



## RESEARCH ARTICLE

### EFFECT OF DIFFERENT BAG OPENING METHODS ON THE GROWTH, YIELD, AND NUTRITIONAL COMPOSITION OF TWO OYSTER MUSHROOMS *PLEUROTUS OSTREATUS* AND *PLEUROTUS CYSTIDIOSUS*

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#### ABSTRACT

The study was conducted to compare the effects of different bag opening methods on the growth, yield, and nutritional composition of oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). Bag opening methods were used including (1) removing ring and cotton plug, (2) opening at the top and bottom of culture bags, (3) opening some site around culture bags, (4) de-plastic culture bag. The culture bag after taking of cotton plug (no removing ring) was used as control. This result obtained showed that different bag opening methods had significant effect on mushroom yield, biological efficiency (BE), and no effect on nutritional composition of mushroom PO and PC. Bag opened with larger surface induced rapidly dried substrate and reducing the number of flushes and total harvesting period. However, it induced increasing the number of effective fruiting bodies of both oyster mushrooms, the yield at the first three flushes of mushroom PO and at the first flush of mushroom PC, except de-plastic culture bag. No removing ring was the best method to get the highest yield (204.3 g/bag) and BE (40.80%) of mushroom PC while removing ring was identified as the best method for cultivation of mushroom PO with the highest yield (298.5 g/bag) and BE (59.62%). Opening at the top and bottom and opening some sites around culture bag were suitable opening methods for mushroom PO to reduce the total harvesting period and increase mushroom weight per flush while total yield and PE were not different with control condition.

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## INTRODUCTION

Oyster mushroom belongs to the family of Tricholomataceae (Neelam et al., 2013). The nutritionally gifted fungi now rank second among the important cultivated mushrooms in the world because of their low cost, easy to grow on a large of agro wastes, high biological efficiency and high nutrition value (Mane et al., 2007; Sánchez, 2010). Oyster mushrooms have a great nutrition value since they are quite rich in protein, with all essential amino acids, mineral, fiber, and poor in lipid (Eva et al., 2013; Purkayastha and Nayak, 1981). Moreover, oyster mushrooms are considered as the most potential edible mushroom with high therapeutic values. Oyster mushrooms accumulate a variety of secondary metabolites used as food and medicine to protect against illness and to maintain human health including phenolic compounds, polysaccharides,

polypeptides, steroids. Oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC) are currently found to possess antioxidant activity (Li et al., 2007; Yang et al., 2002). Many authors studied on the method to improve the production and quality of edible mushrooms including oyster mushrooms such as using the different substrates (Ha et al., 2015; Philippoussis et al., 2007; Taurachand, 2004); using light treating condition (Arjona et al., 2009; Chang and Miles, 2004; Kuforiji and Fasidi, 2009; Miyazaki et al., 2011); using good spawn sources (Kuforiji and Fasidi, 2009).

There are limited data in the literature concerning the mushroom yield and nutritional composition of fruiting body under different bag opening methods. Hence, the aim of our study is to determine the effects of different bags opening methods on the growth, yield, biological efficiency and nutritional composition of fruiting body of two oyster mushroom PO and PC.

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## MATERIALS AND METHODS

### Mushroom material and spawn preparation

Oyster mushroom PC(strain AG 2041) and PO(strain AG 2042)obtained from Plant Physiology and Value Added Microorganisms Laboratory (Department of Plant Industry, National Pingtung University of Science and Technology (NPUST), Taiwan) were maintained in potato sucrose agar medium (PSA) at 28°C for regular subculture and maintained on PSA at 4°C. Spawns were prepared in 850mL polypropylene plastic bottles filled with 600g acacia sawdust supplemented with 9% rice bran, 1% sugar, 1% lime powder, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate (in terms of dry weight basis) and 60-70% water content, and then sterilized at 121°C for 5 h. After cooling to room temperature, 10-20 mycelium discs (diameter 1cm) of each oyster mushroom were inoculated into each bottle of sterilized spawn. The spawn was incubated at 28°C until the substrate fully colonized.

