



Research article



Effect of physiochemical parameters on yield and biological efficiency of *Volvariella volvacea* cultivated on empty fruit bunch pellets

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ABSTRACT

Background: *Volvariella volvacea* is a highly nutritious edible mushroom grown mainly in South-east Asian countries. However, the low yield of *V. volvacea* has discouraged farmers from engaging in its production.

Objective: The study was conducted to observe the improvement of *V. volvacea* yield depending on various physiochemical parameters of *V. volvacea* growth.

Methods: The parameters tested in this study include the weight of the substrate, i.e., 2 kg (W1) and 6 kg (W2); the surface area of the substrate: A1 (1218 cm²), A2 (1530 cm²) and A3 (2000 cm²); and four different substrate formulations (F1, F2, F3 and F4).

Results: Substrate weight and surface area were found to be important, but not critical, factors in determining fruiting bodies formation, total fungal mass, and BE rate. However, the formulation media showed a significant contribution that could help in the induction of fruiting bodies. According to the results, the culture medium with a mixture of EFB substrate and black soil showed the highest BE percentage of 17.75 % (at optimised substrate weights = 2 kg).

Conclusion: The results of this study can be used as a reference for further studies to improve the cultivation of *V. volvacea*, especially when EFB fibres are used as the main substrate. Future studies to identify genes involved in the formation of fruiting bodies are strongly recommended.

1. Introduction

Volvariella volvacea is an edible mushroom that is often found in countries with warm climates, especially in Southeast Asia. It is

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highly nutritious and is usually consumed for health and medicinal purposes [1]. The *V. volvacea* not only contains a high protein and fibre content but is also known as an excellent source of antioxidants and glucan [2]. In addition, *V. volvacea* has been reported to contain a basic protein that is effective against certain human cancer cells [3]. In general, lignocellulosic wastes have always been used as a substrate for the cultivation of *V. volvacea* as they contain important components such as celluloses, hemicelluloses, and lignin [4]. The *V. volvacea* can produce extracellular enzymes that can degrade the components of lignocellulosic wastes to utilise them as its own nutrient source [5]. Different types of agricultural waste are used as substrates in different countries, depending on which raw materials are abundant and easily accessible. For example, rice straws are mainly used in China and India, while empty palm oil fruit bunches are used in Malaysia and Indonesia.

Biological efficiency (BE) and carbon to nitrogen ratio (C/N) are other crucial factors that need to be considered when choosing a substrate for mushroom cultivation. The optimal C/N ratio for the cultivation of *V. volvacea* is between 40 and 80 [6]. On the other hand, a substrate with a higher BE value indicates higher productivity and is more suitable for the cultivation of *V. volvacea* [7]. Therefore, it is important to choose lignocellulosic wastes that have a high BE value for the cultivation of *V. volvacea*. However, a major disadvantage of *V. volvacea* mushrooms is their low yield per harvest cycle, which is of little interest to local farmers. To introduce the *V. volvacea* mushroom as a sustainable crop, it is therefore essential to research a method to increase the yield of the *V. volvacea* mushroom. Based on several studies conducted in the past, factors such as supplementing the substrate with organic fertilisers and chemical substances have been found to be favourable for the growth and biological efficiency of *V. volvacea* [8,9]. A mixture of different agricultural wastes on a substrate can also increase the chances of higher yield of *V. volvacea* fruiting bodies [10].

