*Insight: Cambodia Journal of Basic and Applied Research,* Volume 1 No. 2 (2019)


# Log and bag cultivation of Oyster (Pleurotus ostreatus) and Lingzhi (Ganoderma lucidum) mushrooms in Cambodia

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**To cite this article:** Srey C. (2019) Log and bag cultivation of Oyster (Pleurotus ostreatus) and Lingzhi (Ganoderma lucidum) mushrooms. *Cambodia Journal of Basic and Applied Research (CJBAR), 1(2)*, 32–42.

## សង្ខិត្តន័យ

ការអភិវឌ្ឍឧស្សាហកម្មផលិតផ្សិតនៅកម្ពុជា ដូចជា ផ្សិតអយស្ទ័រ (Pleurotus ostreatus) និងផ្សិតលីងហ្ស៊ី (Ganoderma lucidum) តម្រូវឱ្យមានចំណេះដឹងក្នុងស្រុកស្តីពីការបង្កើនអត្រានៃការលូតលាស់ ការគ្រប់គ្រងគុណភាព ការគ្រប់គ្រងជំងឺ និងលក្ខណៈជីវសាស្ត្រផ្សេងទៀតដែលត្រូវផលិត។ ការសិក្សានេះមានបំណងប្រៀបធៀបអត្រានៃការលូតលាស់ និងទិន្នផលនៃប្រភេទផ្សិតទាំងពីរនេះដោយបណ្តុះក្នុងមជ្ឈដ្ឋានថង់ប្លាសិច និង កំណាត់ឈើ។ ការផលិតផ្សិតត្រូវចាប់ផ្តើមពីការបណ្តុះមេផ្សិត គឺដាក់ជាលិការនៃពូជផ្សិត P. ostreatus និង G. lucidum ទៅក្នុងប្រអប់សារធាតុជំនួយការលូតលាស់ (potato dextrose agar) ហៅកាត់ PDA។ បន្ទាប់មក បំបែកមេពូជដែលបណ្តុះបានក្នុងប្រអប់នេះ ដោយផ្ទេរទៅក្នុងដបស្រូវកាន់តែច្រើន ហៅថាមេស្រូវ។ ចុងបញ្ចប់ មេស្រូវត្រូវបានផ្ទេរទៅក្នុងមជ្ឈដ្ឋានដុំថង់អាចម៍រណា (ដើមកៅស៊ូ) និងកំណាត់ឈើ ដែលបានរៀបចំរួចជាស្រេច។ ចំពោះករណីបណ្តុះជាមួយកំណាត់ឈើ មេពូជនៃ P. ostreatus ចំនួន១៩ករណី និង មេពូជ G. lucidum ចំនួន២២ករណី បានឆ្លងជំងឺ។ ចំណែកករណីបណ្តុះក្នុងដុំថង់អាចម៍រណាវិញ មេពូជ P. ostreatus តែ៥ករណី និង មេពូជ G. lucidum តែ ១៣ករណី ប៉ុណ្ណោះ បានឆ្លងជំងឺ។ ប្រភេទផ្សិតផ្សេងៗគ្នាត្រូវការលក្ខខណ្ឌលូតលាស់ខុសគ្នា។ ឧទាហរណ៍ ពូជផ្សិត P. ostreatus ត្រូវការលូតលាស់ក្នុងសីតុណ្ហភាពពី ២០ទៅ៣០អង្សាសេ ក្នុងសំណើមពី ៧០ទៅ៩0%។ ចំណែក ពូជផ្សិត G. lucidum ត្រូវការលូតលាស់នៅសីតុណ្ហភាពពី ១៨ ទៅ ២៥អង្សាសេ ក្នុងសំណើមពី ៨៥ទៅ៩0%។ ការជ្រើសរើសមជ្ឈដ្ឋានបណ្តុះក៏ជះឥទ្ធិពលដល់ទិន្នផលផងដែរ។ ចំពោះប្រទេសកម្ពុជា ការបណ្តុះផ្សិតពូជ P. ostreatus ដោយប្រើដុំថង់អាចម៍រណា ត្រូវបានផ្សព្វផ្សាយ និងលើកកម្ពស់ដល់ប្រជាកសិករ។

