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Growth performance and mineral analysis of *Pleurotus ostreatus* from various agricultural wastes mixed with rubber tree sawdust in Malaysia

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ABSTRACT

This study aims to contrast the impacts of various agricultural wastes on the development, yield and mineral content of *P. ostreatus*. All substrates were added 5% by weight dry wheat bran and 1.5% by weight dry calcium carbonate and sterilized at 100 °C for 8 h using a sterilization chamber. The best substrate to obtain a greater total weight of fresh mushrooms and biological efficiency was 50% SB + 50% SR (207.87 g/bag; 78.44%) with mycelial growth at 26 ± 2 days. All of the agricultural wastes used in this study have abundant potassium (K) content as it is the mineral element needed for the mushroom growth. Therefore, the potassium (K) was the maximum macroelement in concentration as compared to other nutrients and all heavy metal content were not detected in the first fresh mushroom harvested. Therefore, SB seemed to be a better alternative material to grow *P. ostreatus*.

1. Introduction

Pleurotus is a fleshy fungus with a spore-bearing fruiting body belonging to the family *Pleurotaceae* (Mazidi et al., 2020). It is known as the oyster fungus because its fruiting bodies open in the shape of an oyster during its morphogenesis process (Patil, 2012). *Pleurotus ostreatus* is the second largest cultivated mushroom commercially across the globe, mainly in Asia, America as well as Europe due to cheaper production technology and simple, high biological efficiency, wide range of climate conditions, few environmental controls, shorter growing times, their fruiting bodies are rarely affected by pest and diseases compared to other edible mushroom (Bellettini et al., 2019). The market demand for mushrooms in Malaysia is gradually increasing because of the rise in human population and the impulse in the uses of mushrooms whether in medicine and culinary. It provides a variety of macronutrients and micronutrients for our health. The primary consumption of *Pleurotus* spp. was for either nutritional or dietary benefits such as high in fibres, amino acids, proteins, minerals (Ca, K, Na, Mg, P and Fe) and vitamins (folic acid, C, B-complex, riboflavin), and little in fats, cholesterols and sodium (Samsudin and Abdullah, 2019). In addition to the nutritional attributes of *Pleurotus* spp., beneficial health impacts such as antioxidants, antihypercholesterolemia, antivirals, antibacterials and antidiabetic renders it healthy diet (Roncero-Ramos and Delgado-Andrade, 2017).

Pleurotus spp. is also a white rot mushroom that has the greatest effective ligninolytic activity. The ability of these fungi to break down lignin differs considerably depending on the species and the growth medium (Janusz et al., 2017). Recently, lignocellulosic biomasses have gained increasing research interests and particular significance due to their renewable nature (Adebayo & Martinez-Carrera, 2015). *P. osteatus* species can grow on wide range of agro waste materials like empty fruit bunch and palm pressed fiber (Ali et al., 2013), oil palm fronds (Ali et al., 2018), sugarcane bagasse (Ahmad Zakil et al., 2020), rice straw (Utami and Susilawati, 2017) and corn cob (Rambey et al., 2019). Mycelia

Abbreviations: PVC, Polyvinyl Chloride; SR, Rubber Tree Sawdust; SB, Sugarcane Bagasse; EB, Empty Fruit Bunch; PF, Palm Press Fibre; OPF, Oil Palm Frond; CC, Corncob; B. E., Biological Efficiency; XRF, X-ray Fluorescence; ICPMS, Inductively Coupled Plasma Mass Spectrometry; Na, Sodium; K, Potassium; Mg, Magnesium; Ca, Calcium; P, Phosphorus; Fe, Iron; Mn, Manganese; Zn, Zinc; Cd, Cadmium; Pb, Lead; Cu, Copper; FAO, Food and Agriculture Organization; WHO, World Health Organization; CHNS Analysis, Carbon, Hydrogen, Nitrogen, Sulphur Analysis.

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growth and fruit body formation of fungi are strongly influenced by the type of agricultural waste used as a base substrate and the quality of the spawn. The growing medium provides the physical support and nutrients that is required to fulfill the life period of the fungi (Atila, 2017). For excellent growth of *Pleurotus ostreatus*, there must be carbon and nitrogen sources and other minerals in the substrate like S, Mg, Ca, K, P and certain lower levels minerals like Mn, Fe, Zn, Mo and Cu with ash content which is between 2.5 and 15.7 (Cueva et al., 2017). The *Pleurotus* species degrades complex lignin-cellulose-hemicellulose structure and later uptakes nutrients for its grows, and can grow in a wide variety of agricultural waste which consists mainly of polymers in the plant cell walls, like cellulose 40 to 60%, hemicellulose 15 to 35% and lignin 10 to 30%, being the important source of carbon as well as nitrogen (Cueva et al., 2017). It is possible to supplement the substrate, if required, with additional nitrogen sources, like oat bran, rice bran, wheat bran, millet, sorghum or copra cake (from spent coconut) for producing high quality fresh mushrooms (Masevhe et al., 2016). The acidity of the substrate can be neutralized by a few additives, for example, gypsum, limestone and chalk.