Many previous research papers have emphasised the importance of the type of raw materials used as substrates for *V. volvacea* cultivation. However, most studies differ in terms of the biophysical properties of the substrates used to grow *V. volvacea*. Therefore, it is difficult to determine whether there is an external factor that could influence the outcome of their experiments. Despite numerous studies, there is still limited knowledge on how certain physiochemical parameters such as substrate weight, surface area and formulation affect the biological efficiency (BE) and yield of *V. volvacea*. The present study fills this gap by investigating the interaction of these parameters to optimise mushroom cultivation yields. Therefore, the aim of this study is to investigate different physiochemical parameters that can influence the yield of *V. volvacea* culture. These include weight, surface area and substrate formulation. Ultimately, this study supports sustainable consumption and production (SDG 12) by accurately demonstrating the use of empty fruit bunch pellets as a substrate for the cultivation of *V. volvacea*. This would not only help reduce agricultural waste in the palm oil industry, but also serve as an example for farmers to replicate the method for commercialisation. This study uniquely integrates EFB pellets and innovative substrate formulations to overcome the challenges of cultivating *V. volvacea*. By optimising these parameters, we aim to not only increase yield but also reduce the environmental footprint of agricultural practises.

2. Materials and methods

2.1. Preparation of *Volvariella volvacea* spawn and EFB pellets for cultivation

The *V. volvacea* spawn was obtained from a local *V. volvacea* farm in Kg. Musa Pedu, Kuala Nerang, Kedah, Malaysia. The empty fruit bunch pellets were supplied by a local supplier (Ecobed Sdn. Bhd., Puchong, Malaysia). To produce the empty fruit bunch pellets, water is mixed with the EFB pellets at a ratio of 1:1.5. The mixture was left overnight to allow the pellets to soften. Spawning of *V. volvacea* on the EFB pellet substrate took place the next day at a ratio of 250 g of spawn per 1 kg of substrate.

2.2. Cultivation and harvesting of *Volvariella volvacea* mushrooms

This cultivation method was adapted from a method described by Thuc et al. [7]. The *V. volvacea* was cultivated indoors based on the studies carried out. These growth substrates of *V. volvacea* were self-arranged in a closed structure of a self-modified four-storey metal frame for a greenhouse. To achieve the darkness and humidity required for the growth of *V. volvacea*, the outer layer of the greenhouse was covered with dark, opaque plastic. Temperature and humidity remained constant and were recorded throughout the culture room during the entire four-week or 28-day cultivation process. Temperature and relative humidity remained constant and averaged 37 °C and >80 %, respectively.

2.3. Optimisation of growth through different substrate weights

The growth substrates were prepared using two forms of EFB dry weight (as shown in Table 1). The components used to prepare the substrates for the cultivation of *V. volvacea* included 80 % w/w EFB pellets, 8 % w/w rice bran, 7 % w/w organic fertiliser (processed

Table 1
Substrate weights and surface specifications for the cultivation of *Volvariella volvacea* with EFB pellets.

Samples	EFB pellet (kg)	Surface Area (cm ²)
W1	2 kg	707
W2	6 kg	1798

Note: W = weight of the substrate.

grade) and 5 % w/w of calcium carbonate [11]. EFB pellets are defined as cylindrically shaped biomass processed with an average length of 10–15 mm and a diameter of 6–8 mm. In this study, two levels of EFB pellets weights were used for the cultivation trial: EFB pellets with W1 = 2 kg and W2 = 6 kg (Table 1). The EFB pellets were soaked overnight in a ratio of 1:1.5 (pellets: water) to achieve a moisture content of about 65–70 %. After this step, the pellet was carefully pressed to remove excess water. The oligochitosan booster was prepared by a 1/200 dilution of the stock solution with distilled water. It was sprayed at five-day intervals during the 28-day cultivation phase in a spray bottle with a volume of 20 ml. The self-modified greenhouse with dimensions of 3 m × 3 m × 3 m and had four levels to support the substrate placement. All the above tiers were spaced 30 cm apart to maintain adequate airflow through the tiers. The experiment was conducted in duplicates.

2.4. Optimisation of growth through different cultivation surfaces

To determine the influence of different surfaces on growth and yield, the surface of the cultivation setup was manipulated. At this stage, the media formulation proposed by Triyono et al. (2019) was used [11]. Three types of plastic trays measuring 25 cm × 20 cm (A1), 35 cm × 25 cm (A2) and 45 cm × 35 cm (A3) were used for the cultivation of *V. volvacea*. The surface area of each plastic tray was calculated using the following formula:

$$\text{Surface area (cm}^2\text{)} = \text{length (cm)} \times \text{width (cm)}$$

Each container was then filled with 2 kg of EFB pellet substrate. For the cultures, each size of culture containers was replicated twice.