## Abstract

The development of the mushroom industry in Cambodia through the production of oyster (Pleurotus ostreatus) and lingzhi (Ganoderma lucidum) species requires local knowledge about growth rates, quality control, disease management and other biological characteristics to be produced. This study compares the growth rate and yield of these

species in both bag and log cultures. Mushrooms were cultivated by transferring tissues from P. ostreatus and G. lucidum strains to potato dextrose agar (PDA) plates. Then, these strains were multiplied through transferring their spawn to successive grain bottles. Finally, the spawn was harvested and transferred to either plastic bags filled with sawdust (rubber tree) or prepared logs. In the case of the strains grown in logs, 19 of the P. ostreatus cultures and 22 of the G. lucidum cultures were contaminated; while this occurred for only 5 and 13 of the bag cultures, respectively. Different mushroom species require different growing conditions. For instance, P. ostreatus strains thrive at a temperature between 20°C and 30°C at 70 to 90% humidity. However, G. lucidum strains thrive at a temperature between 18°C and 25°C, at a humidity of 85 to 90%. The choice of substrate also affects yield. In the Cambodian context, a sawdust substrate was found to produce higher yields, in terms of both biological efficiency and the number of fruiting bodies. It is recommended that the cultivation of P. ostreatus on a sawdust substrate is promoted to rice farmers in Cambodia.

**Keywords:** Mushrooms, oyster mushrooms (P. ostreatus), lingzhi (G. lucidum)

## Introduction

Rice accounts for 75% of agricultural production in the wet season in Cambodia—between May to October. Outside of the wet season, many Cambodian families adopt livelihood strategies such as small-scale poultry and vegetable farming or migrating to work in urban centers (Fosbenner et al., 2018). Mushroom farming has significant potential to further supplement incomes during the dry-season in Cambodia.

Humans have consumed mushrooms as part of a normal human diet and for medicine for thousands of years throughout the world (Kozarski et al., 2011). Moreover, edible mushrooms have beneficial effects on health and in the treatment of diseases (Heo et al., 2003; Wachtel-Galor et al., 2004; Kuo et al., 2006) such as hyper-tension, obesity, diabetes, cancerous tumors. Active compounds in mushrooms also protect liver function (Heo et al., 2003; Xu et al., 2016), immunomodulatory actions (Kim et al., 1996), anti-angiogenic and antioxidant activities (Song et al., 2003). Mushrooms are fat and cholesterol-free (Bellettini et al., 2016) (United Nations-N.U., 2007), high in potassium; and very low in sodium and carbohydrates. They also have anti-neoplastic and lipid-reducing properties (Chang and Buswell, 1996; Wasser and Weis, 1999).

Mushrooms grow rapidly as fresh fruiting bodies on agricultural wastes including olive cake, tomato tuff, pine needles, wheat straw, banana leaves, cotton waste, maize stover, palm oil and other wastes. Their production characteristics are different than those of plants. There

are many kinds of edible mushroom species including oyster mushrooms *(Pleurotus sajor-caju)*, straw mushrooms (*Volvariella volvacea)*, lingzhi *(Ganoderma lucidum)*, ear mushrooms *(Auricularia auricular)* and shiitake *(Lentinula edodes)*. In Cambodia, recently the cultivation of oyster (*Pleurotus ostreatus*) and lingzhi mushrooms (*Ganoderma lucidum*) has become popular (Obaa and Nshemereirwe, 2004). While Cambodians have cultivated mushrooms many years and the country has stocks of substrate, mushroom production is dependent on the quality of this substrate. Effective substrates help to minimize cultivation costs and cultures contaminated by disease.

The potential of mushroom cultivation as an effective cash crop to compliment rice production is dependent on the management of risks associated with substrate contamination. This research compares the cultivation of P. ostreatus and G. lucidum mushrooms strains in bag and log cultures, with respect to their growth dynamics, productivity, susceptibility to disease, and other biological characteristics. It aims to provide locally relevant information about mushroom production to farmers in Cambodia.

