# PHYSICOCHEMICAL CHARACTERIZATION OF RUBBERWOOD SAWDUST, MUSHROOM SPENT MEDIUM, RICE HUSK, AND RICE HUSK ASH AS POTENTIAL GROWTH SUBSTRATES FOR WILD *Schizophyllum commune* CULTIVATION

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**Abstract**. Currently, in Malaysia, there was very limited information on the cultivation of wild *Schizophyllum commune* mushrooms as compared to other common and popular species such as *Pleurotus* spp. Hence, the feasibility of utilizing several lignocellulosic biomasses such as rubberwood sawdust (RSD), mushroom spent medium (MSM), rice husk (RH), and rice husk ash (RHA) as a potential growth substrate for optimal mycelial growth rate and yield in wild *S. commune* cultivation was evaluated in this study based on the lignocellulosic contents and morphology surface analysis using Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-ray (EDX). The results indicated that RH (37.82%) contained a large amount of cellulose followed by RSD (34.90%) and MSM (27.21%). There was no cellulose obtained from RHA. Before the cultivation process, the SEM image of RHA showed an even and flat surface with many small holes and RH showed an intact surface. Both RH and RHA contained a high amount of silica. The maximum mycelial growth of 7.71 mm/ day and a yield of 117.76 g were obtained from RH and RSD, respectively. The results demonstrated that RSD with a high amount of cellulose and mild destructed structure in the absence of silica gave best yield under a considerable length of colonization time (21.00±0.24 days). Therefore, RSD should be further explored in the next growth substrate formulation study for various growth responses improvement and enhancements. These findings are vital in sustaining the production of *S. commune* by the local growers and in ensuring continuous supply in the local market.

**Keywords:** Lignocellulosic biomasses, *Schizophyllum commune*, SEM-EDX

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## Introduction

In Malaysia, rubberwood sawdust (RSD) is the most popular and common biomass used for the cultivation of various types of mushrooms such as *Pleurotus* sp. (grey and white oyster), *Auricularia polytricha*, and *Schizophyllum commune* [1]. RSD is the by-product of the milling and cutting process in the rubberwood processing factory. As the second-largest agricultural plantation after oil palm with a total acreage of 1.07 million hectares (Mha), rubber crops are widely distributed over 13 states in Malaysia [2]. Rubber trees are normally replanted every 25–30 years when they are uneconomical for latex production [3]. Since replanting was a regular annual exercise, the supply of RSD for mushroom cultivation was quite consistent.

Paddy production is the third biggest after oil palm and rubber plants. Local paddy production reached about 2.6 million tonnes in 2012 and increased to about 2.615 million tonnes after one year, which was the largest national paddy production since 1980 [4]. As the main food commodity, paddy will always be cultivated regularly to fulfil people's needs. Consequently, the yearly generation of rice husk (RH) and straw will also be regularly and abundantly accumulated. In rice mills, RH is produced after grain milling, which presents in abundant and locally underutilized. In 2009, the output of RH in Malaysia was about 0.44 million tonnes based on an annual grain production of 2.2 million tonnes [5]. Due to the emerging technological development in the agro-industry, the RH generated is expected to increase tremendously and can be estimated by multiplying the amount of paddy production by the residue product ratio of 0.2 considering that 20% of the paddy weight is RH [6-7]. Shafie et al. [6] stated that the potential increase of paddy residue in Malaysia will cause abundant availability of the resources and then create waste management problems. Hanai et al. [8] claimed that RH generated from rice cultivation is one of the most serious wastes in Japan. RH has been disposed of either by incineration or as farmyard manure. Rice husk ash (RHA) was produced after the combustion of RH in the rice mills for heat generation in the drying process of the paddy. Therefore, mushroom cultivation activity might be one of the best solutions in solving the disposal problem related to the aforementioned biomasses waste.