2.5. Substrate formulations for growth optimisation

The study investigated the influence of substrate formulation depending on the type of cultivation medium by exchanging the four different formulations as shown in Table 2. The booster solution was obtained by diluting the oligochitosan stock solution at a ratio of 1/200, i.e., 5 ml of stock solution was added to 1 L of distilled water. It was used with a spray bottle at five-day intervals during the cultivation period of 28 days. The experiment was carried out in duplicate.

2.6. Measurement for fungal growth

In each experiment, the yield of the fruiting body of *V. volvacea*, the weight and the relative humidity of the fruiting body were observed. The measurements were taken daily, and the fruiting body was noted as soon as a fruiting body had developed. The temperature and humidity were measured hourly with a digital hygrometer and a thermometer to maintain the temperature between 23 and 27 °C and the humidity between 85 % and 90 %. The biological efficiency (BE) of each sample was determined using the following equation [12]:

$$\text{Biological efficiency (BE)(\%)} = \frac{\text{Total weight of fresh } V. \text{volvacea (kg)}}{\text{Dry weight of EFB substrate (kg)}} \times 100$$

The total yield of the species *V. volvacea* was measured daily during the harvesting phase. The mean yield quantified the average total number of fruiting bodies harvested per EFB substrate growth block during the 28-day cultivation period. The mean yield for each EFB substrate block was calculated using the following formula [12]:

$$\text{Mean yield} = \frac{\text{Total yields of mushroom of each substrate block}}{\text{Total number of days of cultivation}}$$

Table 2

Summary of the substrate formulations for optimisation of the cultivation of *Volvariella volvacea*.

Sample	Substrate formulation
F1	2 kg EFB pellet
F2	2 kg EFB pellet 8 % (w/w) Rice bran 7 % (w/w) Organic fertilizer 5 % (w/w) Calcium carbonate
F3	2 kg EFB pellet 0.5 kg Black soil
F4	2 kg EFB pellet Oligochitosan

Note: F= Formulation.

2.7. Data analysis

The collected data for yield, total weight and biological efficiency were statistically analysed using IBM SPSS Statistics V28.0. To account for significant differences between treatments, a two-way ANOVA with Tukey's *b*-test was performed. Mean differences were defined by *p*-values of 0.05. Pearson's *r* coefficient was used to determine the correlations between substrate weight, yield and total fungal biomass. The experiment was designed as a completely randomized factorial arrangement with three independent factors. Another evaluation was a Jupyter heatmap analysis with the Jupyter notebook environment, which was used to create a heatmap visualisation and examine the data contained within. The analysis used in the study was to determine the relationship and correlation in the given data set. The libraries used to create the heatmap and perform the analysis were imported from Seaborn and Matplotlib formats. The heat map analysis was further analysed to understand the patterns and trends related to the effects of different weights of substrate, media formulation and acreage size on the percentage of biological efficiency.

3. Results and discussion

3.1. The effect of substrate weight on *Volvariella volvacea* cultivation

The density of the substrate in relation to *V. volvacea* increases the colonisation rate, yield, size of the fungus, moisture content and contamination. To investigate more complex interactions between more than one factor: substrate weight (2 kg, 6 kg), surface area (A1, A2, A3), and substrate formulation (F1, F2, F3, F4), a two-way ANOVA was performed. This approach attempts to determine the direct effects of the variables and their interactions on the BE. In the case of W1 and W2, the total yield and mass of fruiting bodies of *V. volvacea* are shown in Fig. 1. The unit of mushroom yield in Fig. 1 is kilograms (kg). Statistical error bars for the yield and mass data with standard deviations to estimate the effects of the treatments on the absolute yield. The analysis also revealed that W2 had a higher average yield and weight than W1. The result in W2 showed that there was a significant difference in the yield of *V. volvacea* with and without treatment ($p = 0.026$) and the mass production of *V. volvacea* also showed a significant difference ($p = 0.019$).

