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Effective microorganism pre-treatment on oil palm empty fruit bunch fibre for cultivation of *Volvariella volvacea* (Bull.) Singer

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Abstract

Pre-treatment with effective microorganisms (EM) for the cultivation of *Volvariella volvacea* using oil palm empty fruit bunch (EFB) was proposed to increase yield. The effect of different EM doses on the mycelium growth and yield was observed. The treatment was carried out using a combination of two parameters: composting times (5 days (T1), 10 days (T2) and 15 days (T3) and dosages of EM (0% (E1), 10% (E2), 20% (E3) and 30% (E4). While the composition of EFB was analysed to compare the changes before and after the pre-treatments. It was determined that EM pre-treatments of 20% and 30% resulted in significantly faster mycelial growth compared to the other treatments. The highest yield of *V. volvacea* was observed at T2E4 (10d, 30% EM) with 271.5±57.28 g or biological efficiency (B.E) of 9.11%. The highest average weight per fruiting body (FB) was obtained at T1E3 (5d, 20% EM) with 14 g, while T2E4 (10d, 30% EM) yielded the highest number of harvested FB with 42. Cellulose, hemicellulose and lignin were reduced in all treatments tested. Both EM dosages and composting times significantly affected the yield of *V. volvacea*. EFB fibre was a potential substrate for the cultivation of *V. volvacea*.

1. Introduction

The oil palm empty fruit bunch (EFB) is classified as a lignocellulosic compound, which contains cellulose and hemicellulose as well as polysaccharide and lignin in its cell wall. Every year, more than 20 million tons are produced from palm oil processing in Malaysia, most of which are not efficiently utilized (Onoja, 2018). In the last decade, several research and development activities have been carried out to utilizate this biomass in various fields, including agriculture, furniture and energy production (Huzir *et al.,* 2018). The use of EFB either as a sole substrate or as a co-substrate for the cultivation of *Pleurotus* sp., *Volvariella volvacea*, *Auricularia polytricha,* and *Flammulina velutipes* has been recorded previously (Ahlawat and Tewari, 2007; Ali *et al.,* 2013; Lau *et al.,* 2014; Harith *et al*., 2014). The cultivation of *Volvariella volvacea* in Malaysia is gaining attention due to its unique taste and higher price (Azhar *et al*., 2018). This lowland species grows in a temperature range of 28-32°C, which prefers substrates rich in cellulose but poor in lignin and produces extracellular cellulolytic enzymes for bioconversion of cellulosic constituents fruiting bodies (Chang, 1996). Paddy straw, water hyacinth, EFB, oil palm pericarp, banana leaves, sawdust, cotton waste and sugarcane bagasse have been reported to be suitable for growing this fungus with cycles lasting about 4-6 weeks (Belewu and Belewu, 2005; Chang and Hayes, 2013). While the use of EFB fibres for the cultivation of this mushroom in Malaysia is scarce. EFB fibres were obtained by screw pressing, drying and milling to reduce water content and substrate size (Yii *et al.,* 2014).

In Malaysia, *V. volvacea* has been grown outdoors using whole EFB in protected environments or intercropping in plantations (Azhar *et al.,* 2018). The limitations of this cultivation method include low yield and high labor requirement, making it less popular compared to the cultivation of oyster mushrooms, which accounts for 90% of the market in Malaysia (Rosmiza *et*

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al., 2016; Umor *et al*., 2020). Its low biological efficiency compared to other commonly cultivated mushrooms is due to the lack of a ligninol or lignin conversion system, which limits the species ability to grow and bear fruit in a woody substrate (Ahlawat and Tewari, 2007; Bao *et al*., 2013). A common technique to improve substrate quality was composting, usually done before spawning (Rajapakse, 2011). Local growers composted EFB for cultivation of *V. volvacea* by adding a small amount of calcium carbonate ($CaCO_3$) and irrigating with tap water at certain intervals before wrapping them in a polyethylene tarp for 10 days (Azhar *et al.,* 2018). The composting process aims to improve the degradation of the substrate, reduce the substrate's lignin components, and increase the availability of nutrients (Philippoussis *et al*., 2001). Different strategies of composting may provide a better solution to increase the availability of nutrients for fungal growth. For example, the propagation of specialized microorganisms instead of indigenous ones could increase the degradability of the substrate.