### Substrate preparation, mushroom cultivation and harvest

Sawdust (SD) on dry weight basis were supplemented with 9% rice bran, 1% sugar, 1% lime powder, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate. Water content of the final mixture was adjusted to about 65%. Each substrate formula after supplementing nutrient and distilled water was filled into 10 × 23 cm polyethylene plastic bags (1kg/bag) and sterilized in an autoclave at 121°C for 5 h. After substrates were cooled to room temperature, they were inoculated with the 2g spawn per bag and incubated at 28°C and 60-70% relative humidity under dark condition. After the surface of substrates was entirely covered and filled with mycelium, then the cultivation bags were moved to a cultivation room in which temperature was maintained at 24°C and kept at related humidity about 90% or above. The culture bags were opened by sterile scalpel with some methods including (1) removing ring and cotton plug, (2) opening at the top and bottom of culture bags, (3) opening some site around culture bags, (4) de-plastic culture bag. The culture bag after taking of cotton plug (no removing ring) was used as control. Mushroom fruiting bodies were harvested from each of the culture bags when the in-rolled margins of the mushroom caps began to flatten. The mushroom production and biological efficiency (BE) of each treatment were recorded with duration around 1.5-2 months. BE was measured by the weight of fresh mushroom per dry weight of substrate.

### Fruiting body analysis

Mushroom samples were dried by an oven at 40°C to a constant weight to calculate moisture content and then ground into power samples for other analysis. The protein content ( $N \times 6.25$ ) of samples was estimated by the macro-Kjeldahl method (Ezeibekwe *et al.*, 2009). The samples were analyzed for nutritional composition (fat, carbohydrates, fiber, and ash) using the Association of Official Analytical Chemists procedures (AOAC, 1995). The fat was determined by extracting a known weight of powdered sample with ethyl ether, using a Soxhlet apparatus. The ash content was measured by incineration at  $600 \pm 15^\circ\text{C}$ . Total carbohydrates

were calculated by difference. Energy was calculated according to the following equation: Energy (kcal/100g) =  $4 \times \text{protein} + 4 \times \text{carbohydrate} + 9 \times \text{fat}$ . The mineral contents (P, K, Ca, Mg, Fe, Mn, Zn, and Cu) were analyzed by ICP atomic emission spectrophotometry (Horiba Jobin Yvon, Long Jumeau, France)

### Experimental design and data analysis

The experiments were conducted in NPUST, Taiwan during summer season, 2015 (March to July). The experiment was arranged in a randomized complete block design with three replications and eight culture bags per replication. One-way analysis of variance (ANOVA) was conducted with Duncan's multiple range tests to compare the mean significant differences ( $p \leq 0.05$ ) among treatments by using computer software SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSION

### Effect of different bag opening methods on the growth parameters and fruiting body characteristic

Mushroom production was influenced by the substrate, environment in the mushroom cultivation room as well as cultivation technique. Table 1 showed that using different bag opening methods significantly affected on the first harvesting day, the total harvesting period, and fruiting body characteristics of two oyster mushrooms PO and PC except the first harvesting day of mushroom PC. Bag opening stimulated the number and development of fruiting body formation which induced significantly increasing the number of effective fruiting body per flush. Further, using de-plastic bag method, fruiting body of mushroom PO was harvested earlier 3.66 days at the first flush than control treatment (no removing ring). In the control treatment, the number of effective fruiting bodies of mushrooms PO and PC was 9.17 and 2.03 fruiting/flush. When culture bags were opened with different methods, the number of fruiting body was from 26.27 to 56.80 fruiting bodies in case of mushroom PO and from 3.13 to 5.37 fruiting bodies in case of mushroom PC. The larger surface of culture bags was opened, the more number of fruiting bodies obtained. This fact resulted in smaller mushroom size (Figure 1). When culture bags were opened with a larger surface, it induced substrates drying more rapidly so that it reduced the number of flush and decreased total harvesting period of mushroom PO and PC. Total harvesting day of mushroom PO in case of removing ring, opening some sites around culture bag, opening at the top and bottom of culture bag, and de-plastic bag decreased 7.14 days, 8.69 days, 9.35 days, and 11.01 days, respectively in comparison with no removing ring. In case of mushroom PC, opening at the top and bottom and opening some sites around culture bag reduced 14.98 days and 18.39 days respectively in comparison with control treatment (no removing ring). Bag opening with larger surface reduced total flushes and increased the number of effective fruiting bodies of mushrooms PO and PC hence it significantly influenced on mushroom weight per flush. Opening some sites around culture bag had the highest values in mushroom weight of mushroom PO (77.78 g/ flush) while opening some sites around culture bag and opening at the top and the bottom gave the highest mushroom weight (98.59 and 89.70 g/flush, respectively) of mushroom PC.



