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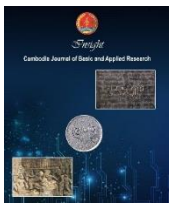

Recycling of spent Oyster mushroom (*Pleurotus ostreatus*) sawdust waste for the production of Straw mushroom (*Volvariella volvacea*)

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សង្ខិត្តន័យ

ការដាំដុះផ្សិតចំបើង *(Volvariella volvacea)* មានប្រវត្តិដ៏យូរលង់ណាស់មកហើយ និងថែមទាំងបានឆ្លងកាត់ការអភិវឌ្ឍជាហូរហែរៀងមក។ ទោះជាយ៉ាងណាក៏ដោយ ការស្រាវជ្រាវដែលកំពុងតែបន្តនេះគឺមានគោលបំណងប្រើប្រាស់កាកសំណល់កសិកម្មផ្សេងៗ ដើម្បីបង្កើននូវប្រសិទ្ធភាពលក្ខណៈជីវសាស្ត្រ។ នៅក្នុងការសិក្សានេះ យើងសង្កេតលើយុទ្ធសាស្ត្រប្រកបដោយនិរន្តរភាព ដែលជាការលើកស្ទួយការបំប្លែងទៅជាសេដ្ឋកិច្ចថ្មីមួយ ដោយការកែច្នៃកាកសំណល់ផ្សិតសឱ្យទៅជាមជ្ឈដ្ឋានមួយសម្រាប់បណ្តុះផ្សិតថ្មីទៀត។ ការស្រាវជ្រាវរបស់យើងបានផ្តល់នូវការយល់ដឹងអំពីការយកកាកសំណល់ផ្សិត(SMSW) នៃផ្សិត *Pleurotus ostreatus* មកប្រើសារជាថ្មីសម្រាប់ការបណ្តុះផ្សិត *V. volvacea* ។ យើងបានរកឃើញថា លក្ខខណ្ឌល្អបំផុតសម្រាប់ការបណ្តុះផ្សិត *V. volvacea* គឺនៅសីតុណ្ហភាព 35.0°C ក្នុងកម្រិតសំណើម 80-85% ជាមួយអត្រាលូតលាស់ជាមធ្យម 10.60 cm និងទំងន់សរុប 0.77 kg ជាមួយនឹងប្រសិទ្ធភាពជីវសាស្ត្រ BE ប្រហែល 15.47% ក្នុងរយៈពេល 15 ថ្ងៃនៃការដាំដុះ។ យើងក៏បានរកឃើញទៀតថា SMSW ជាកាកសំណល់ដ៏មានសក្តានុពលមួយសម្រាប់ជំនួយការដាំដុះ *V. volvacea* ។ របកគំហើញទាំងនេះអាចត្រូវបានអនុវត្តដើម្បីកែលម្អការបណ្តុះផ្សិត *V. volvacea* ផងនិងក្នុងការដាំដំណាំផ្សេងៗទៀតក្នុងវិស័យកសិកម្មផងដែរ។ តាមរយៈការអនុវត្តការគ្រប់គ្រងកាកសំណល់ប្រកបដោយភាពច្នៃប្រឌិត អត្ថបទនេះអាចរួមចំណែកក្នុងការធ្វើឱ្យមានកំណើនសេដ្ឋកិច្ច និងក្បាលវិស្ថានសម្រាប់មនុស្សជំនាន់ ក្រោយ។ ដូច្នេះ ការសិក្សានេះបានរួមចំណែកដល់កំណើនសេដ្ឋកិច្ចសង្គមយ៉ាងសំខាន់ និងគាំទ្រដល់គោលនយោបាយសេដ្ឋកិច្ចបៃតង ដោយពិភាក្សាអំពីការកែលម្អការប្រើប្រាស់ SMSW ។

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ABSTRACT

The cultivation of straw mushroom *Volvariella volvacea* has a long history and has undergone continuous development. Nevertheless, ongoing research aims to utilize various agricultural wastes to improve biological efficiency. In this study, we investigate a sustainable strategy that promotes the conversion to a circular economy by recycling spent mushroom substrate in new mushroom crops. Our research provides insight into reducing spent mushroom sawdust waste (SMSW) of *Pleurotus ostreatus* for application in *Volvariella volvacea* cultivation. We found that the optimal conditions for cultivating *V. volvacea* were at a temperature of 35.0℃ a humidity of 80-85%, resulting in an average growth rate of 10.60 cm and a total weight of fruit bodies of 0.77 kg, with a biological efficiency of approximately 15.47% within 15 days of cultivation. We discovered that the SMSW could be an alternative substrate for *V. volvacea* cultivation. Our findings can be applied to improve *V. volvacea* cultivation and practical applications in agriculture. By involving innovative waste management practices, this article can foster economic growth while preserving the environment for future generations. Therefore, this study contributes to significant socio-economic development and supports green economic policies by discussing the improvements from using SMSW.