Effective microbes (EM), a commercial product containing groups of beneficial microorganisms such as lactic acid bacteria, yeasts, photosynthetic bacteria and actinomycetes, and fungi, have been widely used in agriculture as soil conditioners, fertilizers and plant enhancers (EMRO, 2018). It is expected the application of EM will accelerate the degradation of the cellulosic substrate and convert it into polysaccharides that can support the development of fruiting bodies. This study was conducted to investigate the effect of EM pre-treatment of EFB fibre to the mycelial growth and yield of the *V. volvacea.* The EFB fibres are used as the main substrate for *V. volvacea* instead of whole bundles due to their lighter weight and volume, which is advantageous in handling.

2. Materials and methods

2.1 Preparation empty fruit bunch fibre and Volvariella volvacea spawn

The substrate used in the present study was EFB fibre supplied by the Malaysian Palm Oil Board (MPOB) research facility in Bangi, Selangor. The substrate was screw-pressed, dried and ground. This process reduced the water content and substrate particle size. The EFB was locally produced from an oil palm mill in Klang, Selangor. The physical characteristic of the substrates is presented in Table 1. The spawn of Volvariella volvacea is acquitted locally from the local producer and at suitable age for propagating.

2.2 Effect of different doses of effective microorganism pre-treatment

The effective microorganism used in this study was a commercial product of EMRO (Effective Microorganism Research Organization) of Japan and purchased from the EMRO office at Johor Bahru, Malaysia. The EM solution is activated by adding 1 part of EM to 1 part of molasses and 18 parts of dechlorinated water prior to 14 days incubation. The activated solution is diluted 500 times prior to use (EMRO, 2018). Forty kg of substrate was weighed and divided into four piles of ten kg. All substrate is added with 5% (w/w) $CaCO_3$, 6% (v/w) organic fertilizer and 7% (w/w) rice bran (Triyono *et al.,* 2018). The experiment is conducted as follows; treatment 1 (T1) as control, treatment 2 (T2)- EM added 10% (v/w), treatment 3 (T3)- EM added 20% (v/w), treatment 4 (T4)- EM added 30% (v/w).

2.3 Mycelial run test

About 5 g of the substrate was filled into a 25 mL test tube. Then 0.5 g of spawn grains were added to the substrate before being incubated at 35℃. Growth of mycelium was observed and measured from day 3 onward. The length of mycelia (cm) was measured vertically every 3 days until the substrate was completely permeated with mycelium (Hasan *et al.,* 2010).

2.4 Volvariella volvacea cultivation

After 10 days of composting, about 3 kg of the substrate was added to the basket, measuring 45 cm × 30 cm × 15 cm (L × W × H). The substrate was mixed with 150-180 g of shredded mushroom spawn. The mushroom bed was then brought into the mushroom house and randomly distributed on six shelves. Then the basket was covered with a plastic cover and incubated on the shelf for 10 days at room temperature (28-32^{o}C). On the 10th day, the plastic cover was opened and the shelf was covered with a dark plastic sheet to wait for fruiting. Every day, water mist was applied to the compost and the bottom of the mushroom house was watered to provide adequate moisture. The fruits were harvested daily at egg stage and the weight was recorded.

2.5 Effect of different composting time and doses of effective microorganism pre-treatment

Ninety kg of the substrate was weighed and divided equally into four closed containers. In every container 5% $CaCO^3$, 7% chicken manure and 8% rice bran were added on a weight basis (Triyono *et al.,* 2018). Chicken manure and rice bran were added as sources of nutrient

Table 1. Characteristic of EFB substrates.

Samples	Moisture (%)	Length (L) (mm)	Diameter (D) (mm)	Bulk density (kg.m^{-3})
EFB fibre (S2)	4.21±0.4	30±2.4	1±0.0	0.1150±0.1

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and nitrogen component while $CaCO_3$ was supplemented to stabilize the pH changes during composting. pH was dropped during composting due to acid and ammonia accumulation by adding $CaCO_3$, and the pH can be maintained at a range of 6-8, which was suitable for the growth of this mushroom (Kumar *et al.,* 2017). This would produce about 25 kg of compost in every container. The two treatment parameter were tested in combination as below; composting time : T1(5 d) T2 (10d) T3 (15d) and dosage of EM : E1 (0%) E2 (10%) E3 (20%) E4(30%).

