Effect of bamboo waste replacing cottonseed husk on cultivation of *Pleurotus geesteranus*

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Abstract. Bamboo debris are rich in nutrients and suitable for the cultivation of edible fungi. In order to develop the new raw materials for cultivation of *Pleurotus geesteranus* and the resource utilization of bamboo debris, the mycelial growth, fruiting body growth and economic benefits of *P. geesteranus* was studied in 9 types of bamboo debris replacement medium formulas (A, B, C, D, E, F, G, H, I) and the cottonseed husk formula cultivated for *P. geesteranus* in Leshan City as a control (CK). Results shown that formula G was suitable for the growth of *P. geesteranus*. The composition of the formula G was 64% bamboo debris, 16% cottonseed husk, 8% sawdust, 10% bran, 1% white sugar and 1% plaster. In formula G, the average growth rate of *P. geesteranus* mycelia was 5.39 mm / d, 7.67 mm / d, and 8.42 mm / d in 10 days, 15 days, and 20 days after inoculation, average bag yield was 0.33 kg / bag, biological efficiency was 89.87%, and the ratio of output value to input value was 3.34.

1. Introduction

*Pleurotus geesteranus* belongs to Eumycota, Basidiomycetes, Agaricales, Pleurotaceae, *Pleurotus*, and is one of the rare edible fungi that has been cultivated in a wide area in recent years [1]. Its flavor is strong, the taste is crisp and tender, and the market is good. Although reported that more raw materials are used for the cultivation of *P. geesteranus* [2-10]. However, wood chips and cottonseed husks are still used as the main raw materials for cultivation of *P. geesteranus* [11-15]. Leshan is not the main production area of wood chips and cottonseed husks, and the cost of raw materials fluctuates greatly. Therefore, the development of local raw materials for the cultivation of *P. geesteranus* according to local conditions has become an important way to break through the bottleneck of industrial development. More and more recent researches are concerned about biomaterials, especially the reuse of biomass and related wastes [16-20]. Leshan is rich in bamboo resources, and there are many bamboo and bamboo processing enterprises. About 60% to 70% of waste will be generated during bamboo processing, except for some used in the manufacture of new materials [21, 22], biology fuel [23, 24], medicines [25, 26], fertilizer production [27] and other fields, a large amount of waste is discarded or incinerated. This not only causes environmental pollution, but is also a waste of bamboo resources [28].

The development of methods for cultivating *P. geesteranus* with bamboo waste will not only hope to break through the bottlenecks that restrict the development of *P. geesteranus* industry, but also increase the reuse rate of waste resources for bamboo processing. Previously, there have been reports...
of successful cultivation of *Lentinus edodes*, *Dictyophora indusiata*, *Pleurotus eryngi*, and *Pleurotus geesteranus* using bamboo waste. Zhang et al. [29] used bamboo shavings to successfully cultivate multiple varieties of *L. edodes*. Lu et al. [30] studied the effect of fermentation time and dosage of waste bamboo shavings on the growth and development of bamboo clams, and the results showed that when the fermentation time was 45 d, hyphae of *D. indusiata* had the fastest growth rate, and the hyphae was white, thick, and good growth. Liu [31] had successful cultured *P. eryngii* with moso bamboo shavings. Chen et al. [32] utilized bamboo husks instead of cottonseed husks to cultivate *P. geesteranus*, the best replacement ratio of bamboo husks was 90%, and the formula composition was 58% wood chips, 27% bamboo chips, 3% cotton seed hulls, 10% bran, and 2% lime.

Although there are precedents for the successful cultivation of edible fungi using bamboo waste, it has not been reported whether bamboo waste are suitable for the cultivation of *P. geesteranus*. Bamboo debris is a type of waste generated during bamboo processing. The organic content of bamboo debris is 88.20%, and the total nitrogen content is 0.71%. Its carbon-nitrogen ratio is close to that of cottonseed husks. Therefore, in this experiment, bamboo shavings were used instead of cottonseed husks for the cultivation of *P. geesteranus*. Study the effects of different substitution ratios on the growth of *P. geesteranus* mycelium and fruiting body yields, and comprehensively compare economic benefits, with a view to providing a reference for the development of new *P. geesteranus* substrates, reducing production costs and comprehensive utilization of bamboo processing waste. Therefore, this paper studied the cultivation of *P. geesteranus* by different substitution ratios of bamboo debris, and investigated its effects on the growth of *P. geesteranus* hyphae and the yield of fruiting bodies, and discussed the feasibility of using bamboo debris to cultivate edible fungi.

2. Materials and methods

2.1. The strains used in this experiment

The test strain of *P. geesteranus* was provided by the Microbiology Laboratory of Leshan Normal University.