Mushroom spent medium (MSM) might be one of the alternative biomasses that can be utilized as a growth substrate for the second production of mushrooms. After a certain period of cultivation, the mushroom bags will no longer produce fruiting bodies due to the low nutrient content even under optimal environmental conditions for cultivation [9]. MSM that are lignocellulosic substrate, are not completely degraded and usually contain high organic matter content with low macro- and micro-nutrient concentrations [10]. Therefore, without a proper disposal method, the wastes can cause major environmental pollution and turn into a habitat for some pests such as rats and cockroaches as the remaining mycelium in the bag logs becomes their food source. However, some cultivars used the spend as livestock bedding, soil conditioner, and organic fertilizer. The current yearly generated mushroom spent wastes locally is very difficult to predict and measure because the number of growers keeps increasing with their products ranging from small to large scale. However, the accumulation of waste is expected to increase as the number of growers rises.

In any mushroom cultivation, there are several intrinsic factors such as pH, moisture content, C: N ratio, minerals, medium composition, particle size, level of spawning, and surfactant that can affect the mycelial growth rate, total colonization time, total yield, and biological efficiency [11]. All these factors have a strong relationship with the lignocellulolytic enzymes of the mushroom in cleaving the wall structures of the biomasses

and converting the complex chain of carbohydrate components into smaller compounds via solid-state fermentation. Other important factors affecting the mushroom growth responses are the internal and external porosity of the lignocellulosic biomasses and mixtures of the growth components respectively. Rapid mycelial prefoliation was observed in a well-aerated mushroom bag log that is related to internal empty spaces and porosity within the biomasses matrix [12].

*S. commune* or known as the "split gills" mushroom is one of the most common edible mushrooms that can be found on all continents except Antarctica. In Malaysia, it is commonly called as "cendawan kukur" or "cendawan sisir"; in Sarawak it is known as "kulat kerang" and in Sabah as "kodop". This mushroom is categorized as white-rot fungi which is the most frequently found in soft- and hardwood-rotting processes such as rubberwood and mango trunk. It is belonging to the family Schizophyllaceae and can be easily identified by the peculiar structure of its gills (which cover hymenium during unfavorable climatic conditions) [13-14]. It has a small size (1-5 cm wide), with an elastic and leathery texture. The fruiting bodies resemble fan to shell-shaped with short striped and are grey-white to brown. Herawati et al. [15] reported that *S. commune* can easily be found on the surface of palm oil empty fruit bunches during the natural decomposition process in East Kalimantan. This seasonal mushroom species can also easily be found in decaying woods, especially during the rainy season [16-17]. Due to the seasonal and difficulty in getting this mushroom stock in the local market, the study is embarked to evaluate the feasibility of using several lignocellulosic biomass wastes such as RSD, MSM, RH, and RHA as a growth medium to grow wild *S. commune*. These wastes will be first characterized in terms of their lignocellulosic content and C: N ratio followed by an analysis of their morphology surface using a Scanning Electron Microscope coupled with an Energy Dispersive X-ray. Four important growth responses that are mycelial growth rate, colonization time, total yield, and biological efficiency will be compared from the different biomasses used for the cultivation of wild *S. commune*.

## Materials and Methods

The fresh fruiting body of wild *Schizophyllum commune* was collected from dead rubber trees in a rubber estate at Kampung Musa, Pedu, Kedah 6°13'43.1"N 100°40'46.6"E. The pure culture of the mushroom was maintained on Malt Extract Agar (MEA) in a sterile petri dish (8.6 cm diameter). The strain of "wild split gills" mushrooms were identified and authenticated by Macrogen, Korea using Polymerase Chain Reaction (PCR) technique and Internal Transcribed Spacer (ITS) analysis. The rubberwood sawdust and mushroom spent medium were obtained from a local grower in Perlis, Malaysia whereas rice bran, rice husk, and rice husk were purchased from Dibuk's Rice Mill Factory. The agricultural grade of calcium carbonate was purchased from Beseri's Agricultural Shop.

### *Analysis of Lignocellulosic Content and C: N Ratio*

The analysis of ash, extractives, cellulose, hemicellulose, and lignin of the biomass samples were performed according to Li et al. [18]. The content of carbon and nitrogen in the biomass samples were examined by FELDA Global Venture Laboratory, Pahang using the Dumas-Combustion method. All analytical experiments were completed in triplicates and the average values were calculated.