Malaysia currently produces 44% of oil palms and it can be considered the second largest producer as well as exporter of oil palms (Shevade and Loboda, 2019). The oil palm industry produces a bulky number of solid lignocellulosic biomass like oil palm fronds (OPF), oil palm trunks (OPT), palm pressed fibres (PF), empty fruit bunch (EB) and palm shells (OPS). Oil palm fronds make up 70% from the overall oil palm biomass produced, whereas the EB makes up 10% and OPT makes up only about 5% of the biomass (Khalil et al., 2012). In addition, only a small proportion of the oil palm biomass can be transformed into a higher value-added product, while a huge percentage remains underused. In 2014, 96,000 t of oil palm biomass were generated and this figure is projected to hike up annually (Hassan et al., 2019). The oil palm waste has led to a huge problem of disposal and environmental pollution. EB is the fruit bunch waste generated post the removal of fruits from the fresh fruit bunch (FFB) (Ahmad Yahaya et al., 2017), whereas PF is leftover of fibrous that is recovered from palm fruit during the extraction of palm oil accounting about 11% of the FFB (Riansa-ngawong and Prasertsan, 2011). The OPF is produced through harvesting, pruning and replanting (Ali et al., 2018). Since these palm oil industry biomasses contain cellulose, hemicellulose and lignin, they act as renewable raw materials suitable for bioconversion into value-added products as they are plentiful and readily available.

In addition to the solid waste from the palm oil industry, sugarcane bagasse (SB) is also available abundantly in rural and urban regions in Malaysia. SB is the fibrous by-product remaining after the extraction of the sucrose-rich juice from sugarcane stalks that is generated in large amounts from many industries. A Mt. of sugarcane can produce 280 kg of bagasse (Ameram et al., 2019). SB is usually employed for steam production in sugar plants as well as for alcohol distillation (Nunes et al., 2020). This bagasse can be an environmental pollutant if it is simply eliminated without treatment. To reduce the environmental burden, the usage of SB must be investigated.

Corncob (CC) is also another residue in Malaysia which are abundant wastes left after the removal of corn kernels and are easily available yearly. In 2012, corn production in Malaysia was 52,481 t, and the following year it increased by approximately 5% to 55,000 t (Shariff et al., 2016). Malaysia ranked 113 among 165 corn producing countries in 2013 (Factfish, 2015). Consequently, CC waste will increase every year and it will pollute the environment if it is not properly control. All these types of agricultural waste, particularly oil palm biomass, SB and CC in general, have a high hemicellulose, cellulose and lignin content which can be converted into products with greater value added instead of being eliminated, which can lead to global warming and the creation of green houses.

Currently, in Malaysia, *P. ostreatus* is commercially grown using SR as a base substrate. Nevertheless, a high need for SR causes to a surge in prices and has become a severe issue for mushroom growers specifically *P. ostreatus*. EB, PF, OPF, SB and CC have a high content of hemicellulose, cellulose as well as lignin for carbon source or carbohydrates in ensuring *P. ostreatus* grows well. Interestingly, these agricultural wastes can be easily found at a low cost in Malaysia. Hence, the EB, PF, OPF, SB and CC have great potentials to be used as an alternative substrates to cultivate *P. ostreatus* in the coming days and simultaneously solve the abundance of solid waste as well as problems that arise in the environment in Malaysia.

In this research, five lignocellulosic wastes were obtained locally; oil palm empty fruit bunch (EB), oil palm press fiber (PF), oil palm frond (OPF), sugarcane bagasse (SB) and corn cob (CC) were used to cultivate *P. ostreatus*. This study aims to evaluate the suitability of different types of lignocellulosic wastes that are available abundance in Malaysia in order to replace the rubber tree sawdust (SR) to cultivate oyster mushroom. The influence of these agricultural wastes on the fruit bodies' yield, mycelium development, biological efficiency (B.E.) and mineral content was evaluated.

2. Materials and methods

2.1. Description of the experimental site

This experiment was conducted in an open area near Block Y, under the canopy of 0.6 m × 0.6 m, at the Universiti Malaysia Pahang, Malaysia from the months of March–December 2019. The local climate was hot, humid and tropical with plenty of rainfall. The Universiti Malaysia Pahang campus is located at 3.5437° N, 103.4289° E. The mushroom house was placed under the canopy and 70% of it was covered with solar net to avoid excessive direct sun rays entering the mushroom house. This practice was similar to that used by local farmers.

2.2. Experimental design

Twenty-one experiments with three replications were conducted to achieve the desired objectives and were organized according to completely random design (CRD).

2.3. Source of experimental materials

2.3.1. Source of spawn

Pure cultures of *Pleurotus ostreatus* were obtained from the Department of Agriculture in Kuantan, Pahang, Malaysia.

2.3.2. Source of growth substrates

The PF and EB were collected from Kilang Sawit Panching, Gambang, Pahang, whereas the OPF was collected from the oil palm plant near Universiti Malaysia Pahang. The SB was collected at Local Growers in Kuantan, Pahang and the CC was collected weekly at Night Market, Gambang, Pahang, respectively. These wastes were collected in massive quantity and dried under the sun to remove water content. The samples were then ground by using an industrial grinder at the Laboratory of Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang and sieved to obtain samples within the 1–2 mm size. Calcium carbonate and wheat bran were bought from Pekan Mushroom, Pekan, Pahang.