2.2. Bamboo debris used in this experiment

The bamboo debris was collected from the waste processed by the bamboo paper processing enterprise in Leshan City, Sichuan Province, China and was used after sieving (pore diameter 0.6 cm). Cottonseed hulls, sawdust, bran, white sugar, and gypsum were purchased from Leshan City.

2.3. The test method used in this experiment

According to the nutritional composition of bamboo shavings and cottonseed husks, combined with the nutritional needs of *P. geesteranus*, 9 types of culture medium formulations (A, B, C, D, E, F, G, H, I) for *P. geesteranus* were designed, and the cottonseed husk formula cultivated for *P. geesteranus* in Leshan City was used as a control (CK) (Table 1).

| Treatment | Bamboo debris | Cottonseed husk | Sawdust | Wheat bran | White sugar | Plaster |
|-----------|---------------|----------------|---------|------------|-------------|---------|
| CK        | 0             | 80             | 8       | 10         | 1           | 1       |
| A         | 16            | 64             | 8       | 10         | 1           | 1       |
| B         | 24            | 56             | 8       | 10         | 1           | 1       |
| C         | 32            | 48             | 8       | 10         | 1           | 1       |
| D         | 40            | 40             | 8       | 10         | 1           | 1       |
| E         | 48            | 32             | 8       | 10         | 1           | 1       |
| F         | 56            | 24             | 8       | 10         | 1           | 1       |
| G         | 64            | 16             | 8       | 10         | 1           | 1       |
| H         | 72            | 8              | 8       | 10         | 1           | 1       |
| I         | 80            | 0              | 8       | 10         | 1           | 1       |
Each formula was a treatment, which was prepared with 100 kg of dry material and bagged with 175 × 400 × 0.05 (mm) high pressure polyethylene bag. Each treatment was repeated three times. The mycelial growth length was measured at 10 days, 15 days and 20 days after inoculation. The mycelial growth rate was calculated, and the mycelial growth, hyphal traits, full bag time, mushrooming time, and yield of each fresh mushroom were recorded, and the biological efficiency, ratio of out-value to in-value was calculated.

3. Results

3.1. Effect of bamboo debris on mycelial growth of Pleurotus geesteranus

Bamboo debris had significant effects on the mycelial growth of *P. geesteranus* mycelium (Table 2 and Table 3). From the strength of mycelium growth, the hyphae of F and G formulas were the strongest, and mycelium growing on other formulas was relatively weak. In the three periods of 10 days, 15 days, and 20 days after inoculation, the average growth rate of mycelium grown in Formula G was the highest, and the average growth rate of mycelium grown in Formula A and CK was the lowest. There was no significant difference in hyphal properties, hyphae of all formulas were very dense and tidy.

| Treatment | Mycelium growth potential | Mycelial traits | Full bag time (d) |
|-----------|---------------------------|----------------|------------------|
| CK        | +++                       | Dense, tidy, white | 25               |
| A         | +++                       | Dense, tidy, white | 24               |
| B         | +++                       | Dense, tidy, white | 24               |
| C         | +++                       | Dense, tidy, white | 24               |
| D         | +++                       | Dense, tidy, white | 23               |
| E         | +++                       | Dense, tidy, white | 22               |
| F         | +++                       | Dense, tidy, white | 19               |
| G         | +++                       | Dense, tidy, white | 19               |
| H         | +++                       | Dense, tidy, white | 20               |
| I         | +++                       | Dense, tidy, white | 24               |

| Treatment | 0-10 d | 0-15 d | 0-20 d |
|-----------|--------|--------|--------|
| Control   | 3.64gF | 4.62gF | 5.11hI |
| A         | 3.8gF  | 4.74gF | 5.17hI |
| B         | 4.03fE | 5.00fE | 5.39gG |
| C         | 4.13fE | 5.08fE | 5.44gG |
| D         | 4.59cCD| 5.88cD | 6.37fF |
| E         | 4.83eD | 6.38dC | 6.61eE |
| F         | 5.04bB | 6.62cC | 7.06dD |
| G         | 5.39aA | 7.67aA | 8.42aA |
| H         | 5.33aA | 7.08bB | 7.88bB |
| I         | 4.86cBC| 6.56cC | 7.48cC |

Note: Different lowercase letters in the same column indicate a significant difference when $P < 0.05$, and different uppercase letters in the same column indicate a significant difference when $P < 0.01$. 

Table 2. The effect of bamboo debris on mycelial growth of *Pleurotus geesteranus*

Table 3. The effect of bamboo debris on mycelial growth rate of *Pleurotus geesteranus* (mm/d)
3.2. Effects of bamboo debris on the development of fruit body of Pleurotus geesteranus

Bamboo debris had significant effects on the growth and development of *P. geesteranus* fruit bodies (Table 4). As can be seen from Table 4, Formula G has the highest average bag yield (0.33 kg / bag) and biological efficiency (89.87%).