## Conceptual Framework

All fungi are part of a diverse group of saprophytes (Guillamon et al., 2010), but not all fungi are mushrooms. Mushrooms are found just about everywhere (Bishop et al., 2015). They are produced in both tropical and subtropical regions (Ibekwe et al., 2008). The optimal growing temperature for many mushrooms is between 32$^{o}$C and 35$^{o}$C and they fruit more productively between 28$^{o}$C and 32$^{o}$C (Randive, 2012). Mushroom are the fleshy, spore-bearing fruiting bodies found in grassy meadows and woodland habitats, growing upon lingo-cellulosic substrates, such as straw and wood (Belewu and Belewu, 2005). Numerous types of mushrooms grow in nature, however, less than 25 species are widely accepted as a food; and few have attained commercial importance (Longvah and Deosthale, 1998). In ancient times, mushrooms were harvested from the wild, with their pleasing texture and aroma making them attractive as a fresh food (Longvah and Deosthale, 1998). World production of mushrooms reached 3.4 million tons in 2007 (Guillamon et al., 2010). In this study, the biological characteristics of two species of mushrooms will be studied in the context of Cambodia:

- **Phellinus ostreatus,** which belongs to the fungi kingdom, basidiomycetes class, Hymenochaetaceae sub-class, Agricals order (Shah et al., 2004; Vetayasuporn, 2006), Tricholomataceae family (Ha and Chun-Li, 2015), Pleurotus genus (Gibriel et al., 1996) and Ostreatus species. They grow naturally in the tropics (Vetayasuporn, 2006) on dead and decaying woody biomass or wooden logs (Randive, 2012). P. ostreatus can grow at temperatures ranging from 25 to $35^0$ C, at a humidity of 70 to 90% (Bhatti et al., 2007) with a growing season that may extend to eight months per year (Randive, 2012). Spores need to be stored at a temperature of between 18 and $25^0$ C (Samuel and Eugene, 2012).
- **Ganoderma lucidum,** which is of the basidiomycetes species, polyporaeae order, and Ganodermataceae family (Sullivan et al., 2006). This strain produces shiny *reishi* or *manetake* mushrooms, otherwise known as *lingzhi* in China and Cambodia. Their natural habitat is on a range of dead or dying trees (Wasser, 2005), with mycelia growth occurring at temperatures between 15 and $35^0$ C, at a humidity of 60 to 70%. Optimal primordial initiation occurs between 25 and $30^0$ C. Optimal mycelia growth between 18 and $25^0$ C, at 85 to 90% humidity. The optimal development of fruiting bodies occurs between 24 and $28^0$C, at 85-95% humidity. When the cultivation temperature drops below $20^0$ C, fruiting bodies tend to turn yellow and stop growing (Xuan-Wei et al., 2012).

## Materials and Method

Mushroom strains were grown on potato dextrose agar **(**PDA) plates using a procedure that varied slightly from that outlined in the literature (Oei, 2005; Das and Mukherjee, 2007; Chang et al., 2016). Potatoes (200 g) were peeled and cut into one-centimeter cubes, before being placed into one liter of boiling water and left to simmer for 15 to 20 minutes**.** The broth was then filtered, before water was added to a total volume of one liter**.** Then 20 g of sugar, and 20 g of agar was added and stirred slowly at a constant speed until the materials were completely dissolved. The PDA was then stored at $4^O$C in a refrigerator until use. A pure multi-spore or tissue culture was collected from the middle of fresh fruiting bodies of P. ostreatus and G. lucidum and transferred to the PDA with a sterilized knife.

Mycelia were grown over a period of about 10 days, before being multiplied via transfer to grain bottles containing rice, where they being grown for a further 10 to 15 days (Chang et al.,

2016). The grain was then transferred to a new bottle. The entire spawn production process took 2 to 3 weeks to complete (Peng et al., 2000; Moonmoon et al., 2010; and Chang et al., 2016). A mixture of sawdust (rubber tree) (79%) rice bran (20%), calcium Carbonate ($CaCO_3$) (1%) and water (65%) was prepared for the final stage of production. Spawn harvested from each bottle was transferred to both plastic bags filled with sawdust and logs. The substrate was placed is an autoclave for 20 min at 121$^{O}$C (Ashraf et al., 2013), before being used to grow mycelium to fruiting bodies over a period of about four weeks (Hyunjong and Woo, 2004).Logs were prepared by cutting them at least two weeks prior to inoculation from appropriate species of trees. Bark damage was avoided to minimize other species of fungi from enter the log. They were then cut into 24 cm pieces to enable them to fit into the autoclave at a diameter of between 6 and 8 cm. Small holes (2 to 3 cm deep) were then drilled into the logs at 5 to 6 cm intervals, into which the spawn was added. The results from each experimental run were analyzed statistically, using an inferential t-test to determine if there was a significant difference between the biological characteristics of each mushroom strain. The analysis was conducted using SPSS 16.0. Values were determined to be significant if the p-value was found to be less than 0.05.