1. Introduction

At this time, mushroom cultivation is carried out worldwide, utilizing the widest variety of technologies that are now available, ranging from the most basic to the most cutting-edge (Dhar, 2017). However, after mushroom cultivation, it generates spent mushroom sawdust waste (SMSW) (Ribas et al., 2009). SMSW refers to the residual material left over after the cultivation of mushrooms using sawdust as a substrate (Antunes et al., 2020). Sawdust is a typical substrate used in mushroom cultivation, mainly for oyster mushrooms. During the cultivation process, the sawdust substrate is inoculated with mushroom spores or mycelium and placed in a controlled environment where the mycelium grows and forms fruiting bodies (the mushrooms) (Hultberg et al., 2023). Once the mushrooms have been harvested, the remaining sawdust substrate is considered spent waste. SMSW could be a valuable resource for various applications rather than being discarded as waste, such as a substrate for growing other types of mushrooms, particularly species that are less demanding with regard to nutrient requirements. Using SMSW in new growing cycles has already been reported on (Cunha Zied et al., 2020). As it connects with transforming agricultural and agro-industrial waste into food of high nutritional value, using a circular economy method in producing edible mushrooms is highly appealing. It is an environmentally friendly choice (Pontes et al., 2018).

SMSWs have been utilized as substrates for the cultivation of mushrooms. SMSW has the potential to serve as an alternate substrate for the cultivation of various types of mushrooms. On the other hand, it is yet to be known whether or not the spent sawdust wastes produced by multiple kinds of mushrooms are suitable cultivation substrates for mushrooms (Wu et al., 2020). Mushroom cultivation is the leading solution for utilizing abundant agro-waste (Prasad et al., 2017). Many mushroom species, including *Agaricus*, *Pleurotus*, *Agrocybe*, and *Volvariella*, have been widely commercially produced using different types of agro-waste (Oseni et al., 2012). Straw mushroom (*V. volvacea*) is commonly grown using rice paddy straw as a substrate. There are about 50,000 known species of fungi, and about 10,000 are considered edible. However, only six mushrooms are widely preferred for large-scale cultivation. They are straw mushrooms (*Volvariella* spp.), Oyster mushroom (*Pleurotus* spp.), Button mushroom (*Agaricus* spp.), Milky mushroom (*Calocybe* spp.), Shitake mushroom (*Lentinulla* spp.), and Jew's ear mushroom (*Auricularia* spp.) (Borah et al., 2019). The edible straw mushroom, also known as *V. volvacea* is a nutrient-rich food source widely farmed commercially in many regions of Asia using agricultural wastes as growth substrates (Bao et al., 2013). An edible fungus that can be found growing in tropical and subtropical environments, this mushroom is also known as the straw mushroom or the Chinese mushroom. China is responsible for producing the vast majority of straw mushrooms obtained by artificial cultivation. In the 18th century, Buddhist monks at Nanhua Temple in Guangdong Province enhanced their food by devising a crude method that employed fermented rice straw as a growth substrate. This allowed them to cultivate mushrooms in their diet. Because of the high regard for the mushroom, it was frequently offered as a tribute to the royal families of China (Chang, 1977).

In addition, *V. volvacea* is a tropical fungus that must be exposed to temperatures between 28 and 35℃ to achieve vegetative growth and fruiting. Temperatures lower than 15℃ can cause cold damage to the fruiting body, which, in turn, has a detrimental impact on the viability of the fungal mycelia (Chang, 1978). The oyster mushroom, scientifically known as *P. ostreatus*, is a popular type of edible fungus. It was planted in Germany and is currently produced commercially worldwide to provide food. It grows on various cellulosic agricultural wastes (Borah et al., 2019). However, various substrates have also been used to cultivate this mushroom, including paddy straw, water hyacinth, oil palm bunch, pericarp waste, banana leaves, sawdust, cotton waste, and sugarcane bagasse (Reyes, 2000; Chang & Miles, 1989). *V. volvacea* has some familiar names, such as paddy straw mushroom, Chinese mushroom, or warm mushroom. This mushroom usually produces various lignocellulosic waste materials and has been cultivated with different methods depending on the practical situation (Yella et al., 2021). Culturing *V. volvacea* is easy, less complicated than other types of mushrooms, and economically potent. Before 1970, only paddy straw was used as a substrate for this mushroom cultivation. However, the introduction of ginning mill waste (cotton waste) as heating material in 1971 revolutionized a new method for paddy straw mushrooms. It was a suitable replacement for paddy straw and yielded more (30-40%). After the adoption of cotton waste, the cultivation of paddy straw mushrooms has become semi-industrialized in Hong Kong, Taiwan, Indonesia, Malaysia, China, India, Thailand, and Southeast Asia (Umor et al., 2020). Historically,

V. volvacea is grown using paddy straw as the primary substrate under natural environmental conditions. *V. volvacea* favors high cellulose and hemicellulose but low lignin substrates due to its capacity to secrete cellulolytic enzymes (Ahlawat & Kumar, 2005). A kind of edible mushroom known as *V. volvacea*, it is widely cultivated across East and Southeast Asia and utilized in various Asian foods (Umor et al., 2020). This mushroom was first produced in China in 1822, and production started in India in 1940 (Kaushik et al., 2018; Chang & Miles, 1989). It belongs to the Pluteaceae family and Basidiomycota Phylum, with high nutritional components and a unique taste.