### *Analysis of Morphology Surfaces Using Scanning Electron Microscope Coupled With Energy Dispersive X-ray (SEM-EDX)*

Microstructure of surface morphologies and elemental contents of the biomass samples were carried out at the School of Material Engineering, UniMAP using Scanning Electron Microscope (Model JEOL-JV 5600) coupled with Energy Dispersive X-ray (Model Oxford ISIS LINK 3.2) (SEM-EDX). A thin layer of gold was coated on the samples before measurement to prevent charging on the surface. SEM micrographs with 1000x magnifications were obtained to observe the morphologies of all biomass samples.

### *Evaluation of the Biomass Samples as a Growth Substrate for Wild S. Commune Cultivation*

Four different types of lignocellulosic biomass wastes (rubberwood sawdust, mushroom spent medium, rice husk, and rice husk ash) was first screened as the main component to be used in the growth substrate mixture based on colonization time and yield of wild *S. commune.* All the biomass samples were first sun-dried and the common local formulation of 100:50:1 which corresponds to biomass: rice bran: $CaCO_3$ was used. Converting the ratio value into the mass of components in a 1 kg substrate mixture resulted in 662 g biomass, 331 g rice bran, and 7 g $CaCO_3$. The moisture content of the substrate mixture was adjusted in the range of 40-47%. The prepared substrates were placed in polypropylene (PP) bags with a dimension of 22.86 cm (length), x 33.5 cm (circumference) x 0.05 mm (thickness) at a packing density of 300-500 g of substrate per bag to standardize the height of the bag logs in the range of 110-119 cm. The bag log was first compressed by knocking up and down at a low altitude seven times. The wet weight of each of the bag logs for a particular experiment run was recorded. A set of the neck, cap, and net was attached to the mouth of the PP bags. The paper size of 2.5 cm x 2.5 cm was cut from an A4 paper (weight of 70 g) and placed on the cap before closing with a net. The total dry and wet weight of the substrate mixture in the bag logs was calculated and recorded. The bag logs were sterilized in an autoclave at 121 °C for 30 min and then cooled overnight to room temperature. For each of the sterile mushroom bags, approximately 5 g of matured wheat grain spawn was transferred in the aseptic condition in the laminar flow.

The inoculated bag logs were incubated at ambient in a dark and good ventilated room until fully colonized. The duration for the mushroom bag log to be fully colonized was recorded before 5 vertical slits were made on the mushroom bags from top to bottom using a small clean knife with a sharp tip. The mycelial growth rate (mm/ day) was calculated using the following equation [19]: -

$$Mycelial\ growth\ rate\ \left(\frac{mm}{day}\right) = \frac{height\ of\ colonized\ \ bag\ log\ (mm)}{incubation\ time\ (days)}$$

The mushroom bags with the net were replaced with a stopper and were placed on a recycled pellet in randomized order with an equidistance of 10 cm to the neighboring bags. To provide sufficient air ventilation, the wall of the mushroom house was made from a 90% sunshade polyethylene net. To maintain the desired humidity between 85 to 95%, the mushroom house (15 feet x 15 feet x 9 feet) was equipped with an electrical engine pump (1.0 hp) and 12 fine mist nozzles which were operated intermittently every 2.5 hours for 3 minutes.

On day 6th, the pump was switched off for 12 hours before the harvesting process and was on again after the harvesting process was completed. Each cluster of mushroom fruiting bodies from each slit was collected by cutting the stipe vertically using a sharp knife from above to the bottom part of the bags by leaving behind parts of the stipes. In the next three days, fresh fruiting bodies emerged on the same spot as the remaining stipes. The pump was once again switched off for twelve hours before the harvesting time. The fresh fruiting bodies of wild *S. commune* were harvested before the color turned to dark brown. The total yield of the first and second harvests from all types of biomasses was recorded. The biological efficiency (%) was examined as follows [20]:

$$Biological\ efficiency\ (\%) = \frac{Total\ fresh\ weight\ of\ mushroom}{Dry\ weight\ of\ substrate} \text{x } 100\%$$