Although substrate weight is clearly the determining factor for BE, other factors such as fertiliser and oligochitosan boosters cannot be completely excluded. This is because all the samples and the formulation of each of the samples are completely similar, so the increase in EFB automatically means an increase in rice bran, fertiliser, calcium carbonate and oligochitosan. As pointed out by Biswas and Layak (2014), the performance of *V. volvacea* increases when rice straw substrate is supplemented with 2 % (w/w) rice bran [8]. Supplementing the substrate with micronutrient boosters also has a similar favourable effect on the yield of *V. volvacea* [9]. Khan et al. (2013) also determines the position of calcium carbonate as a soil pH regulator in fungal production [13]. It is also important to note that in addition to a higher substrate dry weight value, the W2 samples also had a higher substrate surface area compared to the W1 samples. This is likely to be another factor that may influence the results.

In agreement with our expectations, W2 gives a higher BE of 81.82 % for *V. volvacea* when 6 kg of EFB pellet substrate is used, in contrast to W1 where only 2 kg of the same substrate was used (BE observed = 28.40 %). Similarly, W2 is three times higher than W1, which is in line with the results of Umor et al. (2021) who found 28 % BE for *V. volvacea* on EFB pellets [14]. However, this contrasts with other studies that indicate that biological efficiency decreases with increasing substrate weight per bag, while substrate weight has no effect on days to fruiting [15]. Thus, even though the BE in this study was 81.82 %, an insight into the effects of specific substrate formulations and environmental conditions could be gained. These results suggest that weight and formulation should be prioritised over surface area, providing a direction for future optimisation and realistic advice for the sustainable cultivation of *V. volvacea*. However, according to the heat map analysis shown in Fig. 2, the substrate had the least impact on the yield and total number of mushrooms produced. This means that the absolute mass of the substrate had no effect on fruiting yield, total mushroom weight or bioconversion efficiency.

As can be seen from the heat map in Fig. 2, the correlation between the total biomass of the mushrooms and the weight of the substrate used to grow the mushrooms was lower (63 % relationship). However, the total yield and total mass of mushroom production showed a very close correlation, with a percentage of 81 %. This result is thus in line with Triyono et al. (2019) and Assan & Mpofu

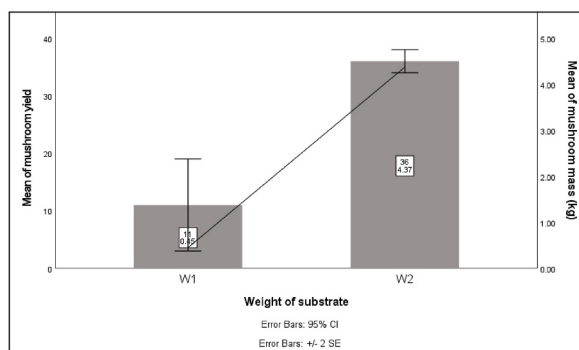


Fig. 1. Comparative mean yield and mass of *Volvariella volvacea* fruiting bodies across substrate weights.

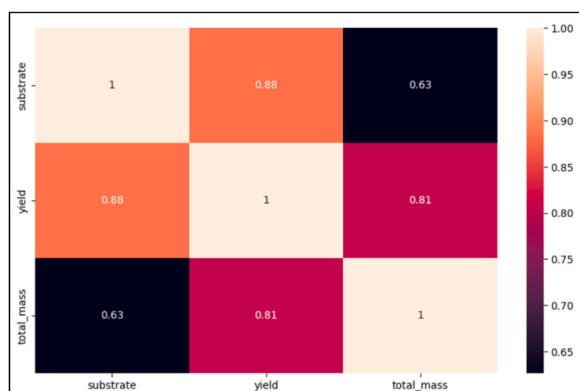


Fig. 2. Heatmap analysis of relationships among tested variables and substrate weight for *Volvariella volvacea* cultivation.

