^{ab}
100WP	22.00 ± 1.41^{c}	25.50 ± 3.54^{a}	28.50 ± 0.71^{c}	33.50 ± 4.95^{b}
50PS: 50EFB	18.33 ± 0.58^{ab}	21.67 ± 1.15^{a}	24.00 ± 1.00^{ab}	29.67 ± 1.15^{ab}
50EFB: 50WP	19.67 ± 1.53^{ab}	23.67 ± 1.15^{a}	26.33 ± 1.53^{abc}	30.33 ± 0.58^{ab}
50PS: 50WP	20.50 ± 2.12^{ab}	24.00 ± 1.41^{a}	27.00 ± 0.00^{c}	30.50 ± 0.71^{ab}

Note: PS = paddy straw, EFB: empty fruit bunch, WP: waste paper. Mean ± SD values with similar letters denote not significantly different (p>0.05).

The harvesting activity of *V. volvacea* over 45 days is shown in Figure 3. The peak harvesting period ranged from days 25 to 34 after inoculation. The harvesting activity was separated into several flushes, with the majority of this activity peaking in the first flush. This was seen for PS, PS:EFB, EFB:WP and PS:WP, with most of the yield harvested in the first flushes and decreasing numbers of fruiting bodies harvested observed in the second and third flushes. In contrast, EFB and WP resulted in the most yield in their second flushes before they stopped producing fruiting bodies. PS and EFB showed the fastest first flushes of harvesting, ranging between days 20 and 24, whereas the rest of the solid waste combinations achieved their first flushes between days 25 and 29, with the slowest first flush observed when cultivated on WP. Even though PS and EFB yielded faster results, they only managed to produce two flushes of yield, which conformed with the reports of Philippoussis et al. [18]. Nonetheless, all the solid waste formulations showed conformity with Pattanayak et al. [19] in estimating that the first harvest duration of *V. volvacea* occurred 28 to 35 days from the inoculation date. On the other hand, the combination solid wastes of PS:WP recorded the highest number of mushrooms harvested (n=17) followed by EFB (n=15), in their first and second flushes, respectively. As previously mentioned for the slowest first flush, WP also recorded the lowest number of harvested mushrooms (n=3) for both flushes combined. The stark difference between the harvesting activity of WP and PS:WP further supported the reports of Apetorgbor et al. and Tripathy [7,20], who found that supplementing substrates with solid wastes improves mushroom development and production. The presence of different wastes may lead to a synergistic activity of solubilising enzymes and cause the wastes to be more effectively utilised by the mycelium for a better spawn run, primordia formation and yield.

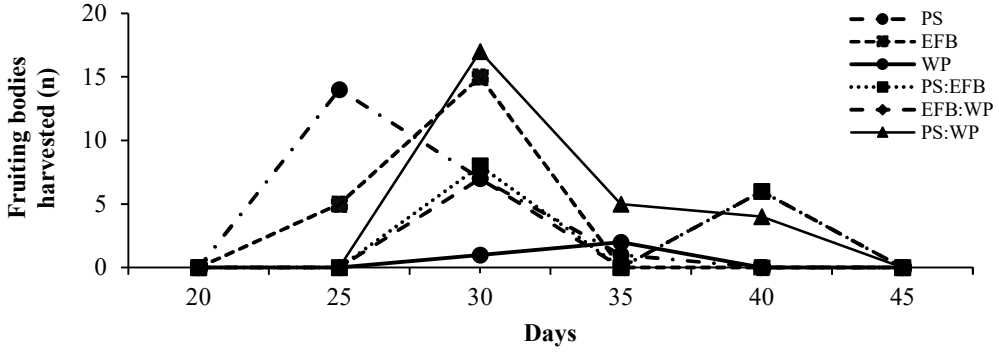

Figure 3 *Volvariella volvacea* harvesting activity for 45 days. PS: Paddy straw, EFB: empty fruit bunch, WP: waste paper.