2.4. Substrate preparation and substrate analysis

As shown in Table 1, five different substrate combinations with various biomass ratio were prepared with three duplicates for every medium formulation. The 100% SR medium was served as a control experiment. The substrates were mixed using the ratio 100:1.5:5 of agricultural wastes, calcium carbonate and wheat bran, respectively. The substrate mixture was made sure to remain moist to prevent the substrate from disintegrating by adding a little water that is 65% of the overall dry weight of the substrate mixture. Approximately 300 to 700 g

Table 1
Formulation of mushroom substrate by using different biomass ratio.

Material combination	Dry material	Supplement	
EB + SR	100% EB, 75% EB + 25% SR, 50% EB + 50% SR, 25% EB + 75% SR	Wheat bran (5% of dry weight used)	Limestone ($CaCO_3$) (1.5% of dry weight used)
PF + SR	100% PF, 75% PF + 25% SR, 50% PF + 50% SR, 25% PF + 75% SR		
OPF + SR	100% OPF, 75% OPF + 25% SR, 50% OPF + 50% SR, 25% OPF + 75% SR		
SB + SR	100% SB, 75% SB + 25% SR, 50% SB + 50% SR, 25% SB + 75% SR		
CC + SR	100% CC, 75% CC + 25% SR, 50% CC +50 % SR, 25% CC + 75% SR		

of each medium was kept in polyethylene bags at a fixed height of 15 cm and a volume of 1, 178 cm^3. The baglogs were later compressed and closed with PVC-necks, and afterwards covered using papers in preventing the entrance of insect and pack firmly. The bags were sampled for pH and moisture content measurement and C/N ratio. In order to obtain the C/N ratio of mushroom substrate used in this study, the CHNS analysis was by performed using CHNS Elemental Analyzer (Elementar, Japan) at the Central Laboratory, Universiti Malaysia Pahang (UMP). Bags filled with substrate were located on the vertical steel shelves and sterilized at 100 °C for 8 h in the sterilization unit. The baglogs were cooled overnight before being inoculated after sterilization process.

2.5. Substrate inoculation, incubation and fruiting

About 7–10 g spawn of the *Pleurotus ostreatus* mushroom was used to inoculate all the bagged substrates at the center of the substrate under a sterile environment after cooling. The *Pleurotus ostreatus* was grown in three replicates for each substrate mixture. To ensure proper distribution of the spawn, the bags were shaken after the spawn was added to the substrates. The inoculated substrates were incubated in the dark room vertically at a consistent temperature of 28 °C with relative humidity between from 80 to 85%. The substrates were moved to a fruiting room after the exterior of baglogs were completely covered with mycelium. The bags were rearranged horizontally and the bags' upper parts were unfolded in order to induce first cropping fruiting. A fine mist of tap water was dispersed on the bags in order to keep the 80–90% relative humidity twice a day using an automatic sprinkler. The fruit mushroom develop at the ambient temperatures. After 3 fruiting flushes, the experiment was terminated. The duration of mycelium growth and the total yield of fruiting bodies from three flushes were observed and recorded. The average yield in three flushes was employed in computing the biological efficiency (B.E.) at the end of harvest cycle.

2.6. Performance/productivity measurement

The measured criteria for the performance of *P. ostreatus* involved the number of days required to complete mycelial growth (days to colonize the substrate), fruiting time (initial of pin heads), fresh mushroom yield as well as biological efficiency (B.E.). The mycelium growth was determined for every 3 days of intervals. The mycelium growth was measured through the use of tape in centimeter until the day the bags are filled up with mycelium (linear length). The mushroom bags' caps were opened to enable the pinhead formation once mycelium has filled up the bags. However, some of the mushroom bags need to slit over the plastic bags in order to induce the pin head formation. It takes about 3 to 4 days for the pin head formation from the day the cap is opened. The fructification body of the oyster mushroom developed 2–3 days after the pin head was formed. The mushrooms were harvested by hand. After the harvesting process, the bags were closed using a cap about 7 to 10 day to allow the mycelium to accumulate the nutrient for a certain period for the subsequent fruiting cycle. The fruiting body formation indicates that the mushroom can be harvested. The weight of fruiting body was weighed using digital scale. Total average yield was computed as the total of average three flushes that are cultivated on various mediums. The computed outcome was expressed in grams per bag. The biological efficiency (B.E.) is the percentage conversion of dry substrates to fresh fruiting bodies, as illustrated in the formula as follows Eq. (1):

$$\text{B.E.}(\%) = \frac{\text{Fresh weight of mushroom per bag (g)}}{\text{Dry weight of substrate per bag (g)}} \times 100\% \quad (1)$$

2.7. Mineral element analysis of raw materials and fruit body analysis of fresh mushrooms

The elemental analysis of all raw materials used in this study such as SR, EB, PF, OPF, SB and CC were performed using the X-ray Fluorescence (XRF) (ZSX PRIMUS II, RIGAKU, United States) at the Centre Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang. The mineral content (Na, K, Mg and Ca) and heavy metal content (Cd, Pb, Cu) in fruit bodies that resulted from the first harvest of the cultivated mushroom were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) at the Central Laboratory, Universiti Malaysia Pahang.