**Figure 1. Fruiting bodies of oyster mushrooms PO (A) and PC (B) in no removing ring (a), removing ring (b), opening at the top and bottom of culture bag (c); opening some sites around culture bag (d); and de-plastic culture bag (e). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus***

**Table 1. Effect of different bag opening methods on first harvesting day, total harvesting period, and fruiting body characteristics of oyster mushrooms PO and PC**

Substrate formula	First harvesting day (day)	Harvesting period (day)	No. of effective fruiting bodies/flush	Mushroom weight (g/flush)
PO				
No removing ring (control)	41.57 ± 1.34 <sup>a</sup>	47.92 ± 0.64 <sup>a</sup>	9.17 ± 0.83 <sup>e</sup>	45.22 ± 0.26 <sup>c</sup>
Removing ring at the top	40.78 ± 0.89 <sup>b</sup>	40.78 ± 1.86 <sup>b</sup>	26.27 ± 2.28 <sup>d</sup>	59.71 ± 0.19 <sup>b</sup>
Opening at the top and bottom,	41.18 ± 1.11 <sup>a</sup>	38.57 ± 1.10 <sup>b</sup>	33.03 ± 2.73 <sup>c</sup>	89.70 ± 0.33 <sup>a</sup>
Opening some sites around culture bag	39.24 ± 0.53 <sup>ab</sup>	39.23 ± 2.53 <sup>b</sup>	43.47 ± 3.48 <sup>b</sup>	89.59 ± 1.31 <sup>a</sup>
De-plastic culture bag	37.91 ± 0.36 <sup>b</sup>	36.91 ± 2.33 <sup>b</sup>	56.80 ± 3.40 <sup>a</sup>	45.51 ± 0.30 <sup>c</sup>
PC				
No removing ring (control)	55.02 ± 1.03 <sup>a</sup>	46.03 ± 0.64 <sup>a</sup>	2.03 ± 0.03 <sup>e</sup>	68.96 ± 1.43 <sup>bc</sup>
Removing ring at the top	56.13 ± 1.03 <sup>a</sup>	46.29 ± 1.10 <sup>a</sup>	3.13 ± 0.09 <sup>b</sup>	56.01 ± 0.65 <sup>d</sup>
Opening at the top and bottom,	55.49 ± 0.57 <sup>a</sup>	31.05 ± 2.94 <sup>b</sup>	2.87 ± 0.23 <sup>b</sup>	72.82 ± 0.83 <sup>b</sup>
Opening some sites around culture bag	56.64 ± 0.64 <sup>a</sup>	27.64 ± 1.39 <sup>b</sup>	5.51 ± 0.36 <sup>a</sup>	77.78 ± 0.50 <sup>a</sup>
De-plastic culture bag	55.72 ± 0.97 <sup>a</sup>	-	5.37 ± 0.33 <sup>a</sup>	66.12 ± 1.79 <sup>c</sup>

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test. Each value is expressed as mean ± standard error (SE) (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*

**Table 2. Effect of different bag opening methods on the yield and biological efficiency of two oyster mushrooms PO and PC**