In addition, *V. volvacea* is a vital source of healthy foods and medicines to regulate immune responses (Li et al., 2021). There are about 38,000 varieties, 300 mushroom species are edible, and pharmacological research is ongoing on its medicinal properties. The traditional Indian medical system extensively uses *V. volvacea*, linked to anti-tumor, immunosuppressive, and immunomodulatory properties. It is known as a rich source of protein, fibers (chitin), vitamins (a large amount of vitamin C, and also water-soluble vitamins like riboflavin, biotin, and thiamine), fats (5.7%), carbohydrates (56.8%), amino acids (essential amino acids like alanine, arginine, glycine, serine), unsaturated fatty acids, essential minerals (potassium, sodium, and phosphorus), and has low calorific value (Roy et al., 2014; Sukara, 1985; Zakhary et al., 1984). *V. volvacea* contains high content levels of minerals, protein (28.1%), polysaccharides (5.8%), fiber (20.7%), and carbohydrate, but little fat or cholesterol (Luu et al., 2022). *V. volvacea* is the sixth most popular mushroom due to its high nutritious value, unique aroma, texture, and delicious taste.

Moreover, it has been reported to be a fast-growing mushroom compared with most other cultivated mushrooms (Chang & Steinkraus, 1982). Although growing mushrooms as a nutritious food has been widely practiced, cultivating mushrooms produces a lot of waste. For every kilogram of mushrooms produced, about three kilograms of waste material containing straw or compost is left behind (Triyono et al., 2019). In Cambodia, we have faced a severe problem with SMSW (Ngoc & Schnitzer, 2009). This study looks at decreasing the environmental impacts of mushroom production, effectively treating spent sawdust wastes, and determining suitable spent sawdust substrates for new cultivation of *V. volvacea*. Therefore, the present study aimed to utilize SMSW productively for cultivating *V. volvacea*. The optimization conditions for the cultivation of *V. volvacea* were monitored. Moreover, the average growth rate analysis of *V. volvacea* and the assessment of fruiting bodies were reported.

2. Material and methods

2.1 Chemicals and spawn preparation

The *V. volvacea* was obtained from Cambodia Mushroom Farm, Phum Prek Lvea, Sangkat Chom Chao, Khan Po Sen Chey, Phnom Penh, Cambodia. It was grown on potato dextrose agar medium (PDA medium), which contained 200 g/l of potato (*Solanum tuberosum*), 20 g/l of glucose, and 15 g/l of agar. PDA media ingredients were purchased from ÆON Mall Phnom Penh. The basic procedure was to remove a piece stem of *V. volvacea* sterilely, place it on an agar plate, and incubate it at 25℃ in dark conditions. Sawdust spawn was prepared in polypropylene plastic bottles filled with 250 g of dry sawdust and supplemented with 10% rice bran and 1% calcium carbonate ($CaCO_3$). The mixture's water content was adjusted to approximately 65% and then sterilized at 121℃ for 25 min. The sterilized sawdust mixture in each bottle was inoculated with one square centimeter of mycelial agar discs. The spawn was incubated at 25℃ until the substrate was fully colonized. In this study, only technical-grade compounds were employed.

2.2 Preparation of substrate for cultivation

The spent mushroom sawdust waste (SMSW) was collected from Samnang Mushroom Farm, Phum Russey, No. 388, St. Lum, Sangkat Stueng Mean Chey, Khan Mean Chey, Phnom Penh, Cambodia. SMSW was ground and dried at 32℃ for 24 hours before substrate preparation, and it was utilized as the main ingredient in cultivating *V. volvacea*. To the SMSW were added glutinous rice flour 3%, $CaCO_3$ 3%, EM fertilizer 1%, and $NaNO_3$ 1%. The $CaCO_3$, EM fertilizer, and $NaNO_3$, which were mixed with water so that approximately 65% of the mixture was water content (**Table 1**). Then, each polyethylene bag was filled with 5 kg of substrate and sterilized at 121℃ for 30 min. After the substrates were cooled to 25℃, they were inoculated with 250 g of sawdust spawn per bag. The SMSW without nutrient supplementation (SMSW-N) and sawdust spawn inoculation (SMSW-SS) was used as the control cultivation. The cultivations were performed at 35℃ and 80% humidity. The average growth rate of mycelium (AGR) was investigated under optimum conditions for 21 days. The experiments were conducted in dark conditions. The growth length of *V. volvacea* determined the mycelium extension rates. The average growth rate of mycelium of *V. volvacea* was calculated using equation (**1**) (Belewu & Belewu, 2005).

$$(\text{AGR})(\text{cm}) = \frac{\text{Shortest growth length} + \text{Longest length}}{2} \quad (1)$$

Table 1: Supplemental ingredients for substrate preparation.

Supplemental ingredients (optimum ratio)	
Spent mushroom sawdust waste (SMSW)	100 kg
Glutinous rice flour	3.0 kg
Calcium carbonate ($CaCO_3$)	3.0 kg
Sodium nitrate ($NaNO_3$)	1.0 kg
EM fertilizer	1.0 litre
Moisture content	65.0%
pH	7.0

2.3 Analysis of the biological efficiency

The total fresh weight of fruit bodies (TWFB) produced by the *V. volvacea* was recorded. The TWFB was observed for each bag. The TWFB of all the fruit bodies of *V. volvacea* from each set of 3 replications was measured as the total yield of mushrooms. The assessment of the total weight of fruit bodies was performed for 21 days. The SMSW-N and SMSW-SS were used as the control cultivations. The cultivations were performed under optimum conditions. The biological efficiency (BE) calculated the effectiveness of *V. volvacea* and was used

to evaluate substrate conversion efficiency in *V. volvacea* cultivation. By definition, it was related to the weight of the harvest and the dry substrate. The BE analysis of the cultivation of *V. volvacea* was calculated using equation (2) (Silva et al., 2020).