3. Results and discussion

3.1. Effect of different biomass mixture ratio on the cultivation of *Pleurotus ostreatus*

3.1.1. Substrate analysis of different biomass mixture ratio

The chemical properties such as pH, moisture content, total N and total C contents, and C/N ratios are displayed in Table 2. All these parameters are extremely crucial factors for mycelium colonization and fruiting bodies development. The uppermost moisture content was found in 100% SR (control substrate) and the lowermost moisture content was found in 50% SB + 50% SR. In the present study, the moisture content in the control substrate was relatively higher as compared to other substrates. This might be due to the fact that the control substrate has a larger water retention capacity than other substrates. According to (Bellettini et al., 2019), moisture content is a very important factor that affects the cultivation of *P. ostreatus* mushrooms, as it affects the yield and production of mushroom. In previous studies, substrate moisture content has been adjusted from 50 to 75% (Chang and MIles, 2004). In this research, the moisture content of the substrate including the control substrate was approximately 46.33 to 62.54%. Furthermore, the pH of the substrate is also one of the important aspects that encourage the achievement of fungi to develop well. The pH values

Table 2
Chemical properties of different biomass mixture ratio.

Biomass mixture ratio	Moisture content (%)	pH	C (%)	N (%)	C/N ratio
100% SR (control)	62.54	6.27	17.71	0.14	126.5
100% EB	50.22	5.86	17.22	0.19	90.63
50% EB + 50% SR	48.66	5.74	15.89	0.2	79.45
100% PF	57.55	6.14	14.16	0.29	48.83
50% PF + 50% SR	48.56	5.95	15.93	0.21	75.86
100% SB	52.64	8.03	13.2	0.12	110.00
50% SB + 50% SR	46.33	6.75	14.26	0.14	101.86
100% CC	53.98	6.27	6.89	0.17	40.53
75% CC + 25% SR	52.14	6.15	13.09	0.32	40.91
50% CC + 50% SR	48.44	5.95	18.48	0.36	51.33
25% CC+ 75% SR	47.68	6.05	19.61	0.21	93.38

of substrates range from 5.74 to 8.03 in this study. These results are consistent with (Nwokoye et al., 2010) where the pH of the substrate must be 3 to 10 to enable mushrooms to develop in the tropics and therefore, mycelia could be tolerating the temperature of 28 °C. The total N and C content of the 50% CC + 50% SR was higher than those of the other substrates. In this study, the C/N ratio of the substrate formulation ranged from 40.56 to 126.5 and the maximum C/N ratio was obtained in the 100% (control medium). These ranges were in agreement with reports showing that the most appropriate C/N ratio for oyster mushroom production is between 32 and 150 (Cueva et al., 2017). The C/N ratio was dependent on the C and N sources. The level of N influenced reproductive time, primary initiation and biological efficiency (B.E.) (Bellettini et al., 2019). Consequently, the substrate types and formulation for the cultivation of *P. ostreatus* must have a balanced ratio of nitrogen and carbon to reach an ideal C/N ratio.

3.1.2. Mycelium colonization time, pin head formation time and first harvest time

Pleurotus ostreatus was grown on numerous biomass mixture ratio as substrates. *Pleurotus ostreatus* could degrade all lignocellulosic formulations either in its pure form or in combination. There was one replicate was contaminated by green mould (*Trichoderma harzianum*) and other bacteria during incubation, such as 100% PF, 75% PF + 25% SR, 50% PF + 50% SR and 50% CC + 50% SR. The growth performance of *Pleurotus ostreatus* either in pure biomass such as EB, PF, OPF, SB and CC or combined biomass with the SR was depicted in Fig. 1. The 100% SR was used as a control substrate in this study. Mycelium growth is the first fruit body formation stage. Mycelium functions to absorb nutrients, organic matter and water from the substrate in stimulating development and growth of *Pleurotus ostreatus*. The number of days required to complete the spawn run varied from 20 ± 7.07 days to 41 ± 4.00 days for 100% PF and 100% CC, respectively. The result for mycelium colonization in this study was shorter time than that reported by Fufa et al. (2021) where the mycelium colonization for their study takes 29–44 days for the cultivation of *Pleurotus ostreatus* using corncob and bamboo waste. This could be due to the height of all mushroom bags used in this study was kept at 15 cm. The spawning cycle for the 100% CC medium was at the slowest speed (41 ± 4.00) might be due to its low C/N ratio (40.53% from Table 2) and lower permeability to air in the substrate. Moreover, 100% OPF substrate used in this study takes the shortest time of 20 ± 7.07 days in completing the spawn running which might be due to the easy digestion and fast decomposition of PPF. Overall, in this research, the substrates containing SB either in sole or mixed with SR showed the consistent mycelium growth (24 ± 7.94–29 ± 1.73 days). This could be due to the excellent mixing between SR and SB which could help optimize the C/N ratio in substrates. On the other hand, SB additionally had a greater content of sugar that favoring the speedy development of mycelium compared to others which were not incorporated into SB. The fast growth of mushroom was also due to supplement addition (wheat bran mixed) (Oseni et al., 2012).

The pinheads are tiny bodies of mushroom fruit bigger than 0.01 mm. They develop after complete mycelial growth of the mushroom. The lowest growth period (27 ± 3.00 days) for pin head formation was obtained in 100% SR (control substrate) and the longer duration (129 ± 20.07 days) for primordial development recorded 100% EB. The 100% EB, 75% EB + 25% SR, 100% PF and 75% PF + 25% SR substrates require the mushroom bags to be slit over after long days in order to induce the pin head formation. Therefore, these substrates required long duration for primordial development. In this study, *Pleurotus ostreatus* have successfully grown by using 100% EB and 100% PF and these result showed some improvements in term of mycelium colonization, pin head formation and first harvest as compared to Ali et al. (2013) that failed to produce any fresh mushroom due to all the replicates used in their study showing a sign of contamination by bacteria during an incubation. Moreover, the particle sizes for 100% EB and 100% PF in this study were sieved at 1–2 mm size in order to get a uniform size and this practice may have had positive effects towards the production, as it lead to greater total surface area of particle. However, the porosity and aeration of the biomass used in this study increased with the addition of the SR thus promoting the delignation of the substrate. The duration of pin head formation in the *Pleurotus ostreatus* may vary depending on the substrates due to differences in nutrient availability of the mediums, relative humidity (RH) and the temperature harvesting room when the baglogs are transferred (Tesfay et al., 2020).