## Results and Discussion

Table 1 summarizes the lignocellulosic (lignin, cellulose, and hemicellulose), carbon, and nitrogen contents in RSD, MSM, RH, and RHA. MSM contained the highest amount of ash (7.42±0.79 %) and extractives (7.37±0.94 %) compared to the rest of the biomasses. These results might be attributed to the remaining mycelium that is still strongly attached to the RSD matrix as a result of the biological process of mushroom cultivation. On the other hand, RSD contained the highest amount of lignin (29.28±0.78 %) followed by RH and MSM. The lowest amount of lignin in MSM might be due to the degradation of lignin during the growth of the mushroom. A similar observation was obtained by Hiyama et al. [21], where the lignin amount decreased from 24.3% to 17.8% after 85 days of shiitake mushroom cultivation using several kinds of wood as growth substrates. On the other hand, Irawati et al. [9] recorded a decrease in lignin, holocellulose, and cellulose content from 23.2 to 11.6 %, 81.1 to 62.7 %, and 44.3 to 31.7 % respectively, using 77 days-spent culture mediums of *Auricularia polytricha* cultivated in a growth mixture containing hardwood meal of *Alnus japonica*. In another study, the lignin, hemicellulose, and cellulose content in the spent culture medium of softwood (*Cryptomeria japonica*) gave a decrease from 32.9 to 22.9 %, 78.2 to 60.3 %, and 51.6 to 36.5 % correspondingly. The highest hemicellulose and cellulose content were obtained from MSM (39.73±1.93 %) and RH (37.82±0.82 %) respectively. The C: N ratio of RSD (365.35±33.05) was the largest followed by RH (167.55±15.07), MSM (40.33±0.76), and RHA (38.15±0.49).

**Table 1:** Lignocellulosic content, and C: N ratio of biomass samples

| Properties | Rubberwood sawdust | Mushroom spent substrate | Rice husk | Rice husk ash |
|---|---|---|---|---|
| Ash (%) | 1.96±0.48 | 7.42±0.79 | 2.05±0.18 | nil |
| Extractives (%) | 3.89±0.33 | 7.37±0.94 | 3.09±0.40 | nil |
| Lignin (%) | 29.28±0.78 | 18.27±1.34 | 25.94±0.82 | nil |
| Hemicellulose (%) | 29.97±1.57 | 39.73±1.93 | 31.09±1.78 | nil |
| Cellulose (%) | 34.90±2.18 | 27.21±2.34 | 37.82±0.82 | nil |
| C | 42.37±0.23 | 39.42±0.09 | 38.34±0.16 | 0.81±0.02 |
| N | 0.12±0.01 | 1.03±0.02 | 0.23±0.02 | 0.02±0.00 |
| C: N ratio | 365.35±33.05 | 38.15±0.49 | 167.55±15.07 | 40.33±0.76 |