$$BE = \frac{\text{Total weight of fruit bodies}}{\text{Total weight of the substrate (compost) of spawning}} \times 100 \quad (2)$$

2.4 Effects of cultivation condition

This study examined culture settings to optimize mycelial growth. The results of temperature and humidity on yield efficiency were assessed. All experiments were studied with an initial ingredient, as listed in Table 1. Yield efficiency as TWFB was weighed daily for 21 days and analyzed for yield efficiency. The cultivation was studied at five different temperatures (30, 33, 35, 37, and 40℃) under a humidity of 85% to evaluate the optimum temperature for yield efficiency. Moreover, the effects of the five different humidity (65, 75, 80, 85, and 90%) on TWFB under a temperature of 35℃ were also assessed. An incubation chamber (JEIOTECH TH-G-180, Korea) determined temperature and relative humidity. The SMSW-N and SMSW-SS were performed as the control experiments. The experiments were carried out in triplicate (n = 3). All results from these experiments were presented as means of triplicate samples ± standard error.

2.5 Statistical analysis

All statistical analyses were performed using R 4.2.2 (www.R-project.org) with the agricolae package. ANOVA and multiple comparisons (HSD test) were performed to assess the effects of SMSW, temperature and humidity, and growth period with its interactions on AGR, TWFB, and BE. A significance level of $p < 0.05$ was set for all analyses. Each parameter's tables and figures (with the interaction plot) were drawn using Microsoft Excel 2019.

3. Results and discussion

3.1 Effects of spent mushroom sawdust waste on average growth rate (mycelium) and biological efficiency

The average growth rate (mycelium) of SMSW-N and SMSW+N was observed for 21 days. Table 2 presents the average growth rate (AGR) of *V. volvacea* on SMSW-N and SMSW+N for 21 days. Fig.1 shows the different growth rates of SMSW-N and SMSW+N of *V. volvacea* observed for 21 days of the cultivation period. Growth was initially slow, but the mycelium grew exponentially after three days. The average mycelium growth rate on compost media was 0.53 cm/day (data not shown). Additionally, the radial growth of the mycelium was faster in the dark, which was linked to the previous study (Shrestha et al., 2006). Biological efficiency, often referred to as BE, is simply a way to calculate the effectiveness of a mushroom and substrate combination when growing mushrooms. The highest biological efficiency of *V. volvacea* was recorded at 15.47% in 15 days. According to the present experiment, it can be concluded that *V. volvacea* was the best in yield potential. Based on other earlier reports and investigations conducted by Indian researchers (15.21%), paddy straw was used as substrate (Biswas & Layak, 2014). In research in Vietnam (14.5%), spent oyster mushroom sawdust, rice straw, rice bran, and corn bran were used as substrate (Luu et al., 2022), and in Nigeria (5.21%), banana leaves were used as the substrate for cultivation (Belewu & Belewu, 2005). The correlation between BE and the TWFB of *V. volvacea* is elucidated in Fig.2. As a result, BE, and TWFB increased from the third day until the fifteenth day of cultivation. However, they were reported to decrease after fifteen days as the TWFB dropped. It was clear that the rate of BE is linked to the total weight of substrate (compost) of spawning and the production of *V. volvacea*. Table 3 demonstrates the TWFB and BE of *V. volvacea* in 21 days. Therefore, when the TWFB decreased, the BE was also reduced.

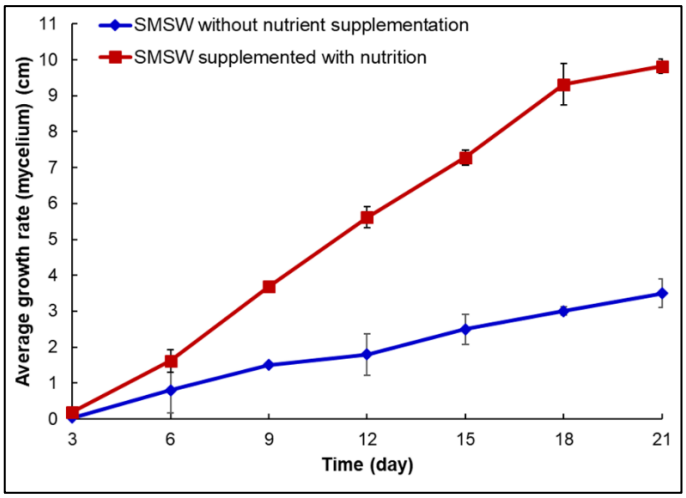

Fig. 1. The average growth rate of *V. volvacea* on SMSW-N and SMSW+N.

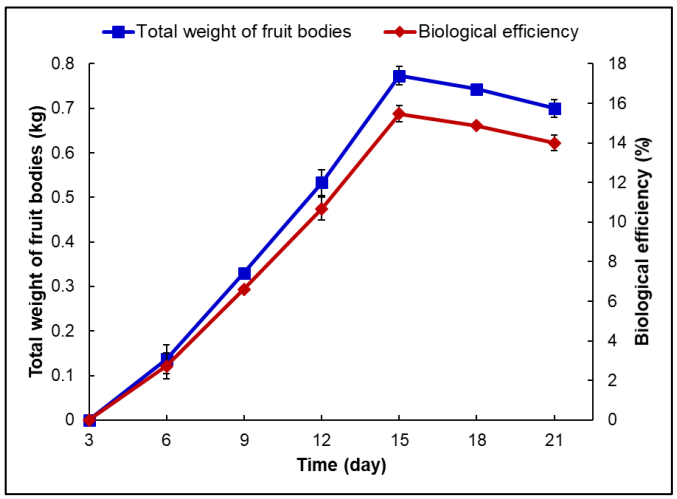

Fig. 2. Correlation between BE analysis and the TWFB.