The final stage in the mushroom cultivation is the fruiting body formation. Days to fruiting body development took place earlier in 100% RS (control substrate) at 29 ± 3.00 days while 100% EB took an extended duration of 131 ± 20.07 days for fruiting body formation. Most of the mushroom bags took around 2 days from primordial formation to mushroom fruiting body maturation. After 2 days, mushrooms can be readily picked. According to Ahmad Zakil et al. (2019), all agro-industrial waste's fruiting body formation acquired between 2 and 4 days. The first harvest in this research is ranging from 29 ± 3.00 days to 131 ± 20.07 days which is contradicting to the outcomes of (Paudel and Dhakal, 2020) that ranged from 38 to 46 days for first harvest yield from oyster mushroom grown on finger millet husk, maize husks, rice straw, banana leaves and black gram pod shell. The different in time for formation of fruiting body could be due to their nutrient compositions and the type of a substrates (Tekeste et al., 2020).

3.1.3. Average yield for all flushes, total average yield and biological efficiency (B.E.) of Pleurotus ostreatus

The quantity of harvest differs greatly for different mediums in all of the three flushes as shown in Fig. 2. Some of replicates from 100% EB and 100% PF are capable to produce fruit body for first and second

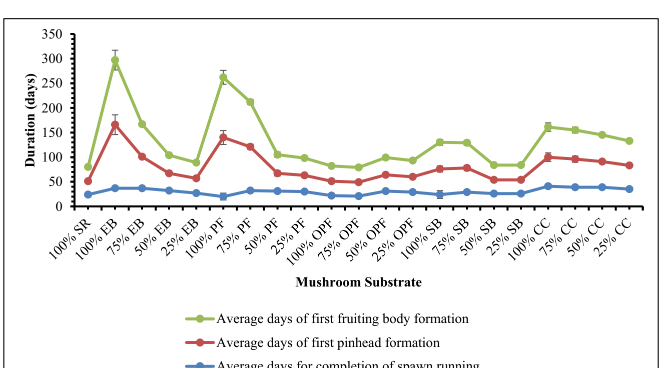

Fig. 1. Number of days taken to complete mycelium growth, number of days taken for pin head formation, and number of days taken for the first fruiting body formation.

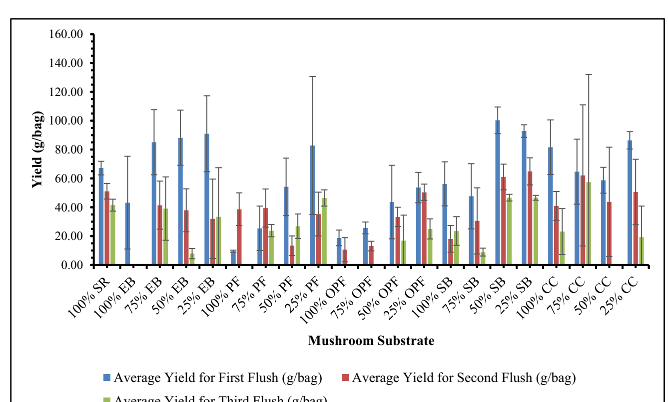

Fig. 2. Average yield for every flush (g/bag).