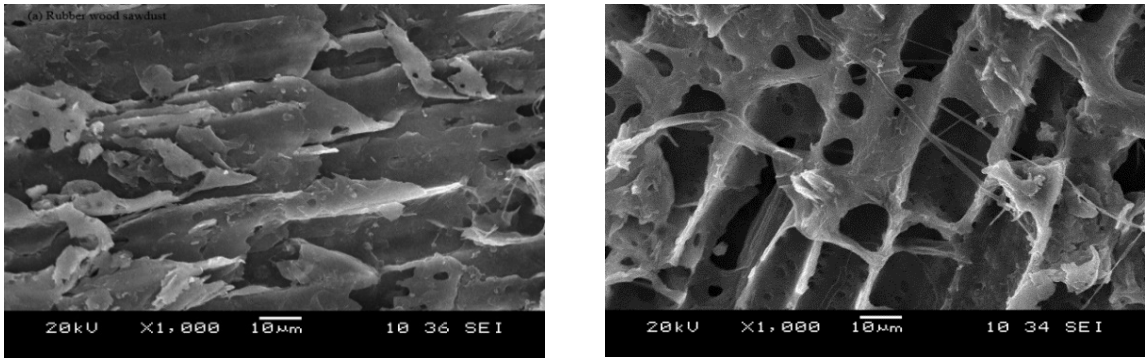
*nil= not included

The morphological changes of RSD, that were induced by biological pre-treatment during the period of cultivation of *S. commune* and RH, which was combusted in the rice mill, were examined by using a Scanning Electron Microscope coupled with Energy Dispersive X-ray (SEM-EDX) to obtain insight into the structural and chemical components modification of the raw materials. The original form of RSD and RH were also observed using the same method. The SEM images and several chemical components of RSD, MSM, RH, and RHA are shown in Figure 1. The SEM image of RSD showed an even and flat surface with broken structure and traces of small holes (Figure 1 (a)). The small holes might be originated from the previous furnishing and lumbering process of the rubber tree. On the other hand, a rugged, rough, and the severely destructed surface was observed from MSM (Figure 1 (b)). The fragments were disconnected from the initial structure and fully exposed. The external surface area and porosity of MSM was increased as a result of the loose structure of the fiber bundles.

A similar observation was reported by Taniguchi et al. [22]. They discovered a loosening structure of cells with a simultaneous increase in porosity of spent rice straw resulting from the cultivation of *P. ostreatus* for a total colonization time of 36 and 60 days. Such a phenomenon was due to the partial degradation of the lignin during the mycelial colonization process. Nevertheless, the morphological surface of RH (Figure 1 (c)) shows a rough and intact structure, whereas, for RHA (Figure 1 (d)), the structure was more porous and broken with increasing area of the external surface as resulted from the combustion process in the rice mill. Both processes of mushroom cultivation and combustion in rice mills caused an increase in internal porosity of MSM and RHA, respectively. As shown in Figure 2 (a-b), some chemical elements such as Mg, P, K, and Ca were increased, whereas C and O were decreased in percentage after the RSD was used as a growth medium for mushroom cultivation. In contrast to RSD and MSM, RH (Figure 2 (c)) and RHA (Figure 2 (d)) contained a substantial amount of Si. Vadiveloo et al. [5] mentioned that RH is rich in silica which is present in the outer epidermal cells. Its outer cell is thick, highly convoluted, and lignified.