The *V. volvacea* hydrolyzed substrate to produce fruit bodies in its cultivation. In this study, the yield of TWFB and BE corresponded with the findings of Thiribhuvanamala et al., 2012. The substrate component in *V. volvacea* compost bed is an essential subject of study for understanding how to increase yield. Previously, a lot of research was carried out to test the set of conditions for high-yield mushroom cultivation; however, it is not a newly significant figure out for the

efficiency of mushroom cultivation, whereas it compares the weight of the harvest to the dry weight of mushroom compost (Umor et al., 2020). Environmental conditions are crucial for creating a fruiting body in mushroom cultivation (Scrase & Elliott, 1998). However, every mushroom has a different behavior regarding the fructification stage, and there is no general set of conditions for all mushrooms. Substantial research is being conducted to test the conditions for high-yield mushroom cultivation; however, it only focuses on a specific species (Biswas & Layak, 2014; Oseni et al., 2012). As discussed, a common strategy to improve yield in *V. volvacea* cultivation supplements the compost bed with additional sources of C and N plus the micronutrient. This strategy effectively increases the BE activities while at the same time speeding up the formation of fruiting bodies (Hennicke et al., 2022; Biswas & Layak, 2014; Zied et al., 2011).

Table 2. The average growth rate (AGR) of *V. volvacea* on SMSW-N and SMSW+N for 21 days.

Time (Day)	AGR (cm)	
	SMSW-N	SMSW+N
1	0.00 ± 0.00	0.00 ± 0.00
2	0.01 ± 0.01	0.11 ± 0.01
3	0.04 ± 0.01	0.36 ± 0.01
4	0.05 ± 0.02	1.42 ± 0.06
5	0.70 ± 0.02	1.82 ± 0.06
6	0.80 ± 0.02	2.55 ± 0.00
7	1.20 ± 0.02	3.60 ± 0.09
8	1.30 ± 0.02	4.43 ± 0.03
9	1.51 ± 0.03	5.63 ± 0.12
10	1.62 ± 0.04	6.72 ± 0.06
11	1.75 ± 0.02	7.45 ± 0.09
12	1.82 ± 0.04	8.55 ± 0.00
13	1.84 ± 0.01	9.65 ± 0.09
14	2.05 ± 0.09	10.57 ± 0.14
15	2.51 ± 0.03	11.15 ± 0.09
16	2.71 ± 0.03	12.22 ± 0.06
17	2.92 ± 0.04	12.93 ± 0.03
18	3.05 ± 0.09	14.12 ± 0.06
19	3.22 ± 0.04	15.00 ± 0.00
20	3.42 ± 0.04	16.05 ± 0.09
21	3.52 ± 0.04	16.18 ± 0.12
SMSW	***	
Time	***	
SMSW x Time	***	

ns, *, **, *** meant not significant and significant at 5%, 1% and 0.1%. ± standard deviation.

3.2 Effects of temperature and humidity on yield efficiency

Temperature and humidity are essential aspects of the cultivation environment and were assessed in relation to their impacts on the yield efficiency of *V. volvacea*. **Table 4** represents the TWFB of *V. volvacea* on SMSW-N and SMSW+N

Table 3. TWFB and BE of *V. volvacea* in 21 days.

Time (Day)	TWFB (kg)	BE
1	0.00 ± 0.00	0.00 ± 0.00
2	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00
4	0.00 ± 0.00	0.00 ± 0.00
5	0.07 ± 0.06	1.33 ± 1.15
6	0.14 ± 0.03	2.73 ± 0.64
7	0.23 ± 0.03	4.60 ± 0.53
8	0.32 ± 0.02	6.33 ± 0.31
9	0.33 ± 0.00	6.60 ± 0.00
10	0.35 ± 0.01	7.00 ± 0.20
11	0.43 ± 0.03	8.67 ± 0.58
12	0.53 ± 0.03	10.67 ± 0.58
13	0.55 ± 0.00	11.00 ± 0.00
14	0.66 ± 0.01	13.20 ± 0.20
15	0.77 ± 0.02	15.47 ± 0.42
16	0.77 ± 0.02	15.33 ± 0.31
17	0.76 ± 0.02	15.20 ± 0.35
18	0.74 ± 0.01	14.87 ± 0.12
19	0.72 ± 0.02	14.47 ± 0.42
20	0.70 ± 0.02	14.07 ± 0.42
21	0.70 ± 0.02	14.00 ± 0.40
Time	***	***

ns, *, **, *** meant not significant and significant at 5%, 1% and 0.1%. ± standard deviation.