harvest after slitting over the mushroom bags and these might be due to the presence of oil and inability of the fungus to extract the cellulose entrapped in the fiber. From the first to the third harvest cycle, the yield has been on a downward trend except those from substrates 100% PF and 75% PF + 25% SR which had the highest yields from the second harvest. This downward trend of the yield over the flush might be because of reducing nutrient content of substrates that the mushrooms taken in throughout the development. In the first flush, the largest quantity harvest (100.30 ± 9.23 g/bag) was observed on 50% SB + 50% SR followed by 25% SB + 75% SR (92.83 ± 4.35 g/bag), 25% EB + 75% SR (90.97 ± 26.36 g/bag) and other mushroom substrate ratio as shown in Fig. 2. The lowest yield was obtained in 75% PF + 25% SR (25.33 ± 15.40 g/bag) and 100% PF (9.5 ± 0.71 g/bag). In the second flush, 25% SB + 75% SR (64.87 ± 9.41 g/bag) produced the highest yield followed by 75% CC + 25% SR (62.03 ± 48.98 g/bag) and 50% SB+ 50% SR (60.97 ± 8.91 g/bag). In the same flush, the smallest yield was obtained on 100% EB (0 g/bag) and 100% OPF (10.53 ± 8.41 g/bag). In the third flush, the greatest yield was obtained by 75% CC + 25% SR (57.40 ± 74.65 g/bag), 50% SB + 50% SR (46.60 ± 2.34 g/bag) and 25% SB + 75% SR (46.57 ± 1.69 g/bag), respectively. In the same flush, there are no yield produce from 100% EB, 100% PF, 100% OPF, 75% OPF + 25% SR and 50% CC + 50% SR. 100% EB, 100% PF100% OPF, 75% OPF + 25% SR and 50% CC + 50% SR cannot produce any yield for the third flush might be due to the inherent low nutrients' availability as well as low capacity of water holding. Overall, in terms of production, the biomass mixture ratio from SB either in its pure form or in combination with the SR could be considered as best substrates. Higher yield of mushroom in 50% SB + 50% SR and 25% SB + 75% SR is due to greater ease of nutrient solubilization in cellulosic substances. The total average yield of fresh oyster mushroom harvested using different biomass mixture ratio is shown in Fig. 3. The highest total average yield (207.96 g/bag) was obtained from substrate 50% SB + 50% SR, which is followed by 25% SB + 75% SR (204.27 g/bag), 75% CC + 25% SR (184.09 g/bag), 75% EB + 25% SR (165.53 g/bag), 25% PF + 75% SR (164.45 g/bag) and 100% SR (159.67 g/bag) while substrate 100% OPF resulted in the least amount of yield (29.22 g/bag). In this research, the substrate with higher C/N ratios caused in greater total average yield except for 75% CC + 25% SR substrate, which shows that low nitrogen content in the substrates can encourage the yield of *Pleurotus ostreatus*. Similarly, 50% SB + 50% SR substrate resulted in greater yield of *Pleurotus ostreatus* (Ahmad Zakil et al., 2020). The substrates' different nutrient composition may lead to different yield. Furthermore, a research show that the substrates C/N ratio employed for the cultivation impacted the *Pleurotus ostreatus* mushroom's yield performance (Osunde et al., 2019). Another factor that might affect the yield is the lignin content of a substrate. The lignin content in substrate may impact the organisms when lignin is employed as a source of energy. High lignin amount, could have resulted in the low yield of fresh mushroom as it cannot be degraded by the enzymes produced by *Pleurotus ostreatus*. Hoa et al. (2015) indicated that cellulose rich inorganic substance can be one of the best substrates to cultivate oyster mushrooms. Therefore, SB biomass mixture ratio can be one of the best substrates to replace the 100% SR. Moreover, substrates having high lignin as well as phenolic content reduced the cellulose activity, but lower lignin would increase enzymatic activity and therefore lead to greater production of mushroom and B.E. (Hoa et al., 2015).

Biological efficiencies (B.E.) which is conversion efficiency of substrate in mushroom cultivation, was calculated as the fresh mushroom harvested every bag to the dry weight of every substrate. Fig. 3 showed that biological efficiency was impacted by various substrates tested. The largest biological efficiency (B.E.) of 78.48% was obtained for 50% SB + 50% SR substrate followed by 75% CC + 25% SR (70.81%). Nevertheless, the lowest biological efficiency of 17.97% was observed for 100% EB substrate. The resulting values for biological efficiency depicted that biological efficiency is directly proportional to the total average yield. These observations agree with the findings from Jamil et al. (2019) where the biological efficiency (B.E.) values varied from 17 to 79%. Jin et al. (2018) reported that lignocellulosic materials are good to cultivate *Pleurotus ostreatus* species. The substrates biological efficiency (B.E.) may differ because of the differences in the substrates' characters. Furthermore, oyster mushroom's biological efficiency differs with varying composition of substrates (Islam et al., 2009). Low cellulonitic and ligninolytic activity of the substrates employed for mushroom production leads to varying biological efficiency of various substrates (Tekeste et al., 2020). As conclusion, in this study, the differences of yield as well as B.E. grown on various substrate were due to differing in physical as well as chemical composition of substrate formulas like mineral contents, cellulose/lignin ratio, moisture content, pH of substrate and particularly C/N ratio.

3.1.4. Composition of the mineral components of the raw materials and fruit body of the mushroom

Table 3 shows the macronutrients and micronutrients found in the SR, EB, PF, OPF, SB and CC samples. Calcium (Ca), potassium (K), Magnesium (Mg) and phosphorus (P) were the major mineral elements while sodium (Na), iron (Fe), zinc (Zn) and manganese (Mn) were the minor elements found in all of the raw materials employed to prepare the cultivation substrate. The presence of both macronutrients and micronutrients is very low which is less than 22,000 mg/kg as shown in Table 3. The amount of macronutrients present in the raw materials that was analyzed followed the order K > Ca > Mg > P while micronutrients followed the order Fe > Mn > Zn > Na. The mineral elements in these raw materials could support the growth of the mushroom considering that crucial elements such as potassium (K) has a key role to synthesize amino acids and proteins, whereas copper and manganese combination, also has a crucial role in enzymatic catalyzes in all biological and

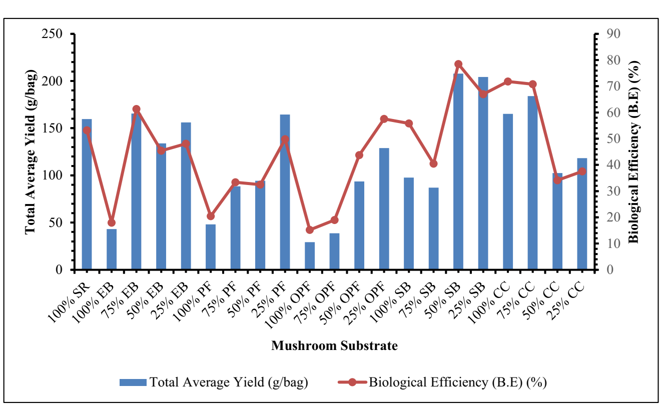

Fig. 3. Total average yield (g/bag) and biological efficiency (B.E.) (%).