The dosage of EM was determined by diluting the activated solution according to the designed concentration of EM v/v (10%, 20%, 30%). Then the solution was added initially during the composting process. In all EM pre-treatment, the composting process was initiated within 5 days before being prolonged to 10 and 15 days. Temperature changes was recorded throughout the composting process. On day 5, every container was opened and compost was removed and filled in three loose baskets with 45 cm × 30 cm × 15 cm (L × W × H). . Every full basket can attain approximately 2.7-2.8 kg of compost in dry weight. These samples were labelled as T1. This step was repeated for day 10 (T2) and day 15 (T3). Similar steps for mushroom cultivation were then repeated. All experiments were done in triplicate with a total of 36 units of the experiment. The setup of the experiment is shown in Figure 1.

2.6 Compositional analysis of empty fruit bunch fibre

Samples of EFB fibre, others from different pre-treat ment after composting and post harvest, were subjected to compositional analysis to determine cellulose, hemicellulose and lignin changes. The determination procedures followed the modified Chesson-Datta method by Isroi (2017). Figure 2 shows the simplified procedure for the compositional analysis.

2.7 Statistical analysis

Cultivation experimental work and compositional analysis are carried out in triplicate. Data for these

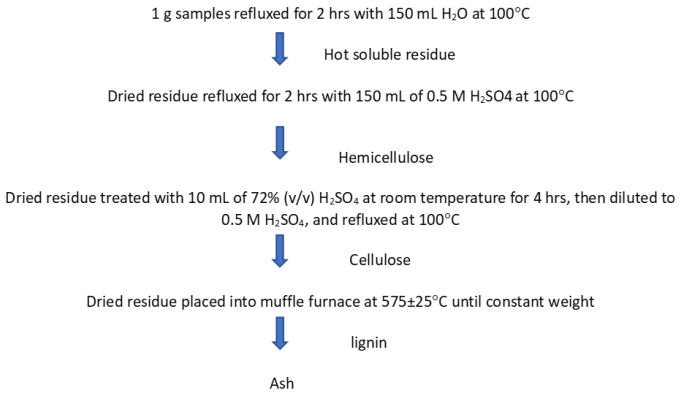

Figure 2. Overall steps for fractionation of EFB composition (Isroi, 2017)

experiments are analyzed using IBM SPSS Statistic 26. Means comparison with LSD and Tukey`s significant test at a level of $\alpha = 0.05$ was performed to determine the significant difference between the treatments.

3. Results and discussion

3.1 Effect of different dose of effective microogranism pre-treatment

Four treatments (T1-T4) of EFB with different concentrations of EM were applied for 10 days of composting, with T1 serving as a control. Temperature changes occurred at the beginning of composting, with the maximum temperature of 50°C measured on day 4. However, towards the end of the 10 days, the temperature levelled off at 30-33°C. Normally, the compost temperature increases at the beginning of composting and within 48 hrs and gradually decreases as the composting phase subsides (Wan Razali *et al*., 2012). The final temperature measured on day 10 was 33°C. A similar trend in temperature changes during composting was observed in all treatments, where the temperature peaks at the early stage of composting and low afterwards. However, the rise in temperature, which peaked at 50ºC, was lower than the common range of 52-65°C (Onwosi *et al.,* 2017). This might be due to the low amount of substrate used in the composting process. In this study, only 25 kg of EFB fibre was used and composted in a small closed container. Thus, requires a longer time to reach a higher temperature as the energy

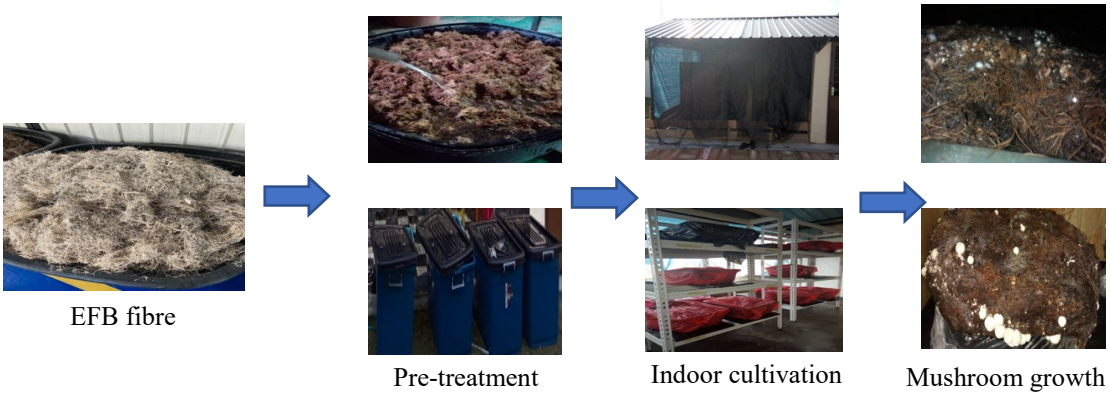

Figure 1. Setup of experiment.