Bag opening method	1 <sup>st</sup> flush (g/bag)	2 <sup>nd</sup> flush (g/bag)	3 <sup>rd</sup> flush (g/bag)	4 <sup>th</sup> flush (g/bag)	5 <sup>th</sup> flush (g/bag)	6 <sup>th</sup> flush (g/bag)	Total yield (g/bag)	BE (%)
<b>PO</b>								
No removing ring (control)	59.43 ± 1.72 <sup>d</sup>	52.93 ± 1.04 <sup>d</sup>	48.18 ± 1.07 <sup>c</sup>	42.56 ± 0.92 <sup>a</sup>	39.71 ± 1.07 <sup>a</sup>	28.50 ± 1.40	271.3 ± 1.55 <sup>b</sup>	54.18 ± 0.31 <sup>b</sup>
Removing ring	80.01 ± 2.85 <sup>c</sup>	69.85 ± 0.58 <sup>c</sup>	57.15 ± 2.80 <sup>b</sup>	50.51 ± 2.64 <sup>a</sup>	41.02 ± 4.03 <sup>a</sup>	-	298.5 ± 0.93 <sup>a</sup>	59.62 ± 0.19 <sup>a</sup>
Opening at the top and bottom,	119.7 ± 1.08 <sup>a</sup>	81.38 ± 0.99 <sup>b</sup>	68.01 ± 1.31 <sup>a</sup>	-	-	-	269.1 ± 0.99 <sup>b</sup>	53.74 ± 0.20 <sup>b</sup>
Opening some sites around culture bag	113.1 ± 1.68 <sup>b</sup>	89.18 ± 0.82 <sup>a</sup>	66.54 ± 4.85 <sup>a</sup>	-	-	-	268.8 ± 3.93 <sup>b</sup>	53.68 ± 0.78 <sup>b</sup>
De-plastic culture bag	80.04 ± 1.36 <sup>c</sup>	38.67 ± 1.76 <sup>e</sup>	17.83 ± 1.40 <sup>d</sup>	-	-	-	135.7 ± 1.17 <sup>e</sup>	27.10 ± 0.23 <sup>c</sup>
<b>PC</b>								
No removing ring (control)	73.49 ± 0.97 <sup>c</sup>	66.98 ± 3.29 <sup>a</sup>	66.41 ± 0.37 <sup>a</sup>	-	-	-	204.3 ± 2.51 <sup>a</sup>	40.80 ± 0.50 <sup>a</sup>
Removing ring	74.42 ± 1.33 <sup>c</sup>	63.61 ± 1.88 <sup>a</sup>	30.02 ± 4.81 <sup>b</sup>	-	-	-	168.1 ± 1.96 <sup>b</sup>	33.56 ± 0.39 <sup>b</sup>
Opening at the top and bottom,	79.96 ± 1.61 <sup>b</sup>	65.68 ± 0.55 <sup>a</sup>	-	-	-	-	145.6 ± 1.67 <sup>d</sup>	29.09 ± 0.33 <sup>d</sup>
Opening some sites around culture bag	98.66 ± 0.30 <sup>a</sup>	56.90 ± 1.19 <sup>b</sup>	-	-	-	-	155.6 ± 1.00 <sup>e</sup>	31.07 ± 0.20 <sup>c</sup>
De-plastic culture bag	66.12 ± 1.79 <sup>d</sup>	-	-	-	-	-	66.12 ± 1.79 <sup>e</sup>	13.20 ± 0.36 <sup>c</sup>

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test. Each value is expressed as mean ± standard error (SE) (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*

**Table 3. Effect of different bag opening methods on nutritional composition of two oyster mushrooms PO and PC fruiting body**