3.2 Characteristics of volvariella volvacea

The characteristics of *V. volvacea* cultivated on different types of solid wastes were evaluated by measuring and recording each harvested fruiting body's weight, height and length. The data were measured and tabulated as shown in Table 2. The mean weight of the *V. volvacea* fruiting body harvested from WP and PS: EFB was recorded as by far the heaviest and the only formulation that weighed over 20 g. Based on the ASEAN Standard for Straw Mushroom commercial guidelines [21], *V. volvacea* is classified into three sizes based on weight. *V. volvacea* weighing more than 40 g are classified in Class 1, meanwhile those within the range of 20 to 40 g fall into Class 2. Class 3 is for *V. volvacea* weighing less than 20 g. The higher the size code, the lower its market value. The mean weight of the *V. volvacea* cultivated on WP and PS:EFB was classified as size 2, while the remaining formulations' mushrooms were classified as size 3. This shows that the mean weight of the *V. volvacea* cultivated on WP and PS:EFB produced a higher size quality for commercial purposes than the rest of the solid waste formulations.

Table 2 Characteristics of *Volvariella volvacea* fruiting bodies cultivated at the egg stage on different formulations of solid wastes.

Formulation	Height (cm)	Length (cm)	Average weight (g)	Classification [21]
100PS	3.42 ± 0.76^{ab}	2.70 ± 0.72^{a}	10.46 ± 5.97^{a}	3
100EFB	3.58 ± 1.10^{ab}	3.07 ± 0.67^{a}	10.60 ± 6.13^{a}	3
100WP	4.73 ± 2.16^{b}	3.70 ± 0.20^{a}	24.00 ± 10.58^{c}	2
50PS: 50EFB	3.80 ± 0.89^{ab}	4.93 ± 0.78^{b}	21.33 ± 6.62^{bc}	2
50EFB: 50WP	3.49 ± 1.27^{ab}	3.28 ± 0.86^{a}	13.29 ± 7.04^{ab}	3
50PS: 50WP	2.63 ± 0.97^{a}	3.07 ± 0.97^{a}	13.57 ± 9.54^{ab}	3

Note: PS (paddy straw), EFB (empty fruit bunch), WP (waste paper). Mean ± SD values with similar letters denote no significant difference at $p>0.05$.

The height and length of the *V. volvacea* cultivated on WP and PS:EFB (4.73 ± 2.16 cm and 4.93 ± 0.78 cm) were significantly taller and longer, and this was the only formulation that produced a fruiting body whose height and length exceeded 4 cm, while the rest of the solid waste formulations ranged between 2.6 and 3.8 cm. Meanwhile, in terms of the smallest fruiting body characteristics, PS:WP presented the highest number of fruiting bodies harvested, as well as the highest yield and BE. The differences in the characteristics of the *V. volvacea* using different formulations may have been due to the cellulose, hemicellulose, and lignin content of the solid wastes. PS is composed of approximately 35% cellulose, 18% hemicellulose and 15% lignin [22], whereas EFB contains 24 to 65% cellulose, 21 to 34% hemicellulose and 14 to 31% lignin [23]. A paper-based product typically contains 90 to 99% cellulose, 13 to 19% hemicellulose and 20% lignin [24,25]. However, the cellulose, hemicellulose and lignin content vary in different types of paper. Even though the *V. volvacea* developmental duration, yield and BE cultivated on WP were reported as inferior compared to other solid wastes, its fruiting body characteristics were superior to those of its supplemented counterparts.