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Table 2. Mushroom yield in different treatment.

Treatment	Mycelium growth (cm) after 12 days	DFPF	DFFH (day)	No. FB	Yield (g)	Biological Efficiency (%)
T1	5.00±0.02^{bc}	15.33±1.154	17.33±1.154	12.33	282.0±142.85	9.14
T2	4.67±0.13^{c}	12.77±0.404	14.77±0.404	11.00	201.3±70.54	6.71
T3	5.33±0.166^{ab}	10.3 ±0.5773	12.33±0.577	19.33	304.3±145.78	10.14
T4	5.53±0.12^{a}	14.0±1.732	16.0±1.732	13.00	187.7±90.0	6.21

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). DFPF: Day for pinhead forming, DFFH: Day for first harvest.

balance is affected by a large surface-to-volume ratio. Heat loss is relatively higher in small-scale composter than in large-scale (Sundberg *et al.,* 2013). In addition, this study did not add any insulating material to the composter to eliminate heat loss.

The result of the experiment is summarized in Table 2. In the first experiment, it was found that the treatment of EM significantly affected the growth of the mycelium of this fungus but not the fruiting bodies. This study produces a similar trend to a previous report by Sopit (2004) for mycelia growth of *Pleurotus ostreatus*. Sopit (2004) suggested that the contribution of EM to the mycelia growth of *Pleurotus* sp. is due to the ability of the latter to limit the competitors' growth by creating an acidic environment. All substrates reached their full growth after day 15. It can be seen that the higher dosage (T3 and T4) of EM treatment significantly improved the growth of mycelium in the composted substrate ($p=0.0014$) compared to the other treatments. Sopit (2004) suggested that the contribution of EM to the mycelial growth of *Pleurotus* sp. is due to its ability to limit the growth of competitors by creating an acidic environment for mycelial growth. However, the development of mycelium into fruiting bodies of *Volvariella volvacea* required completely different conditions (Biswas *et al.,* 2014). Some important factors affected fruiting including quality of spawn and growing environment such as light conditions and air exposure (Sakinah *et al*., 2019). On the contrary, another research by Mapanao *et al.* (2016) on the effect of EM on the growth of *Pleurotus florida* concluded that the EM application did not improve both mycelium growth and yield when compared to the non-treated substrate.

Pinhead began appearing in T3 on day 10.3±0.5773 as the fastest-growing bed, while T4 recorded a four-day delay. In comparison, DFFH shows a similar trend following DFPF. The fruiting stage may be affected if a suitable environment cannot be achieved. It was observed that fruiting was delayed in the different treatment types, with the first fruiting bodies appearing after days 12 to 17. This observation could be due to the environmental factor affecting the formation of fruiting bodies. In this study, the conditions in the mushroom house were controlled manually. For example, the humidity was maintained by spraying the mushroom bed and area daily while temperature was not regulated. The recorded temperature in the mushroom house was between 28-32°C. All these constraints may be the cause of the inconsistent onset of fruiting of the fungus. In general, there are no significant differences between the treatments and the mushroom yield, as shown by the statistical analysis. However, it was found that T3 gave the highest yield with biological efficiency (B.E.) of 10.14%, followed by T1; 9.4% and others. This result is better in terms of biological efficiency when whole EFB are used as a substrate, producing only 6-7% as previously reported (Azhar *et al.,* 2018; Triyono *et al.,* 2019). The use of EFB fibres for the cultivation of *Volvariell*a *volvacea* holds some potential that requires further investigation on treatment methods to achieve high yield other than EM

3.2 Temperature profile during composting

The aim of composting was to produce a selective medium for a certain mushroom to grow and treat substrate from contaminating microorganisms that may curb the growth of mycelium (Belewu and Bababola, 2009). Table 3 depicts the temperature of different treatments during composting, which produce a similar trend as in experiment 1. Changes in the temperature of compost piles are common trends in composting process. Extending the composting time contribute to the lower temperature in the compost. Temperature build-up

Table 3. Temperature profile during composting.

Sample / Day	Temperature (°C)				
	Day 1	Day 4	Day 7	Day 10	Day 15
T1E1	33	50	-	-	-
T1E2	32	52	-	-	-
T1E3	33	51	-	-	-
T1E4	32	49.5	-	-	-
T2E1	32	50	41	36	-
T2E2	33	52	45	35	-
T2E3	32	51	43	34.5	-
T2E4	32	53	44	35.5	-
T3E1	32	48	39	36	35
T3E2	32	49.6	38	37	34
T3E3	33	50.5	41	33.6	33.6
T3E4	32	51	40	33.8	33.8

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during composting is expected and important in the elimination of pathogenic organisms in waste (Onwosi *et al.,* 2017).

