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Study on Physiological and Cultural Requirements of *Pleurotus giganteus*

Soytong, K.* **and Asue, T.**

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Abstract Mushrooms are popular due to their nutritional and medicinal properties. The demands for mushrooms are increasing due to their popularity. As new strains and species are discovered and domesticated, the need to study their physiological and cultural requirements is needed. This research aimed to determine some physiological requirements for the growth and cultivation of giant mushroom. Specifically to: determine the effect of pH on the growth of mycelia; test different culture media for mycelial production; and test the effect of nutrient supplementation for fruit production. Results of this study revealed that pH 7 is the optimum pH for *Pluerotus giganteus* to produce big colony size as well as high mycelial fresh and dry weights. The use of broth containing corn flour, corn flour dextrose, coconut, coconut dextrose, ricebran or rice bran dextrose can be used as alternative media in growing mycelium. The use of the product "nano-KS1" produced mushroom fruiting bodies which were more heavy than in the control and supplemented with amino and *Rhodopsuedomonas.*

Keywords: mushroom, pH, culture media

Introduction

Mushrooms are fruiting bodies produced by fungi belonging to family Basidiomycetes. Traditionally, edible mushrooms were harvested or taken from the forest where they grow naturally. In ancient China, mushrooms were regarded as food only for kings and commoners were not allowed to eat them. Mushrooms are sought after due to their distinct flavor and nutritional contents (Stamets, 2000). Mushrooms contain considerable amounts of proteins, vitamins and minerals with little or no fat at all (Tewari, 1986). Some mushroom species are also known to contain active components that can cure certain diseases and health disorders such as diabetes, tumors, cancers and hypertension (Alam *et al*., 2010). Due to these characteristics of mushrooms, the demand had increased rapidly but nature cannot supply much due to the seasonality of mushroom species growing in the wild. As such, there is a need to domesticate and cultivate these mushrooms using alternative substrates to be able to meet the demands. Chinese history showed that Chinese had been consuming mushroom either as food or medicine several hundred of years ago

* **Corresponding author:** Soytong K.**; E-mail:** ajkasem@gmail.com

(Boa, 2004). The earliest record of mushroom cultivation was in France where they grow mushrooms in caves. Today, there are many species of mushrooms under extensive cultivation in industrial scale. Examples are *Agaricus bisporus, Auricularia sp., Lentinus edodes, Pleurotus sp, Volvariella volcacea* and *Flamullina sp.* In 2006, China was the number one producer and consumer of mushroom with an annual production of 14 million tons (Chang, 2006). In 2007, Thailand was reported to have an annual production of 10 thousand tons which was consumed locally or even exported to neighboring countries. The most common mushroom species being cultivated in Thailand are: *Auricularia sp.* (ear mushroom), *Pleurotus sp*. (Oyster mushroom) and *Volvariella volvacea* (straw mushroom).

As new species of edible mushrooms are being discovered, there is a need to study the physiological requirements of the newly discovered mushroom species for possible domestication and subsequent commercialization. One of the most promising species is the giant mushroom or *Pleurotus giganteus*.

Pleurotus giganteus, formerly known as Panus giganteus (Berk) Corner, is a culinary mushroom that is gaining popularity for its organoleptic properties and commercial prospects. Infact, consumption of this used-to-be wild mushroom has been a long tradition in the indigenous villages in Peninsular Malaysia (Lee *et al*., 2009). A variety of this mushroom from China is now being cultivated in Malaysia and the common commercial name in Malay language is "Seri Pagi" (morning glory). In China, P. giganteus is widely referred as "Zhudugu" (swine's stomach) (Deng *et al*., 2006).

Significance of the study

Due to the high demand for giant mushroom as food and source of medicinal substances, the need to know its physiological requirements for mycelial growth as well as fruiting body production should be studied, thus this research. This research aimed to determine some physiological requirements for the growth and cultivation of giant mushroom. Specifically to: determine the effect of pH on the growth of mycelia; test different culture media for mycelial production; and Test the effect of nutrient supplementation for fruit production.

The study was conducted at King Mongkut's Institute of Technology Ladkrabang.

Materials and methods

The pure culture of mushroom

The pure culture of giant cup mushroom (*Pleurotus giganteus*) was made from tissue culture technique. A healthy fruiting body was taken from a commercial farm and brought to the laboratory. Pieces of mushroom tissues (1x1 mm.) were cut from pileus and stipe of the mushroom. The tissues were placed in sterilized water agar in petri plate. When mycelia started to come out or radiate from the tissues, a portion of the medium containing the growing mycelia was taken aseptically and transferred in to fresh sterilized potato dextrose agar then incubated at room temperature.