### Table 4. Effects of bamboo debris on the development of fruit body of Pleurotus geesteranus

| Treatment | Number of bags | Dry weight per bag(kg) | Average yield (kg/bag) | Biological efficiency (percent) |
|-----------|----------------|------------------------|------------------------|--------------------------------|
| CK        | 256            | 0.39                   | 0.20dC                 | 52.33eC                        |
| A         | 258            | 0.39                   | 0.21cdC                | 54.18eC                        |
| B         | 261            | 0.38                   | 0.22cdBC              | 56.27deC                       |
| C         | 264            | 0.38                   | 0.24bcdBC              | 62.49cedBC                     |
| D         | 268            | 0.37                   | 0.24bcdBC              | 65.50cedBC                     |
| E         | 270            | 0.37                   | 0.26bcABC             | 70.20bcBC                      |
| F         | 274            | 0.36                   | 0.28abAB              | 77.93abAB                      |
| G         | 276            | 0.36                   | 0.33aA                | 89.87aA                        |
| H         | 280            | 0.36                   | 0.27bABC              | 76.22bcAB                      |
| I         | 285            | 0.35                   | 0.24bcdBC             | 69.03bcdBC                     |

Note: Different lowercase letters in the same column indicate a significant difference when *P* < 0.05, and different uppercase letters in the same column indicate a significant difference when *P* < 0.01.

3.3. Effect of bamboo debris on cultivation efficiency of Pleurotus geesteranus

Bamboo debris had significant effects on average bag yield, output value, and ratio of output value to input value (Table 5). As can be seen from Table 5, the ratio of output value to input value (3.34) in formula G was the highest, followed by treatment H (2.89) and F (2.83). The ratios of output value to input value for 9 formulas of bamboo debris were higher than of the control.

### Table 5. Effects of bamboo debris on cultivation efficiency of Pleurotus geesteranus.

| Treatment | Replacement rate | Input value (Yuan/bag) | Average yield (kg/bag) | Output value (Yuan/bag) | Ratio of output value to input value |
|-----------|------------------|------------------------|------------------------|------------------------|-------------------------------------|
| CK        | 0%               | 2.00                   | 0.20                   | 3.27                   | 1.63                                |
| A         | 20%              | 1.89                   | 0.21                   | 3.36                   | 1.78                                |
| B         | 30%              | 1.83                   | 0.22                   | 3.45                   | 1.88                                |
| C         | 40%              | 1.78                   | 0.24                   | 3.79                   | 2.13                                |
| D         | 50%              | 1.72                   | 0.24                   | 3.91                   | 2.28                                |
| E         | 60%              | 1.67                   | 0.26                   | 4.16                   | 2.50                                |
| F         | 70%              | 1.61                   | 0.28                   | 4.55                   | 2.83                                |
| G         | 80%              | 1.56                   | 0.33                   | 5.21                   | 3.34                                |
| H         | 90%              | 1.51                   | 0.27                   | 4.36                   | 2.89                                |
| I         | 100%             | 1.46                   | 0.24                   | 3.88                   | 2.66                                |
4. Discussions
Bamboo debris were rich in nutrients and suitable for the cultivation of edible fungi. There had been reports of using bamboo debris to cultivate *L. edodes*, *D. indusiata*, *P. eryngi*, and *P. geesteranus*. In this study, the bamboo shavings were used to replace some cottonseed husks. The results suggested that compared with the conventional cottonseed hull formula, it was found that the proper substitution ratio increased the yield of *P. geesteranus*.

The results of this experiment indicated that formula G was suitable for the growth of *P. geesteranus*. The composition of the formula G was 64% bamboo debris, 16% cottonseed husk, 8% sawdust, 10% bran, 1% white sugar and 1% plaster. In formula G, the average growth rate of *P. geesteranus* mycelia was 5.39 mm/d, 7.67 mm/d, and 8.42 mm/d in 10 days, 15 days, and 20 days after inoculation, average bag yield was 0.33 kg/bag, biological efficiency was 89.87%, and the ratio of output value to input value was 3.34.

The nutrition required for the growth and development of edible fungi is mainly carbon and nitrogen sources. The suitable carbon-nitrogen ratio for the growth and development of *P. geesteranus* was 30:1 ~ 40:1 [33, 34], which determined the biological efficiency high or low.

5. Conclusion
In this study, formula G had the highest biological efficiency, which was significantly higher than the other formulas, indicating that the carbon-to-nitrogen ratio of this formula was closest to the appropriate carbon-to-nitrogen ratio for the growth and development of *P. geesteranus*, but its specific ratio needs to be further determined.

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