(2014) who argue that substrate weight is not a key factor in determining the yield and total mass of mushrooms [11,15]. The results of the heat map analysis are consistent with those of previous studies [11] and with some findings which were not identified in other studies but only in this investigation, such as a negative relationship between surface area and BE. Our studies suggest that substrate weight does not necessarily lead to maximum yields and that factors such as nutrient distribution and colonisation rate may also play an important role. These factors should be further investigated in future research. Accordingly, we agreed to use 2 kg EFB fibres as the standard substrate weight for the other parameters.

3.2. The effect of substrate surface area on *Volvariella volvacea* cultivation

In relation to Fig. 3, it was explained how the yield and total mass of fruiting bodies were affected by different substrate surfaces. It is also important to note that even though A1 has a lower surface area compared to A2 and A3, the highest mean total mass was obtained. Compared to the other substrates, A2 resulted in the highest fruiting of *V. volvacea*. On the other hand, A3 showed the lowest average yield and mass, but had the largest surface area.

The data also show no difference in yield and mass of fruiting bodies of *V. volvacea* in all tested plots (Table 3). This result proves that surface area does not play the main role in determining the yield of mushrooms during cultivation.

After calculating the BE of the samples cultivated on different surfaces, the highest BE (17.75 %) was found in the fungus cultivated on the small surface (A1). Table 4 shows the percentage of BE, where it was found that the percentage of BE increased with decreasing surface area of the EFB substrate. Thus, with higher productivity of BE rates, a higher percentage of BE was observed, namely A1, followed by A2 and A3.

3.3. The effect of substrate formulation on *Volvariella volvacea* cultivation

Using the different formulations, the productivity of *V. volvacea* was determined and it was found that the total mass of *V. volvacea* fruiting bodies produced the highest yield in formulation F3, followed by F4, F2 and F1, as shown in Fig. 4.

Using two-way analysis of variance, the result was shown to have a different level of significance compared to all formulations tested for yield and total mass production. According to the data presented in Table 5, there are also obvious differences in F3 compared to F1 and F2 in terms of total mass production. However, the results showed no difference between F1, F2 and F4. This result confirms the previous finding that the formulation of culture media with F3 components could increase mushroom production.

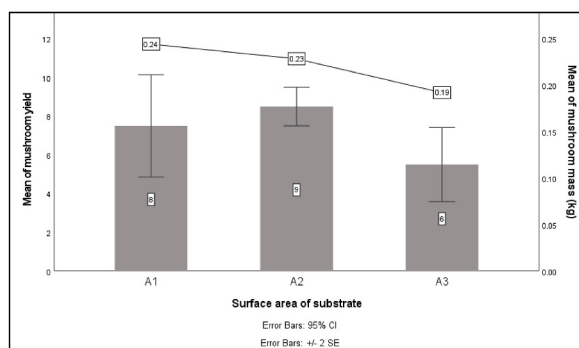


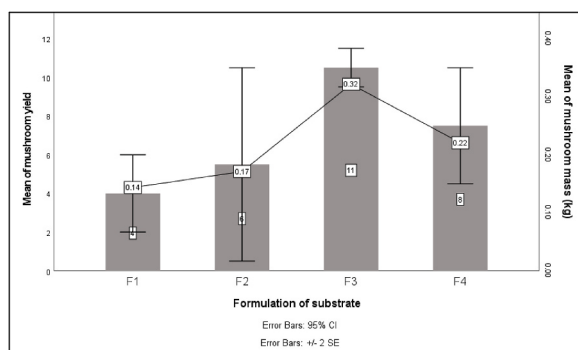
Fig. 3. Effect of substrate surface area on yield and mass of *Volvariella volvacea* fruiting bodies.