Substrate	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Ash (%)	Energy Kcal/100g
<b>PO</b>							
No removing ring (control)	87.04 ± 0.80 <sup>a</sup>	20.56 ± 1.22 <sup>a</sup>	3.08 ± 0.09 <sup>a</sup>	21.93 ± 1.32 <sup>a</sup>	48.99 ± 1.95 <sup>a</sup>	5.43 ± 0.19 <sup>a</sup>	306.0 ± 4.94 <sup>a</sup>
Removing ring at the top	85.09 ± 0.53 <sup>a</sup>	20.85 ± 1.74 <sup>a</sup>	3.18 ± 0.37 <sup>a</sup>	22.00 ± 0.29 <sup>a</sup>	48.00 ± 1.68 <sup>a</sup>	5.97 ± 0.09 <sup>a</sup>	304.1 ± 1.97 <sup>a</sup>
Opening at the top and bottom,	85.54 ± 0.61 <sup>a</sup>	22.39 ± 0.86 <sup>a</sup>	3.17 ± 0.28 <sup>a</sup>	19.83 ± 5.24 <sup>a</sup>	48.71 ± 6.14 <sup>a</sup>	5.90 ± 0.25 <sup>a</sup>	312.9 ± 19.17 <sup>a</sup>
Opening some sites around culture bag	85.44 ± 0.58 <sup>a</sup>	21.51 ± 1.77 <sup>a</sup>	3.38 ± 0.20 <sup>a</sup>	19.70 ± 1.65 <sup>a</sup>	49.44 ± 3.23 <sup>a</sup>	5.97 ± 0.09 <sup>a</sup>	314.3 ± 6.92 <sup>a</sup>
De-plastic culture bag	84.76 ± 0.49 <sup>a</sup>	19.18 ± 0.51 <sup>a</sup>	3.35 ± 0.13 <sup>a</sup>	20.53 ± 0.80 <sup>a</sup>	51.47 ± 0.81 <sup>a</sup>	5.47 ± 0.17 <sup>a</sup>	312.8 ± 2.48 <sup>a</sup>
<b>PC</b>							
No removing ring (control)	89.55 ± 0.13 <sup>a</sup>	16.77 ± 1.34 <sup>a</sup>	3.52 ± 0.32 <sup>a</sup>	21.50 ± 1.16 <sup>a</sup>	50.98 ± 1.15 <sup>a</sup>	7.23 ± 0.34 <sup>a</sup>	302.7 ± 7.04 <sup>a</sup>
Removing ring at the top	88.46 ± 0.78 <sup>a</sup>	16.84 ± 0.58 <sup>a</sup>	3.63 ± 0.33 <sup>a</sup>	19.72 ± 1.49 <sup>a</sup>	52.67 ± 1.31 <sup>a</sup>	7.13 ± 0.09 <sup>a</sup>	310.8 ± 4.40 <sup>a</sup>
Opening at the top and bottom,	88.74 ± 0.59 <sup>a</sup>	16.04 ± 0.57 <sup>a</sup>	3.27 ± 0.41 <sup>a</sup>	20.92 ± 1.16 <sup>a</sup>	52.48 ± 2.04 <sup>a</sup>	7.30 ± 0.06 <sup>a</sup>	303.5 ± 2.40 <sup>a</sup>
Opening some sites around culture bag	87.84 ± 1.20 <sup>a</sup>	16.12 ± 0.82 <sup>a</sup>	3.58 ± 0.10 <sup>a</sup>	20.98 ± 0.69 <sup>a</sup>	52.12 ± 0.13 <sup>a</sup>	7.30 ± 0.26 <sup>a</sup>	304.9 ± 4.33 <sup>a</sup>
De-plastic culture bag	88.97 ± 0.43 <sup>a</sup>	15.97 ± 0.46 <sup>a</sup>	3.43 ± 0.27 <sup>a</sup>	19.22 ± 1.35 <sup>a</sup>	54.31 ± 1.16 <sup>a</sup>	7.07 ± 0.30 <sup>a</sup>	312.1 ± 4.41 <sup>a</sup>

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's multiple range test. Each value is expressed as mean ± standard error (SE) (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*