3.3 Yield and biological efficiency (BE)

Zhang et al. [26] defined composting as a biological process that depends on the population of a variety of microorganisms converting the waste from organic substances into less complex compounds by breaking down the organic residue. By composting different solid waste formulations, different activities and communities of microorganisms may be formed [11]. This may result in different degrees of readiness of the solid wastes for mushroom cultivation. Composting is an important pre-treatment for solid wastes prior to inoculation with substrate spawn as it helps to make the nutrients from the growth substrate readily available for the use of mycelium for the primordia development which will form the fruiting body. Thus, using different solid waste formulations in mushroom cultivation directly affected the yield and BE. *V. volvacea* is known to have low BE compared to the other edible mushrooms in the world, and efforts are ongoing to maximise the ability of *V. volvacea* to utilise lignocellulosic substrates. Here, BE was used as one of the parameters to evaluate the effectiveness with which solid wastes produce mushroom fruiting bodies. The BE of each formulation of solid waste was calculated using the formula shown below, based on [27]. The harvest weight of each form of solid waste was compared to its dry weight.

$$\text{Biological efficiency (BE)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100\% \quad (1)$$

The yield and BE results are shown in Table 3. The highest yield and BE were observed when cultivated on the PS:WP, PS:EFB and EFB:WP combinations. The yield and BE of these three formulations showed improvement compared to the control PS. Despite showing the best developmental performance and fastest

harvesting duration, PS and EFB showed poor yields of 76.67 ± 53.35 and 70.67 ± 50.14 grams, respectively, compared to the rest of the solid waste formulations. However, in this study, the yield and BE of PS and EFB showed improvements compared to the findings of Apetorgbor et al. [7]. The BE recorded for WP was significantly lower (3.60 ± 0.00) compared to the BE when supplemented with PS and EFB (12.40 ± 3.20 and 9.50 ± 4.10, respectively). Similar results were reported by Tesfay et al. and Chhatbar et al. [10,30], who achieved higher BE values for *Pleurotus ostreatus* and *Pleurotus florida* cultivated on WP supplemented with paddy straw, cornstalk and wheat bran.

Table 3 Yield and BE of *V. volvacea* cultivated on different formulations of solid wastes for 35 days.

Formulation	Total average yield (g)	Total average biological efficiency (%)
100PS	76.67 ± 53.35	7.67 ± 5.34
100EFB	70.67 ± 50.14	7.07 ± 5.01
100WP	36.00 ± 0.00	3.60 ± 0.00
50PS:50EFB	106.67 ± 40.70	10.67 ± 4.07
50EFB:50WP	95.00 ± 41.01	9.50 ± 4.10
50PS:50WP	124.00 ± 32.05	12.40 ± 3.20

Note: PS (paddy straw), EFB (empty fruit bunch), WP (waste paper). Mean ± SD values with similar letters denote no significant difference at $p>0.05$.

The yield and BE improvements identified with this combination of solid wastes may have been due to the differences in population and microbial dynamics formed by combining and composting different types of solid wastes, providing enzymes and nutrients, stimulating growth, and protecting them against harmful pathogens [1,11]. Edible mushrooms can establish beneficial interactions with bacteria, not only as competitors for nutrients and as pathogens. A study conducted by Sharma et al. [28] showed how the positive interactions of bacteria during the button stage of *Phellorina inquinans* initiated the formation of primordia and the enlargement of fruiting bodies. Meanwhile, the cultivation method also possibly contributed to the BE improvement of *V. volvacea*. There are multiple methods of cultivating *V.* volvacea, including the bed method, cage method, spiral method, heap method, plastic crates (PC) and the polyethylene (PE) bag system [29]. Research by Nath et al. [29] reported a higher number of fruiting bodies and higher yield when cultivating *V. volvacea* using a PE bag system compared to using plastic crates or the bed method.

4. Conclusion

Despite showing the least favourable developmental duration, WP showed improvements in the characteristics of the fruiting bodies, yield and BE when combined with PS or EFB. More studies on the effects of different substrate compositions will clarify the relationship of the substrate with the production of high-yield and high-quality fruiting bodies, which would further assist in the efforts to commercialise *V. volvacea* on a global scale. Given the lack of previous studies, it is hoped that this study sheds some light on the usage of waste paper as an alternative for *V. volvacea* cultivation.

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