Table 3Comparative analysis of yield and mass of *Volvariella volvacea* across different surface area.

Surface area of substrate		Yield of <i>V. volvacea</i>			Mass of <i>V. volvacea</i>		
		Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.
A1	A2	−1.000	1.394	0.491	0.016	0.053	0.775
	A3	2.000	1.394	0.185	0.052	0.053	0.353
A2	A1	1.000	1.394	0.491	−0.016	0.053	0.775
	A3	3.000	1.394	0.060	0.036	0.053	0.511
A3	A1	−2.000	1.394	0.185	−0.052	0.053	0.353
	A2	−3.000	1.394	0.060	−0.036	0.053	0.511

*The mean difference is significant at $p < 0.05$ level.**Table 4**Impact of substrate surface area on biological efficiency (BE) of *Volvariella volvacea* cultivation.

Samples	Biological Efficiency (%)
A1	17.75
A2	15.50
A3	12.45

**Fig. 4.** Total yield and mass (kg) of *Volvariella volvacea* fruiting bodies following different substrate formulations.

This is also supported by Table 6, which shows the highest percentage of BE, F3 (17.75 %), F4 (12.75 %), F2 (10.9 %) and the lowest percentage of BE at F1 (7.45 %). These data explain and suggest that supplementation increases the yield of fruiting bodies, BE and total mass of *V. volvacea*, as found in previous work and literature [16,17]. However, nutrients from rice bran, fertiliser, soil (F2) and oligochitosan (F4) could influence the growth of mycelia per se. Another assumption could be attributed to the effect of the micro-biome present in the substrate (as the substrate used in this experiment was not sterilised prior to cultivation). This is shown, for example, by the fact that substrate F3 contains black soil and other bacterial taxa that can contribute significantly to fungal production.

Table 5Comparative analysis of yield and mass of *Volvariella volvacea* across different substrate formulations.

Formulation of substrate		Yield of <i>V. volvacea</i>			Mass of <i>V. volvacea</i>		
		Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.
F1	F2	−1.500	2.208	0.534	−0.027	0.048	0.595
	F3	−6.500*	2.208	0.042	−0.178*	0.048	0.020
	F4	−3.500	2.208	0.188	−0.077	0.048	0.182
F2	F1	1.500	2.208	0.534	0.027	0.048	0.595
	F3	−5.000	2.208	0.086	−0.151*	0.048	0.034
	F4	−2.000	2.208	0.416	−0.050	0.048	0.358
F3	F1	6.500*	2.208	0.042	0.178*	0.048	0.020
	F2	5.000	2.208	0.086	0.151*	0.048	0.034
	F4	3.000	2.208	0.246	0.101	0.048	0.102
F4	F1	3.500	2.208	0.188	0.077	0.048	0.182
	F2	2.000	2.208	0.416	0.050	0.048	0.358
	F3	−3.000	2.208	0.246	−0.101	0.048	0.102

*The mean difference is significant at <0.05 level.

Table 6
Biological efficiency of *Volvariella volvacea* based on different formulations of substrate.

Samples	Biological Efficiency (%)
F1	7.45
F2	10.9
F3	17.75
F4	12.75

This agrees well with the statement by Noble et al. (2003) that the soil bacteria - *Pseudomonas* bacteria; *Pseudomonas putida* - could be involved in the fruiting of fungi [18]. Liu et al. (2017) also pointed out that some microbial groups such as protobacteria, chloroflexi, bacteria and various other soil microbes were also important for the induction of fungal primordia in the cultivation of fungi [19].