## Results and Discussion

### *Comparing the presence of contamination in each mushroom culture*

After one month, the rate of contamination for the bag cultures was compared (Table 1). Five P. ostreatus cultures had been contaminated compared to 13 G. lucidum cultures. This result was significantly different. This result for both P. ostreatus G. lucidum strains grown on logs was also significantly different, with 19 and 22 cultures found to be contaminated, respectively.

### *Mycelium growth characteristics for bag and log cultures (P. ostreatus and G. lucidum)*

P. ostreatus mycelium grown in bag cultures was observed 1 to 2 days after inoculation. Mycelium growth ended after 28 to 30 days, with fruiting occurring on 4 to 6 occasions over a period of 6 months. G. lucidum spawn took 4 to 5 days to start growing, which did not reach completion until 2 or 3 months later. These cultures had still not produced fruit 6 months after inoculation. The same was observed for the P. ostreatus spawn grown on logs, where mycelium growth was not completed until 3 to 6 months later. No fruiting was observed within a 6 month period. The G. lucidum cultures grown on logs took between 7 and 10 days for mycelium growth

to be observed. Again, no fruiting had occurred after a period of 6 months. Different mushrooms species require different conditions, while the quality of substrate also affects yield (Sanchez, 2010). In the context of this study, P. ostreatus cultures were found to be more productive than G. lucidum cultures. Bag cultures also tended to produce better results than log cultures (Table 2).

**Table 1.** The presence of contamination of P. ostreatus and G. lucidum cultures.

| **Strain** | **Contamination present** | | **$X^2$** | **P-*value*** |
|---|---|---|---|---|
| | **Yes** | **No** | | |
| P. ostreatus (bag) | 5 (16.7%) | 25 (83.3%) | 4.995 | 0.025* |
| G. lucidum (bag) | 13 (43.3%) | 17 (56.7%) | | |
| P. ostreatus (bag) | 5 (16.7%) | 25 (83.3%) | 13.384 | 0.000*** |
| P. ostreatus (log) | 19 (63.3%) | 11 (36.7%) | | |
| G. lucidum (bag) | 13 (43.3%) | 17 (56.6%) | 5.462 | 0.019** |
| G. lucidum (log) | 22 (73.3%) | 8 (26.7%) | | |
| P. ostreatus (bag) | 5 (16.7%) | 25 (83.3%) | 19.137 | 0.000*** |
| G. lucidum (log) | 22 (73.3%) | 8 (26.7%) | | |
| P. ostreatus (log) | 19 (63.3%) | 25 (36.7%) | 2.371 | 0.124 |
| G. lucidum (bag) | 13 (43.3%) | 17 (56.7%) | | |
| P. ostreatus (log) | 19 (63.3%) | 11 (36.7%) | 0.682 | 0.409 |
| G. lucidum (log) | 22 (73.3%) | 8 (26.7%) | | |

## Conclusion

This research compared the growth characteristics of two mushroom species in Cambodia. P. ostreatus*;* and G. lucidum strains were grown on both sawdust bag and log substrates. P. ostreatus were found to be less susceptible to contamination and grew more quickly, producing good quality mushrooms. Sawdust bags were found to be the most productive substrate with the highest yield, biological efficiency, and number of fruiting bodies. It is recommended that oyster mushroom cultivation in bag cultures is recommended to Cambodian rice farmers as to produce a supplementary income.

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**Table 2.** Growth characteristics for P. ostreatus and G. lucidum bag and log cultures.

| **Cultivation medium** | **Mushroom type** | **Time for mycelium to appear** | **Time to completemycelium growth** | **Number of harvests** | **Months to produce fruit** | **Months before harvest** |
|---|---|---|---|---|---|---|
| Plastic bag filled with sawdust | P. ostreatus | 1 - 2 days | 28 - 30 days | 4 - 6 | 4 – 5 months | 5 - 6 months |
| | G. lucidum | 4 - 5 days | 2 - 3 months | n/a | n/a | n/a |
| Log | P. ostreatus | 4-5 days | 3 - 6 months | n/a | n/a | n/a |
| | G. lucidum | 7-10 days | 6 - 12 months | n/a | n/a | n/a |