Table 3
Macronutrients and micronutrients present in the SR, EB, PF, OPF, SB and CC fiber.

Raw material	Macronutrients				Micronutrients			
	Ca	Mg	P	K	Na	Fe	Zn	Mn
	(mg/kg)							
Rubber tree sawdust (SR)	3,350	904	367	2,760	ND[a]	134	17	68
Empty fruit bunch (EB)	2,020	692	584	16,100	ND[a]	901	20	28
Palm press fiber (PF)	3,320	1,140	101	21,800	67	767	26	53
Sugarcane bagasse (SB)	1,090	270	782	5,960	ND[a]	111	20	27
Corn cob (CC)	1,110	688	2,050	11,800	ND[a]	248	48	43

[a] ND: not detected.

physiological processes (Odunmbaku and Adenipekun, 2018).

Pleurotus ostreatus also has the ability to gather minerals in their substrate media. The highest amount of macroelements found in *Pleurotus ostreatus* were potassium (K), which is followed by sodium (Na), magnesium (Mg) and calcium (Ca) as presented in Table 4. Minerals are needed for metabolic responses, formation of rigid bone, water regulation and saline balance, sensory stimulation and other function (Srikram and Supapvanich, 2016). In the present research, potassium (K) accounted or the largest concentration of macroelements as compared to other nutrients, which is followed by Mg, Na and Ca for the all agricultural wastes that utilized in this study. The values reported here are comparable or lower than the values of Mg (1,271–1,754 mg/kg), K (19,496–20,388 mg/kg), Ca (391–821 mg/kg) and Na (615–1,093 mg/kg) for *Pleurotus* fruiting bodies investigated by Jin et al. (2018). The potassium content was highest in both substrates, consistent with the findings described by (Jin et al., 2018). High potassium (K) content in fruit bodies grown on 100% PF as compared to others substrate could be due to greater absorption and accumulation of K from substrates in the fresh mushroom. The ratio between potassium and sodium (K/Na) in vegetables as well as fruits is typically greater than 2 according to the report from Liu et al. (2016), while ratios for mushroom specimens that grown in this study ranged from 10.31 to 69.94. In terms of nutritional perspective, high ratios of K/Na found in *P. ostreatus* are perfect diets for patients with high blood pressure and heart disease (Alananbeh et al., 2014). The Na, Ca and Mg content were higher in fructiferous bodies than other micronutrients, which can be inferred that *Pleurotus ostreatus* in this study has a tendency to accumulate a higher amount of these minerals. Moreover, Ahmed et al. (2016) reported that the Mg plays an important role to regulate potassium fluxes and calcium metabolism. The results in this study were in agreement with (Alananbeh et al., 2014). Sardar et al. (2017) reported that these differences in the mineral contents of the fresh mushroom depend on the chemical composition and biological nature of the substrate employed for growing. The mineral compositions of *Pleurotus ostreatus* vary and this can be attributed to the accumulation as well as adsorption of these components from the substrate used in this study. Table 5 shows the mushroom heavy metals of *Pleurotus ostreatus* fruit bodies first harvested from 100% SR, 100% EB, 100% PF, 100% SB and 100% CC. Copper (Cu), lead (Pb), and cadmium (Cd), as the main toxic in foods, which may result in progressive toxicity. The allowable limit of heavy metals in vegetables and fruit suggested by the WHO/FAO such as Cu, Pb and Cd are 40, 0.3 and 0.2 mg/kg, respectively (Elbagermi et al., 2012). In this research, Cd, Pb and Cu were not found in any of the fructiferous bodies harvested from 100% SR, 100% EB, 100% PF, 100% SB and 100% CC. Therefore, the fungi examined here would not present a risk for the health of consumers.

3.2. Comparison to other studies and recommendations for future work

The shortage and high price of rubber tree sawdust (SR) to grow *Pleurotus ostreatus* particularly in Malaysia are the major concerns of the use of other abundant local agricultural waste. Most of the local agricultural waste available in Malaysia is sugarcane bagasse (SB), paddy straw, corn cob (C), kenaf fiber and palm products and majority of these wastes are hazardous to the environment. These available lignocellulosic biomasses can be considered the most promising alternative growing substrate and cost-effective for *Pleurotus ostreatus* mushroom production because they are most abundant renewable materials on earth (Chukwurah et al., 2012). Mohd Tabi et al. (2008) has shown the potential of empty fruit bunch (EB) and palm press fiber (PF) as viable substrates for mushroom growing either in pure form or combined substrate in particular for the cultivation of *Pleurotus ostreatus*.