Preparation of media with different pH

Potato dextrose agar was prepared by boiling 200g diced potatoes in 1L. of water. When the potatoes were soft, they were taken out and the remaining liquid or infusion was filtered. Exactly 20g. dextrose powder was then added to the potato infusion. The mixture was allowed to cool. The mixture was then allocated in to the different treatments. The pH of the media was then adjusted. To reduce the pH, 1 M lactic acid was added drop by drop until the desired pH was achieved. To increase the pH, 1 M NaOH was used. After adjusting the pH agar for each concentration, 15g/L agar was added to each medium. The mixture was then heated to dissolve the agar. The mixture was then sterilized by autoclaving at 121℃ for 30 minutes. The experimental was performed by using completely randomized design (CRD) with 4 replications. Treatment were done as follows:- pH3, pH5, pH7 and pH9.

An agar plug of mushroom pure culture was cut at peripheral colony which measured 0.4 cm , then transferred to PDA with adjusted pH levels. All plates were incubated at room temperature. Data was collected by measuring the colony diameter (cm) and statistically computed using analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05.

Preparation of different media

The different media used were: corn flour (20g/l), rice bran(20g/l), coconut water (200ml/l), dextrose (20g/l) and agar (15g/l). They were prepared separately in flasks. The media were sterilized at 15 psi for 30 minutes and then poured into sterile petri plates.

Grain spawn and spawn bag preparation

Mixed grain composed of sorghum, corn and beans was brought from the market. One kilogram of grain was soaked in water overnight. The grains were then drained and put into bottles, plugged with cotton and sterilized by autoclaving at 121oC for 30 minutes. When the grains cooled, 1x1 mm pure culture of P. giganteus was transferred into each bottle. The bottles were incubated until the mycelia fully covered all the grains. This served as the grain spawn.

Sawdust was used as main substrate for the culture of the mushroom. Sawdust was soaked overnight, added with 1% lime and 1% ricebran, then mixed thoroughly. The mixture was placed in polypropylene bag. Each bag weighed approximately 1 kg each. The bags were autoclaved for 3 hours. When the bags cooled, grain spawn was transferred into them. The bags were incubated until the mycelia fully covered the substrate.

Casing and nutrient supplementation

Ordinary garden soil was used as the main casing material for growing the giant mushroom. Plastic pot measuring 12-inch diameter was used. A fully ramified bag was placed in the center of the pot then covered with the soil approximately 1 inch thick. Nano-KS1 is a product offered by Assoc. Prof. Dr. Kasem Soytong. This product was prepared from nano-chitosan, amino acid and *Rhodopseudomonas.*

The experiment was laid out in CRD with five treatments and four replicates. The treatments were:

T1= Ordinary garden soil with water
T2= Ordinary garden soil with 1% of nano-KS1
T3= Ordinary garden soil with 0.5% of nano-KS1
T4= Ordinary garden soil with 1% of amino and *Rhodopseudomonas*
T5= Ordinary garden soil with 0.5% of amino and *Rhodopseudomonas*

On the first day, 0.5 ml of water containing the treatments was used to drench the respective pots. Watering the pots was done every week. The pots were placed in a dark area and the moisture was maintained by spraying water around the pots two to three times per day. The fruit that developed were harvested when the cap started to curl. The mushrooms were weighed and dried. The data gathered were statistically analyzed.

Results and discussions

The effect of pH

The effect of pH on the colony size, mycelial fresh and dry weights at 5 days after incubation was determined. The result is presented in Table 1.

Table 1. Effect of pH on the colony size, mycelial fresh and dry weights at 5 days

Treatment	Average colony diameter (cm)	Average fresh weight of mycelia (g)	Average dry weight of mycelia(g)
pH3	0.50d	0.20^{d}	0.012^{d}
pH5	4.03b	8.06^{c}	0.324^{c}
pH7	5.95a	11.36^{a}	0.802^{a}
pH9	3.21c	8.68^{b}	0.371^{b}

Statistical analysis revealed that there were significance differences among treatments in all parameters studied. As to average colony diameter, pH 7 produced the biggest colony diameter with 5.95 cm, this was followed by pH 5 and pH 9 with average colony diameter of 4.03 and 3.21. The least colony diameter was observed in pH 3 which is significantly lower than all other treatments, actually no growth occurred. As to average fresh weight of mycelia, pH 7 still got the highest value with 11.36 which were significantly higher than all other treatments. The same was observed in terms of average dry weight of the mycelia.