3.3 Effect of different composting time and doses of EM pre-treatment

The results of the cultivation of *V. volvacea* are summarized in Table 4. In the second experiment, both EM pre-treatment and composting time significantly affected mushroom yield and substrate composition. Pre-treatment of EM 30% with 10 days of composting of the substrate (T2E4) produced highest yield with 9.11% biological efficiency. The fungus started the fruitification phase on the 12th day after spawning and onward. The fruiting body is harvested until the 26th day after spawning. It was found that the transition from pinning to fruiting body took between two and three days for all samples. Statistical analysis revealed a significant difference between treatments and mushroom yield. T2E4 gave the highest yield, 271.5±57.67, with biological efficiency (B.E.) of 9.11%. While T1E3 and T1E4 yielded significantly higher than the other treatments with biological efficiency of 8.68% and 8.31%, respectively. It was found that in treatments T1 and T2, high dosages of EM (20% and 30%) resulted in higher yields, while this was different in treatment T3, which recorded lower yields. Overall, the result of this study was better than that of the cultivation with whole EFB as substrate, where an E. B. of 6-7% was obtained, as previously reported (Azhar *et al*., 2018; Triyono e*t al*., 2019). It was found that both EM pre-treatment and composting duration had a significant effect on the yield of *V. volvacea* (p-value<0.05).

Another important result was that the average individual fruiting body weight ranged from 4 to 14 g. This was relatively higher than report from Biswas (2014), which recorded only 2.4 g per fruiting body. However, a study by Zikriyani *et al.* (2018) gave a comparable result to this study, where the weight of a single fruiting body was in the range of 7.85 to 10.7 g. The highest average weight per fruiting body (14 g) was observed in sample T1E3. T2E4 produced the most fruiting bodies with 42, while only 8 were harvested from each T3E1 and T3E4.

3.4 Compositional changes of EFB fibre during Volvariella volvacea cultivation

Table 5 presents data on EFB fibre composition analysis in *V. volvacea* cultivation. As previously reported, the initial concentration of chemical composition was in the standard range (Mohammad *et al.,* 2020). Compositional analysis showed that cellulose, hemicellulose and lignin were reduced to different degrees in the composting and postharvest samples in all treatments. For cellulose, there was a positive correlation between the dosage of EM and the degradation process, as the highest reduction was obtained in the T2E4 sample. On the other hand, no clear trend was observed for hemicellulose and lignin. No significant changes in lignin composition during composting. While other compounds mostly reduced significantly after composting and post-harvest. From the results, about 70% of the cellulose, hemicellulose and lignin of the spent substrate for cultivation of *Volvariella* volvacea were still intact compared to the cultivation showing potential high residual nutrients for bioconversion. *V. volvacea* grows well in substrates with high cellulose but low lignin content because it secretes various cellulolytic enzymes but no lignin-degrading enzymes (Suwannarach *et al.,* 2022). Philippoussis *et al.* (2001) also reported a positive correlation between the yield of *V. volvacea* and the cellulose content of the growing medium.

Table 4. Yield performance of *V. volvacea* cultivation

Sample	DFPF	DFFH	FB No.	Av. Weight/FB (g)	Yield (g)	B.E %
T1E1	10.5± 1.14	12.5±1.14	22	6.4	139.5±38.1^{ac}	4.65
T1E2	11.77±0.75	13.77±0.75	17	11.5	195.3±40.51^{aa}	6.51
T1E3	12.0±1.32	14.0±1.32	18	14	260.35±13.4^{ab}	8.68
T1E4	11.33±0.56	13.33±0.56	20	12.03	249.5±10.7^{ab}	8.31
T2E1	10.8±0.611	12.8±*0.61*	29	4	112±1.4^{bc}	3.73
T2E2	13.7±1.56	15.7±1.56	16	8.75	140±65.03^{ba}	4.67
T2E3	11.44±0.356	13.44±0.36	20	8.2	163.5±8.73^{bb}	5.45
T2E4	10.55±0.456	12.55±0.46	42	6.2	271.5±57.28^{bb}	9.11
T3E1	13.1±0.81	15.1±0.88	8	8.38	66.8±7.18^{bc}	2.22
T3E2	10.77±1.35	12.77±1.35	22	11.2	169.3±17.67^{ba}	5.64
T3E3	11.8±1.57	13.8±1.57	16	11.6	186±32.5^{bb}	6.2
T3E4	12.7±1.35	14.7±1.56	8	7.5	60±14.142^{bb}	2.3

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05). DFPF: Day for pinhead forming, DFFH: Day for first harvest.