There are some studies shown that the cultivation of mushroom is one of the easiest and convenient ways to reduce agricultural wastes and resulted in edible fresh mushrooms compared to using rubber tree sawdust (SR) (Ali et al., 2018; Hoa et al., 2015; Rambey et al., 2019). In this study, twenty-one different substrates made from five base substrates EB, PF, OPF, SB, and CC were compared for their performance in the cultivation *of Pleurotus ostreatus* for spawning and harvesting periods as well as for yield and biological efficiency (B.E.). The yield of cultivated mushroom was varied with different growing substrate. The SB base medium was excellent growing substrate either sole or in combination with SR compared to the others in term mycelium growth, pinhead formation, maturity of fruiting bodies, yield and biological efficiency (B.E.). The maximum yield of fresh mushroom weight basis for this study was obtained from 50% SB + 50% SR (207.87 g/bag) with 78.44% of B.E. and relatively lowest duration mycelial growth (26 ± 2 days) and maturity of first fruiting bodies (30 ± 2 days). The total colonization period and first harvest duration for the 50% SB + 50% SR mushroom substrate in this study was relatively faster compare to the finding from Hoa et al. (2015) that found the 50% SB + 50% SR mushroom substrate capable to colonize the growing substrate within around 32 days and first harvest period within around 43 days while the total weight of fresh mushroom and B.E. was lowered quantity which is only 181.29 g/bag and 58.94%, respectively. The outcome of this research is also consistent with Hasan et al. (2015) who found a mushroom substrate containing SB with 10% of wheat bran showed relatively higher on the growth, yield, B.E. and cost-benefit ratio for pink oyster mushroom (*Pleurotus djamor*). In addition, Iqbal et al. (2016) reported that *Pleurotus sajor caju* appeared earlier on SB followed by cotton waste, sunflower heads and chicken pea straw and this finding comply with this study where all SB base mushroom substrate showed relatively minimum period to first harvest of fresh mushroom. The increase in the yield of fresh mushroom in SB base substrate is might be due to easier way of obtaining sugars from the cellulosic materials (Sitaula et al., 2018). Consequently, 50% SB + 50% SR mushroom substrate in this study can be considered the best substrate to replace the current commercial substrate which is 100% SR.

Overall, the yield and biological efficiency (B.E.) were varied with different mushroom substrate may be due to some reasons. Firstly, the increasing spawn levels and nutrient available at higher rates would provide more energy for the growth of the mycelium, primordial formation, and time for the first harvesting of fresh *Pleurotus ostreatus*. Second, the wheat bran supplement in this study increases the ability to retain water and decreases mortality of young fruit bodies because of the shortage of water. This statement in agreement with the finding from Wang (2010) reported that 28–56% (w/w) of the shell of the cotton seed was added to the wheat substrate, significantly improving yield and B.E. by 15–18%. The differences in terms of yield and B.E. of oyster mushrooms grown on different substrates also were due to the differences in

Table 4
Mushroom macroelements of *Pleurotus ostreatus* fruit bodies first harvested from 100% SR, 100% EB, 100% PF, 100% SB and 100% CC.

Macroelements (mg/kg)	100% SR	100% EB	100% PF	100% SB	100% CC
Na	289.2	156.0	208.0	91.4	35.41
K	2,980.6	2,449.25	7,379.4	2,175	2,466.12
Mg	133.6	265.0	489.2	141.9	118.13
Ca	59.3	54.5	130.1	248.9	2.93
K/Na	10.31	15.7	35.48	23.80	69.64

Table 5
Mushroom heavy metals of *Pleurotus ostreatus* fruit bodies first harvested from 100% SR, 100% B, 100% PPF, 100% SB and 100% CC.

Heavy metals (mg/kg)	100% SR	100% EB	100% PF	100% SB	100% CC
Cd	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Pb	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Cu	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]

[a] ND: not detected (less than 0.1 mg/kg).

physical and chemical composition of substrate formulas such as cellulose/lignin ratio and mineral contents, pH, moisture content of substrate and especially C/N ratio (Narh Mensah et al., 2018; Yang et al., 2013). Muswati et al. (2021) also reported that mixing substrates can help optimize compositional characters, such as water retention capability, enhanced substrate structure, porosity that improves water penetration and gas exchange, and an optimal C/N ratio that improves substrate efficiency. By considering the popularity of this mushroom to the consumers, further studies can be carried out to thoroughly modify the environmental conditions which make the temperature and humidity suitable for better growth and development of *Pleurotus ostreatus*. Moreover, the sterilization of mushroom substrate can be considered using an autoclave in order to increase the current temperature used from 100 °C to 121 °C. Consequently, contamination of the mushroom substrate with green mould (*Trichoderma harzianum*) can be reduced in the future.

4. Conclusions

From the current study, it shows that SB biomass mixture either in sole or in combination with the SR was found to be the best substrates for oyster mushroom cultivation. 50% SB + 50% SR yielded a relatively greater yield as well as biological efficiency (B.E.) in shorter first harvest duration. Hence, 50% SB + 50% SR is suggested as the most suitable substrate for the oyster mushroom cultivation. Nevertheless, the other substrate combinations also have great potentials to be employed as an alternative substrate for mushroom cultivation in Malaysia.

CRediT authorship contribution statement

The corresponding author i.e. Fathie Ahamd Zakil was responsible for designing,conducting the experiment, written the original draft of the manuscript and also responsible for making the final revisions.

The second, third, fourth, fifth author i.e. Lim Hui Xuan, Norafira Zaman, Nurul Idayu Alan, Nurul Adila Aida Salahutheen were responsible for conducting the experiment and also helping in written the original draft of the manuscript.

The sixth and seventh author Mohd Shafiq Mohd Sueb and Ruzinah Isha were responsible for supervision and review and editing of the final original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2021.100873.

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