under five different temperature conditions in 21 days. In the 15 days of culture, the 35℃ temperature produced the maximum production efficiency, with an approximate TWFB of 0.77 kg; this decreased to 0.73 kg at 33℃ and then to 0.66 kg at 37℃ (**Fig.3**). Likewise, the yield efficiency of *V. volvacea* dropped to 0.56 kg at 30℃ and 0.54 kg at 40℃. In contrast, at the optimum temperature of 35℃, the SMSW-N was recorded at 0.15 kg, and at approximately 0.11 kg at 30 and 37℃. Additionally, there is no TWFB efficiency from SMSW-SS. The SMSW+N reportedly had five times higher yield efficiency than the SMSW-N. The pattern of the temperature effect was comparable throughout the cultivation period. It has been reported that 35℃ is more favorable for the optimum temperature, amenable to maximum mycelial growth and chlamydospore production of *V. volvacea* (Sakinah et al., 2019; Kumar et al., 2016). The optimal temperature impairs the lignocellulolytic enzymes produced by *V. volvacea* (Chang & Steinkraus, 1982). In addition, the cultivation humidity profoundly influenced the yield efficiency of *V. volvacea*. The optimum humidity for yield efficiency was 80%, with

Table 4: TWFB of *V. volvacea* on SMSW-N and SMSW+N under five different temperature conditions for 21 days.

Time (Day)	TWFB (kg)									
	30°C		33°C		35°C		37°C		40°C	
	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.02	0.10±0.10	0.00±0.01	0.01±0.02	0.00±0.00	0.00±0.00
4	0.01±0.01	0.02±0.01	0.01±0.01	0.04±0.02	0.01±0.00	0.04±0.01	0.01±0.00	0.04±0.01	0.01±0.00	0.05±0.00
5	0.01±0.00	0.06±0.00	0.01±0.01	0.06±0.00	0.01±0.00	0.07±0.00	0.01±0.01	0.07±0.02	0.01±0.00	0.06±0.01
6	0.02±0.00	0.12±0.02	0.03±0.01	0.13±0.02	0.02±0.01	0.15±0.01	0.03±0.00	0.17±0.02	0.02±0.00	0.11±0.01
7	0.03±0.00	0.15±0.01	0.05±0.01	0.18±0.04	0.04±0.00	0.24±0.01	0.04±0.00	0.22±0.03	0.04±0.00	0.19±0.01
8	0.03±0.00	0.16±0.00	0.06±0.01	0.21±0.09	0.05±0.00	0.31±0.01	0.04±0.01	0.25±0.03	0.06±0.00	0.29±0.01
9	0.05±0.00	0.22±0.02	0.06±0.01	0.25±0.06	0.05±0.00	0.33±0.01	0.04±0.01	0.26±0.04	0.06±0.00	0.30±0.00
10	0.07±0.01	0.32±0.01	0.07±0.01	0.33±0.02	0.06±0.00	0.36±0.01	0.06±0.01	0.34±0.04	0.06±0.00	0.31±0.00
11	0.07±0.00	0.33±0.00	0.08±0.01	0.36±0.05	0.07±0.01	0.42±0.02	0.07±0.01	0.37±0.04	0.08±0.00	0.41±0.00
12	0.07±0.00	0.35±0.01	0.10±0.01	0.41±0.10	0.09±0.00	0.54±0.01	0.08±0.01	0.41±0.06	0.09±0.00	0.43±0.00
13	0.09±0.00	0.42±0.02	0.11±0.01	0.46±0.07	0.09±0.00	0.55±0.01	0.09±0.01	0.50±0.05	0.09±0.00	0.45±0.00
14	0.11±0.00	0.52±0.02	0.13±0.01	0.56±0.07	0.11±0.00	0.67±0.01	0.10±0.01	0.55±0.05	0.09±0.00	0.45±0.00
15	0.12±0.01	0.55±0.02	0.14±0.01	0.60±0.09	0.12±0.01	0.76±0.01	0.11±0.00	0.61±0.02	0.11±0.00	0.53±0.00
16	0.11±0.00	0.55±0.00	0.15±0.01	0.61±0.10	0.12±0.01	0.77±0.01	0.12±0.00	0.65±0.01	0.11±0.00	0.53±0.00
17	0.11±0.00	0.55±0.00	0.14±0.01	0.61±0.10	0.13±0.01	0.77±0.01	0.12±0.00	0.68±0.01	0.11±0.00	0.53±0.00
18	0.11±0.00	0.55±0.01	0.14±0.01	0.6±0.10	0.12±0.00	0.75±0.01	0.12±0.00	0.66±0.01	0.10±0.00	0.52±0.00
19	0.11±0.00	0.54±0.01	0.14±0.01	0.59±0.09	0.12±0.00	0.73±0.01	0.11±0.01	0.64±0.01	0.10±0.00	0.52±0.00
20	0.11±0.00	0.53±0.00	0.14±0.01	0.58±0.09	0.12±0.00	0.72±0.01	0.11±0.00	0.62±0.02	0.10±0.00	0.50±0.01
21	0.11±0.00	0.53±0.00	0.13±0.01	0.57±0.08	0.12±0.01	0.71±0.01	0.11±0.00	0.60±0.01	0.10±0.00	0.49±0.01
SMSW					***					
Temperature					***					
Time					***					
SMSW x Temperature					***					
SMSW x Time					***					
Temperature x Time					***					
SMSW x Temperature x Time					***					

ns, *, **, *** meant not significant and significant at 5%, 1% and 0.1%. ± standard deviation.

approximately 0.77 kg TWFB by day 15 of cultivation. **Table 5** confirmed the TWFB of *V. volvacea* on SMSW-N and SMSW+N