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Table 5. Weight of cellulose, hemicellulose and lignin in substrate during mushroom cultivation.

Sample	Cellulose (g)		Hemicellulose (g)		Lignin (g)	
	PC	PH	PC	PH	PC	PH
Control	0.53±0.013		0.32±0.0056		0.15±0.195	
TIE1	0.535±0.08^{bc}	0.52±0.003^{c}	0.19±0.0280^{a}	0.16±0.069^{a}	0.1±0.016^{a}	0.089±0.003^{a}
T1E2	0.57±0.02^{c}	0.48±0.01^{bc}	0.184±0.008^{a}	0.16±0.008^{a}	0.14±0.078^{a}	0.119±0.074^{ab}
T1E3	0.409±0.11^{a}	0.43±0.06^{abc}	0.259±0.078^{ab}	0.22±0.028^{ab}	0.12±0.059^{a}	0.107±0.049^{ab}
T1E4	0.421±0.03^{abc}	0.391±0.033^{ab}	0.245±0.023^{ab}	0.21±0.071^{ab}	0.165±0.02^{a}	0.143±0.017^{ab}
T2E1	0.42±0.047^{abc}	0.35±0.049^{a}	0.267±0.0002^{ab}	0.33±0.1^{b}	0.11±0.072^{a}	0.09±0.009^{ab}
T2E2	0.488±0.06^{abc}	0.47±0.085^{abc}	0.227±0.062^{ab}	0.18±0.018^{a}	0.137±0.03^{a}	0.14±0.031^{ab}
T2E3	0.481±0.088^{abc}	0.46±0.015^{bc}	0.251±0.018^{ab}	0.21±0.039^{ab}	0.14±0.058^{a}	0.14±0.047^{ab}
T2E4	0.3712±0.03^{a}	0.351±0.032^{a}	0.259±0.039^{ab}	0.235±0.027^{ab}	0.213±0.03^{a}	0.201±0.023^{b}
T3E1	0.4±0.05^{ab}	0.35±0.015^{a}	0.312±0.0565^{b}	0.26±0.025^{ab}	0.164±0.05^{a}	0.12±0.03^{ab}
T3E2	0.47±0.087^{abc}	0.45±0.091^{abc}	0.268±0.027^{ab}	0.206±0.019^{a}	0.133±0.06^{a}	0.133±0.062^{ab}
T3E3	0.43±0.001^{abc}	0.41±0.014^{abc}	0.255±0.056^{ab}	0.284±0.038^{ab}	0.170±0.05^{a}	0.136±0.053^{ab}
T3E4	0.41±0.001^{abc}	0.39±0.021^{ab}	0.265±0.026^{ab}	0.224±0.013^{ab}	0.169±0.04^{a}	0.151±0.021^{ab}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). PC: Post-composting, PH: Post-harvest.

Therefore, it is suggested to increase the enzymatic activity during the cultivation of the substrate to improve the B.E further. Moreover, due to the different cell wall structures of the biomass feedstock, it is worthwhile to look for a possible extended pre-treatment strategy, as a single method is not suitable for all applications. (Zulkifli *et al.,* 2019). Thirubhuvanamala *et al.* (2012) found that the addition of micronutrient boosters can increase biological efficiency by up to 25% while increasing yield. According to the study by Triyono *et al.* (2019), adding fertiliser increased the yield and quality of *Volvariella volvacea.* So, it is also suggested for extended work to explore the different strategies to improve yield, including additional organic boosters during cultivation.

4. Conclusion

This study analysed the effects of different EM pre-treatment and composting of a substrate on the mycelial growth and crop yield. EM pre-treatment at 20 and 30% did improve the mycelium growth, yet it was unrelated to the yield. The best dosage and composting time for the highest yield of *V. volvacea* was observed at T2E4 (10d, 30% EM) with 271.5±57.28 g or biological efficiency (B.E) of 9.11%. The overall yield was improved compared to the control and other previous studies. However, it was lower than the yield of other substrates, such as rice straw and cotton waste. Thus, a better approach for yield improvement was required.

Conflict of interest

The authors declare no conflict of interest.

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