To find out how two variables under study could influence each other, the analysis was carried out in Jupyter notebook format. It was found that F1 & F2 (media formulation) and A1 & A2 (surface area of cultivation area) were significantly related to BE rate (Fig. 5). However, media composition and cultivated area size showed a more significant correlation. Given this pattern identification, the Jupyter heat map analysis showed the correlation of selected variables with the effect of surface area and media formulation on BE rate. However, the heatmap analysis for media formulation F2 (15 %) and F3 (12 %) showed a positive correlation with BE rate. In addition, A1 showed a strong correlation strength with the BE rate of 33 %, while A2 showed a weakness of 1.7 %. On the other hand, the media formulations of F1 and F4 as well as the surface (A3) showed an inverse correlation with the BE rate. A negative sign means that when the value of one variable increases, the value of the other variable decreases. Therefore, the negative correlations observed for F1, F4 and A3 can be seen as the result of a lower correlation contributing to the value of the BE rate.

These results also confirm that the larger surface area of the substrate does not necessarily contribute to a higher yield in *V. volvacea*. Furthermore, this result refutes the hypothesis from the previous experiment that a larger surface area of the substrates increases the yield of *V. volvacea*. However, the content and composition of the EFB substrate are some of the factors that could influence the productivity rate in the cultivation of the fungus [20,21]. This study provides some insights into improving the production of *V. volvacea* through cheaper and more effective formulation of substrate and cultivation parameters that can also be applied by smallholders. However, future work should also investigate the effects of gene expression and microbial associations in the substrates on increasing BE and yield.

Although the results suggest that microbial involvement is essential in increasing BE, especially in the formulation of black soil substrates, the conclusions are only hypothesis-generating and further research is needed.

4. Conclusion

It can be concluded that a higher amount of EFB in the culture media also leads to a higher production of *V. volvacea*. However, from the correlation analysis performed, it appears that the weight of the substrate is not a sensitive variable for the yield and total mass of the fungi. From the calculated surface area data, it was found that there is no strong relationship between the surface area available for cultivation and the yield of *V. volvacea*. This means that the area and space required to grow *V. volvacea* is not proportional to the additional yield of the fruiting body. The most influential factor in relation to the BE rate was the identified media formulation. In this study, the EFB substrate with the addition of black soil had found to have the highest BE value compared to the others. This suggests that a complex formulation mixture containing other sources such as nitrogen (organic fertiliser), calcium carbonate and oligochitosan promotes mycelial growth but not fruiting body formation. In addition, the fungus might prefer the natural presence of soil to initiate fruiting body formation. However, the simple component was suitable as it will reduce the cost of cultivation in the future. For future research, it was recommended to focus on the expression of the gene as well as the genes responsible for fruiting bodies formation.

CRedit authorship contribution statement

N.F. Amir: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Mohd-Aris:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **T.N. Tuan-Zakaria:** Validation, Software, Formal analysis. **N.A. Umor:** Resources, Conceptualization. **A. Mohamad:** Resources. **N.M. Razali:** Validation, Formal analysis. **FZ Mohd Yusof:** Resources. **S. Abdullah:** Resources.

Ethics declaration

This study did not require informed consent or review and approval by an ethical committee because it did not involve any direct experimentation or studies on living beings that required ethics approval.

Data availability statement

Data will be made available on request. For requesting data, please write to the corresponding author.

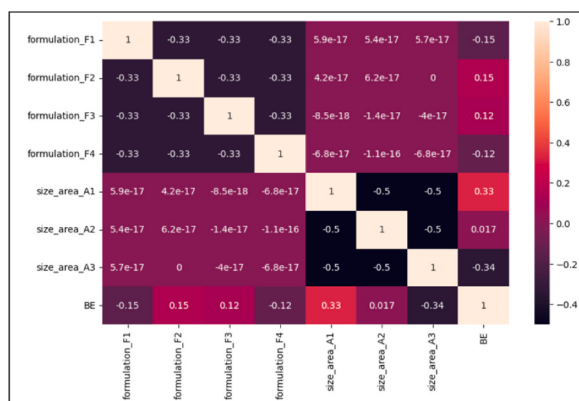


Fig. 5. Heatmap showing the interaction between substrate formulation and cultivation area on biological efficiency of *Volvariella volvacea*.

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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 in this paper.

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