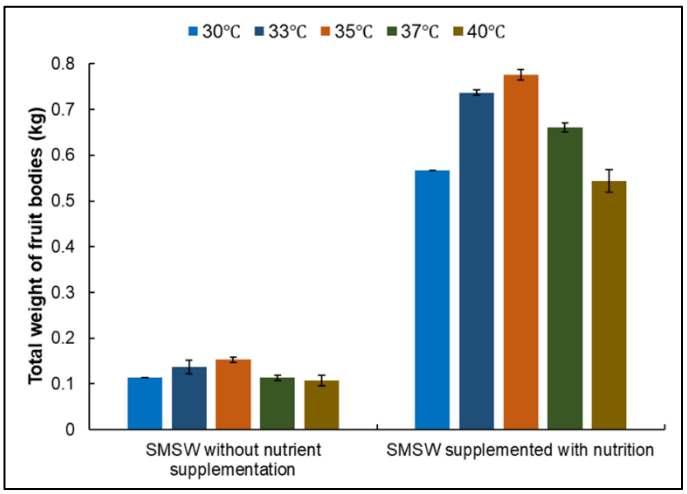

Fig. 3. Effects of temperature on yield efficiency in 15 days.

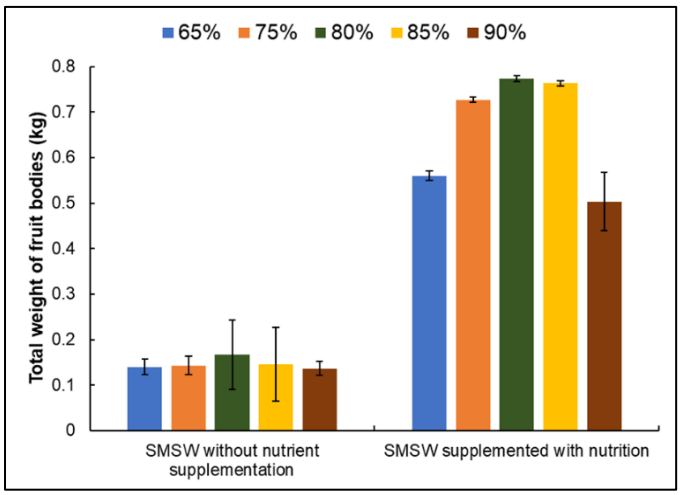

Fig. 4. Effects of humidity on yield efficiency at day 15.

under five different humidity conditions in 21 days. The yield efficiency of 0.56 kg and 0.50 kg were attained at 65% and 90%, respectively (**Fig.4**). Also, the SMSW-N was noted at 0.16, 0.14, and 0.13 kg at humidity levels of 80, 85, and 90%, respectively.

The *V. volvacea* can be cultivated in various temperatures ranging from 33 to 35°C and with a relative humidity of 80-85% (Thiribhuvanamala et al., 2021; Yen, 1992). Relative humidity is necessary to maintain moisture levels and prevent the fruiting bodies from drying out. It is also related to the activity changes of enzymes involved in the respiration and deterioration of *V. volvacea* (Li et al., 2017). In general, when a mushroom substrate is employed, *V. volvacea*'s productivity is attributed to the hydrolytic enzyme production potential of the substrate used, substrate preparation method, and growing conditions (Ahlawat & Tewari, 2007). Environmental factors related to the temperature and humidity of the growth bed must be suitable for growing the mushrooms, as changes in these factors may contribute to adverse effects. Both factors directly affect the yield in a closed-door system or in growing outdoors. Using outdoor cultivation systems is cheaper and easier but with some limitations, as this system produces low yields in subsequent growing seasons due to high contamination (Umor et al., 2020). Moreover, supplementation with various organic additives upgraded the yield of *Volvariella* spp. and formed fruiting bodies within 10-12 days of spawning when analogized to non-supplemented crops (Nannapaneni & Subbiah, 2016). Over the years, many attempts have been made to improve *V. volvacea* cultivation using different

Table 5: TWFB of *V. volvacea* on SMSW-N and SMSW+N under five different humidity conditions for 21 days.