(a) Rubberwood sawdust

(b)Mushroom spent medium




(c) Rice husk

(d)Rice husk ash

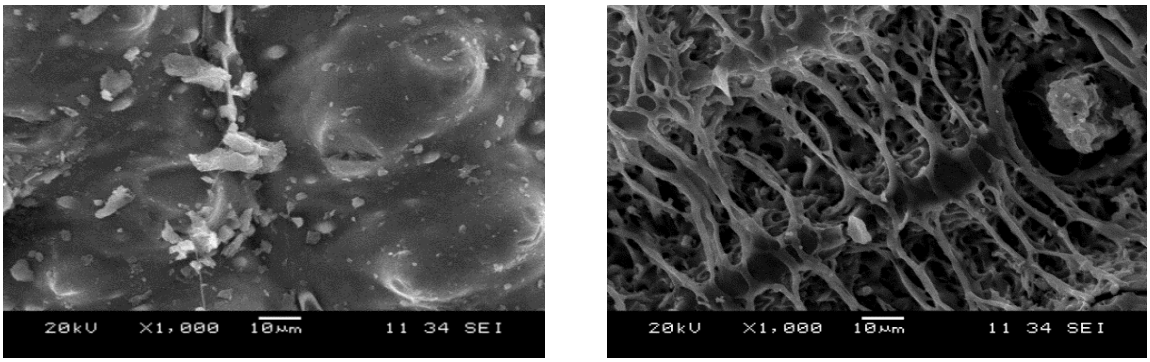
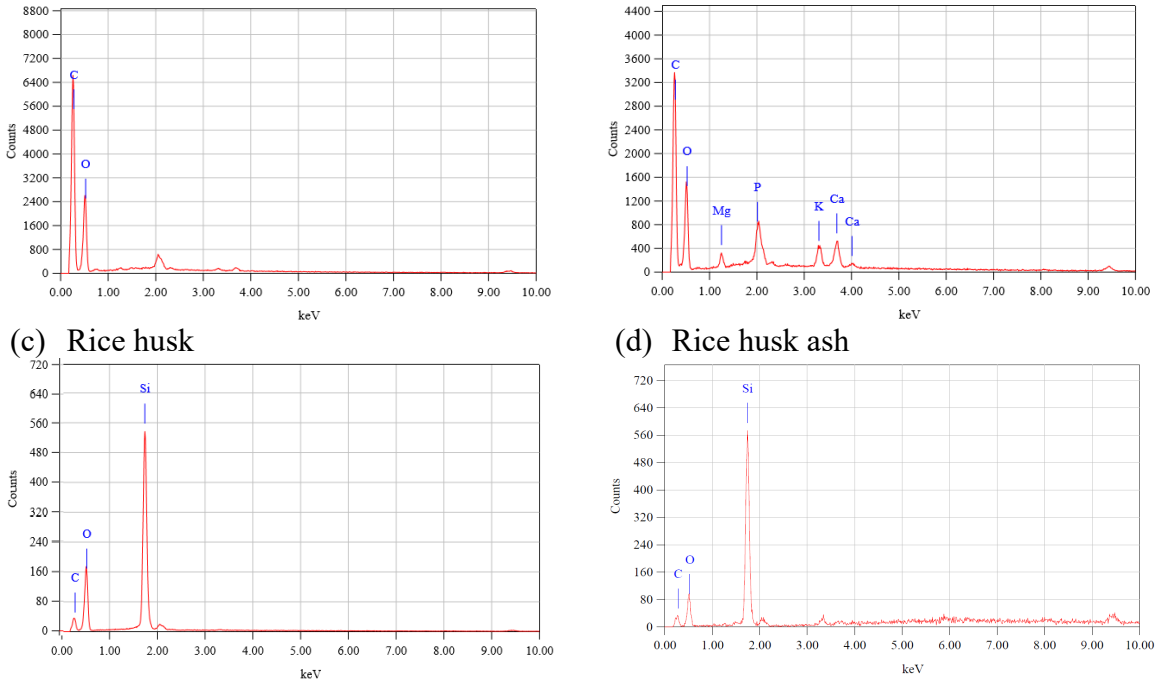



**Figure 1:** Image of morphology surfaces and element contents in (a) Rubberwood sawdust, (b) Mushroom spent substrate, (c) Rice husk, and (d) Rice husk ash**.**

(a) Rubberwood sawdust

(b) Mushroom spent medium




(c) Rice husk

(d) Rice husk ash

**Figure 2:** EDX spectrums of (a) Rubberwood sawdust, (b) Mushroom spent substrate, (c) Rice husk, and (d) Rice husk ash.

Table 2 shows the results of mycelial growth rate, colonization time, total yield, and biological efficiency of wild *S. commune* grown on RSD, MSM, RH, and RHA supplemented with rice bran and $CaCO_3$ in the ratio of 100:50:1 accordingly. In each of the growth substrates, 100% of each different lignocellulosic biomass was employed. The highest yield and biological efficiency of wild *S. commune* were obtained from RSD as growth substrate was 117.76±3.19 g and 29.07±1.02 % respectively, followed by MSM (72.88±3.43 g, 17.52±1.00 %), RHA (5.15±0.13 g, 1.71±0.13 %), and RH (3.10±0.13 g, 1.21±0.13 %). High C: N ratio in RSD and MSM with an appropriate amount of supplemented rice bran might be the reason for the high yield and biological efficiency obtained from these cultivated growth substrates. The different results of yield and biological efficiency among these four different biomasses could be also attributed to the variations in chemical composition that correspond to lignocellulosic content [23]. In addition, the low yield and biological efficiency obtained from RH and RHA might be due to the high content of silica that hinders the cleavage of the ligninolytic structure by the mushroom enzymes. Vadiveloo et al. [5] also mentioned that RH was not utilized in the mushroom industry as the main growth substrate due to its low protein and high lignocellulose contents. Although RH has been used experimentally as a substitute or an additive for sawdust used for the culture media of mushrooms globally, these attempts appear to be not successful to date [8].