### Effect of different bag opening methods on the yield and biological efficiency

Mushroom yield and BE were the main purpose of mushroom grower. Nowadays there are so many studies to improve yield and BE of different mushroom species. In our study, different bag opening methods were examined to find the best method for oyster mushroom PO and PC cultivation. With different bag opening methods, the yield and BE of mushrooms PO and PC were significantly different (Table 2). At the first flush, the best mushroom yield of mushroom PO was obtained in the method of opening at the top and bottom of culture bag (119.7 g/bag), following by the method of opening some sites around culture bag (113.1 g/bag). No removing ring had the lowest yield (59.43 g/bag) of mushroom PO. In case of mushroom PC, at the first flush, opening some sites around culture bag gave the highest yield (98.66 g/bag), following by the method of opening at the top and bottom (79.96 g/bag). De-plastic culture bag had the lowest yield (66.12 g/bag). In general, the larger surface culture bag was opened, the less number of flushes was obtained. This fact was due to large surface opening of culture bag resulted in the substrate drying more rapidly so it reduced the number of flushes. However, except de-plastic culture bag, all cases of bag opening had higher yield at the first, second, and third flush when compared to no removing ring in case of mushroom PO. On the contrary, in case of mushroom PC, opening some sites around culture bag and opening at the top and bottom of culture bag only gave the

higher yield at the first flush. De-plastic culture bag also had the lowest yield at the first flush as well as total yield and BE of mushroom PC. Regarding to the total yield and BE of mushrooms PO, the highest total yield (298.54 g/bag) and BE (59.62%) was obtained in the method of removing ring method. The highest total yield was higher 27.23 g/bag (10%) than that obtained in control treatment (no removing ring) and the highest BE was 5.44% higher than that obtained in control treatment. Although opening at the top and bottom of culture bag and opening some sites around culture bag had three flushes, the total yield and BE of mushroom PO obtained were the same as those in control treatment. This result was due to the highest yield of the first, the second, and the third flush. Regarding to the total yield and BE of mushroom PC, the highest yield (204.3 g/bag) and BE (40.80%) were obtained in control treatment. All cases of examination had lower values of total yield and BE. De-plastic bag showed the lowest total yield and BE (66.12 g/bag, 13.20%, respectively). It was lower 27.6% than BE of mushroom PC obtained in control treatment. It was due to after de-plastic bag, surface substrate of culture bag became rapidly drying which resulted in only one flush formed and developed with smaller size during whole cultivation time.

### Effects of different bag opening methods on nutritional composition of fruiting body

Nutritional composition is one of the most important characteristics of fruiting body and it helps increasing the values

of mushroom. Nowadays, oyster mushrooms have been known as excellent food for human health with low in caloric diet. Nutritional composition of oyster mushrooms was influenced by so many factors. Under different bag opening methods, nutritional composition of oyster mushrooms PO and PC was shown in Table 3. In general, oyster mushrooms PO and PC were rich in protein, fiber, ash, and carbohydrate content, and low in fat content (Table 3). In our previous research, substrate and mushroom species were identified as significant effect on nutritional composition of oyster mushrooms PO and PC (Ha *et al.*, 2015). In other researches, nutritional composition of oyster mushrooms was also influenced by environment condition, the harvesting stage, mushroom age, as well as mushroom storage conditions after harvest (Tao *et al.*, 2006; Vetter and Rimoczi, 1993). However, in this our study, bag opening method had no significant effect on nutritional composition of mushrooms PO and PC. It was due to the same condition of substrates used, temperature and dark condition during incubation for mycelium growth, moisture, CO<sub>2</sub> concentration, and light in cultivation room. Moreover, fruiting bodies of mushroom PO and PC were harvested at the same mushroom age when the in-rolled margins of mushroom cap began to flatten hence, nutritional composition of mushroom PO and PC under different bag opening method was not significant different.

## Conclusion

This result obtained showed that different bag opening methods had significant effect on mushroom production of mushroom PO and PC. Bag opened with larger surface induced rapidly dried substrate and reducing the number of flushes and total harvesting period. However, it induced increasing the number of effective fruiting bodies of both oyster mushrooms, the yield at the first three flushes of mushroom PO and at the first flush of mushroom PC except de- plastic culture bag. Different bag opening method had no effect on nutritional composition of mushroom PO and PC. No removing ring was the best method to get the highest yield (204.3 g/bag) and BE (40.80%) of mushroom PC while removing ring was identified as the best method for cultivation of mushroom PO with the highest yield (298.5 g/bag) and BE (59.62%).

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