Time (Day)	TWFB (kg)									
	65%		75%		80%		85%		90%	
	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N	SMSW-N	SMSW+N
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.02	0.11±0.12	0.01±0.01	0.02±0.01
4	0.01±0.00	0.03±0.00	0.01±0.01	0.04±0.02	0.01±0.01	0.03±0.05	0.01±0.00	0.05±0.00	0.01±0.00	0.05±0.00
5	0.01±0.01	0.06±0.00	0.01±0.01	0.08±0.01	0.02±0.00	0.11±0.01	0.01±0.00	0.07±0.00	0.01±0.00	0.05±0.00
6	0.03±0.01	0.12±0.03	0.03±0.01	0.14±0.02	0.03±0.00	0.15±0.01	0.03±0.00	0.15±0.01	0.03±0.00	0.13±0.01
7	0.10±0.04	0.41±0.18	0.03±0.00	0.16±0.01	0.04±0.01	0.24±0.02	0.04±0.01	0.24±0.01	0.06±0.01	0.23±0.03
8	0.05±0.00	0.22±0.02	0.03±0.01	0.18±0.02	0.06±0.01	0.30±0.01	0.06±0.00	0.33±0.01	0.08±0.01	0.35±0.05
9	0.05±0.00	0.23±0.01	0.04±0.01	0.25±0.02	0.06±0.00	0.33±0.00	0.06±0.00	0.34±0.01	0.09±0.02	0.37±0.06
10	0.07±0.01	0.30±0.03	0.06±0.00	0.33±0.02	0.07±0.00	0.35±0.00	0.06±0.00	0.35±0.00	0.09±0.02	0.38±0.07
11	0.09±0.01	0.37±0.03	0.07±0.01	0.40±0.01	0.08±0.01	0.42±0.03	0.08±0.00	0.44±0.01	0.10±0.02	0.41±0.08
12	0.09±0.01	0.41±0.02	0.09±0.01	0.48±0.04	0.09±0.01	0.51±0.03	0.10±0.00	0.53±0.01	0.10±0.02	0.43±0.08
13	0.10±0.01	0.44±0.01	0.10±0.00	0.54±0.01	0.10±0.00	0.54±0.01	0.10±0.00	0.55±0.01	0.11±0.02	0.48±0.08
14	0.10±0.01	0.45±0.00	0.12±0.00	0.65±0.01	0.12±0.01	0.63±0.03	0.12±0.01	0.67±0.01	0.13±0.01	0.55±0.03
15	0.13±0.00	0.56±0.01	0.13±0.00	0.72±0.01	0.14±0.01	0.75±0.06	0.14±0.00	0.77±0.00	0.13±0.01	0.56±0.01
16	0.13±0.00	0.57±0.00	0.13±0.01	0.73±0.01	0.14±0.00	0.77±0.01	0.14±0.00	0.76±0.00	0.13±0.01	0.55±0.01
17	0.13±0.00	0.56±0.00	0.13±0.01	0.73±0.01	0.14±0.00	0.77±0.00	0.14±0.00	0.76±0.00	0.13±0.00	0.55±0.00
18	0.13±0.01	0.55±0.01	0.13±0.00	0.72±0.01	0.14±0.00	0.76±0.01	0.14±0.00	0.75±0.01	0.13±0.00	0.55±0.00
19	0.12±0.00	0.53±0.01	0.13±0.00	0.71±0.01	0.14±0.00	0.74±0.00	0.13±0.00	0.72±0.00	0.13±0.00	0.55±0.00
20	0.12±0.00	0.52±0.02	0.13±0.01	0.70±0.01	0.13±0.01	0.72±0.01	0.13±0.00	0.71±0.01	0.13±0.00	0.53±0.00
21	0.12±0.01	0.51±0.01	0.12±0.01	0.68±0.01	0.13±0.00	0.72±0.00	0.13±0.00	0.71±0.01	0.13±0.00	0.53±0.00
SMSW					***					
Humidity					***					
Time					***					
SMSW x Humidity					*					
SMSW x Time					***					
Humidity x time					ns					
SMSW x Humidity x time					ns					

ns, *, **, *** meant not significant and significant at 5%, 1% and 0.1%. ± standard deviation.

approaches. As discussed, a common strategy to improve yield in *V. volvacea* cultivation is supplementing the compost with additional sources of C and N plus the micronutrient. This strategy effectively increases the enzymatic activities while at the same time accelerating the forming of fruiting bodies. Many published studies describe the role of organic additives in *V. volvacea* mushroom compost (Thiribhuvanamala, 2012).

4. Conclusion

Recycling spent *P. ostreatus* sawdust waste for the production of *V. volvacea* is an eco-friendly and sustainable agricultural practice. It's important to note that cultivating *V. volvacea* requires careful attention to environmental conditions. Recycling spent oyster mushroom substrate for straw mushroom cultivation is a sustainable practice that reduces waste and maximizes the use of available resources. However, it's crucial to maintain proper sanitation, monitor environmental conditions, and address any contamination issues to ensure the success of each cultivation cycle. The recycling of SMSW into nutritious mushrooms is suggested as an excellent alternative with potentially significant ecological and economic benefits to the regions where sawdust waste is produced. The SMSW supplemented with additional ingredients is an appropriate medium for cultivating *V. volvacea*. The mycelia started to cover the SMSW in about three days, while complete substrate colonization was observed in 21 days. The TWFB was 0.77 kg. The BE analysis was recorded at 15.47%. Although cultivating *V. volvacea* is easy and requires less technology, the current practices still fail to produce a high yield. Research on *V. volvacea* is considered inadequate to answer the issues related to low yields. Following this, a deeper understanding of the mechanisms of enzymatic activities could overcome the barriers to efficient bioconversion of the cellulolytic substrate to fruiting bodies of *V. volvacea*. Another possible solution is to design a suitable compost comprised of the primary substrate supplemented with organic compounds or nutrients.

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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. All authors have read and approved the final, published version of the manuscript.

Credit authorship contribution statement

CHEM Chanchao: Conceptualization, experimental design, data interpretation, visualization, drafting, reviewing, and editing. Y Phoura: Data interpretation, visualization, reviewing, and editing. NHIM Sreyneang: Data interpretation, reviewing, and editing. CHHEANG Lita: Reviewing and editing. OURN Eneang: Reviewing and editing. UK On Norong: Supervision, funding acquisition, reviewing and editing. SREY Chansorphea: Experimental design, Supervision, funding acquisition, reviewing and editing.

All authors have read and agreed to the published version of the manuscript.

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