A study by Manso et al. [24] for *P. ostreatus* cultivation showed that the mycelial growth rate (5.8 cm/ week) was higher and the spawn run period was shorter (20 days) than the control (sawdust of *T. scleroxylon K. Schum* (Wawa)) when 100 % of rice husk was utilized as the growth substrate. However, low yield and poor biological efficiency were obtained that were 23.3 g and 7.8 % respectively. In contrast, the highest yield of 226.1 g and biological efficiency of 75.3 % of *P. ostreatus* were obtained when only 2 % of rice husk was added to the growth substrate. Cultivating the similar aforementioned mushroom species, Thongklang & Luangharn [25] obtained the highest yield and the fastest mycelial growth rate when the growth substrate utilized was RSD and RH in the same ratio. A study by Postemsky et al. [26] proved that a high yield of *Ganoderma lucidum* (56 kg dry weight per ton) could be obtained by using rice straw and RH in the same proportion. On the other hand, the highest mycelial growth rate (7.71±0.04 mm/ day) and colonization time (15.00±0.08 days) were obtained from mushroom bag logs, that contained 100 % RH. These results were supported by a study by Lina et al. [27], where a rapid mycelial growth rate was obtained from the substrate mixture of orange peel, and RH either with (0.0753 km/ h) or without the addition of bran (0.0720 cm/ h). RHA mushroom bag log was fully colonized by the mycelium of wild *S. commune* in 16.90±0.05 days with a growth rate of 6.84±0.04 mm/ day.

**Table 2:** Mycelial growth rate, colonization time, total yield, and biological efficiency of rubberwood sawdust, mushroom spent medium, rice husk, and rice husk ash.

| | **Rubberwood sawdust** | **Mushroom spent medium** | **Rice husk** | **Rice husk ash** |
|---|---|---|---|---|
| Mycelial growth rate (mm/ day) | 5.50±0.06 | 5.51±0.04 | 7.71±0.04 | 6.84±0.04 |
| Colonization time (days) | 21.00±0.24 | 21.00±0.13 | 15.00±0.08 | 16.90±0.05 |
| Total yield (g) | 117.76±3.19 | 72.88±3.43 | 3.10±0.13 | 5.15±0.13 |
| Biological efficiency (%) | 29.07±1.02 | 17.52±1.00 | 1.21±0.13 | 1.71±0.13 |

The mycelial growth performance after 15 days of spawn inoculation and fruiting bodies formation of wild *S. commune* is shown in Figure 3. From the figure, the mycelial propagation in RH was the fastest followed by RHA. This phenomenon might be attributed to the high porosity within the RH and RHA particles in the bag log compared to the one in RSD and MSM, which were more compact. The smaller particles of rice bran might interlock within the RSD and MSM particles and further cause poor aeration and gas exchangeability in the bag log. These statements were also supported by Alam et al. [12], where the high level of rice bran had reduced the internal empty spaces and porosity and further resulted in poor gas exchange and degrading activity of *Calocybe indica*.


**Figure 3:** Wild S. commune mushroom bags on 15th days of incubation on different biomass waste (a) Rubberwood sawdust, (b) Mushroom spent substrate, (c) Rice husk, (d) Rice husk ash

## Conclusions

In conclusion, the most suitable biomass that can be used as a growth substrate for wild *S. commune* cultivation was RSD, followed by MSM. As obtained from the SEM-EDX analysis, the presence of a high amount of silica in RH and RHA was the main reason for the poor yield and very low biological conversion of the wild *S. commune*. Further optimization studies on using RSD as the main growth substrate for wild *S. commune* cultivation can be investigated to sustain the fresh mushroom supply and meet the high demand from local consumers.

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## Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

## Disclosure of Conflict of Interest

The authors have no disclosures to declare.

## Compliance with Ethical Standards

The work is compliant with ethical standards.

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