According to Khan *et al*. (2013) pH is an important factor for good production of Oyster mushroom. Most of the mushrooms grow and perform well at pH near to neutral or light basic. Lime (CaCO3) is an important constituent in mushroom cultivation, commercial cultivation
of mushroom depends upon proper adjustment of pH of substrate. Most of the substrates used for the cultivation of mushroom have pH approximately near to neutral (pH7)

The effect of media

The result of the study on the effect of media on the colony size, mycelial fresh and dry weights at 5 days of incubation is presented in Table 2.

Table 2. Effect of media on the colony size, mycelial fresh and dry weights at 5 days

Treatment	Average colony diameter (cm)	Average fresh weight of mycelia (g)	Average dry weight of mycelia(g)
Potato dextrose broth	5.5750b	11.495[a]	0.765[a]
Corn flour broth	6.3375a	3.776[g]	0.154[e]
Corn flour dextrose broth	6.5a	4.715[f]	0.178[d]
Coconut broth	6.25a	9.341[c]	0.193[d]
Coconut dextrose broth	6.3875a	9.571[b]	0.185[d]
Ricebran broth	6.3875a	7.894[e]	0.640[c]
Ricebran dextrose broth	6.3750a	9.207[d]	0.727[b]

Results revealed that the colony diameter in corn flour, corn flour dextrose, coconut, coconut dextrose, ricebran and rice bran dextrose produced colony which are not significantly different from each other. The control PDB got the least which was significantly lower than all other treatments. However, in terms of fresh and dry mycelial weights, PDB got the highest value which is significantly better than all other treatments. This observation indicates that although the mycelia produced in PDB were smaller than the other media used, the mycelia were thick and heavy.

Effect of nutrient supplementation

The effect of nutrient supplementation on the total number of fruiting bodies, fresh and dry weighs of *P. giganteus* is presented in Table 3.

Table 3. Effect of nutrient supplementation on the fruiting body production of *P. giganteus*

Treatment	Total number of fruiting bodies	Total fresh weight (g)	Total dry weight (g)	Average fruit weight (g)
Control	2.250	62.2016[c]	6.3008[c]	21.9160[b]
Ordinary garden soil with 1% of nano-KS1	4.250	178.9710[a]	18.6695[a]	68.9388[a]
Ordinary garden soil with 0.5% of nano-KS1	3.500	128.7754[ab]	14.1154[ab]	46.2923[ab]
Ordinary garden soil with 1%of amino and *Rhodopseudomonas*	3.75	81.1039[bc]	11.4601[bc]	25.4360[ab]
Ordinary garden soil with 0.5% of amino and *Rhodopseudomonas*	2.500	75.1648[bc]	10.8906[bc]	29.5588[ab]

No significant difference was observed as to the number of fruits in the different treatments. However, the addition of 1% and 0.5% KS1 produced the highest fresh fruit weights which were not significantly different from each other. The control got the least fresh and dry fruit weighs as well as average fruit weight which was significantly lower than all other treatments.

According to Upadhyay *et al*. (2002), Oyster mushrooms Pleurotus spp draw their nutritional requirement from a host substrate or from the agricultural wastes rich in lignin, cellulose and hemicellulose used for their cultivation. Due to varying nutrients in the substrates, different mushroom yields have been recorded by various workers. Nitrogen is an essential element for cellular functions for growth and various metabolic activities particularly protein and enzymes synthesis. The nitrogen content of mycelium ranges between 3 to 6%. Cereal straw used for cultivation of oyster mushroom is a poor source of nitrogen (0.5 to 0.8%) and at the time of fructification when most of the nitrogen is utilized for mycelial growth, the depleted nitrogen in the substrate becomes inadequate and limits mushroom yield. In the present studies seven different organic nitrogen sources: wheat bran, rice bran, soybean floor, de-oiled soybean meal, mustard cake, cotton seed cake and cotton seed meal were evaluated for their effect on mushroom yield. Cotton seed cake and de-oiled soybean meal gave significantly higher yield than unsupplemented bags.

Conclusion

Results of this study revealed that pH 7 is the optimum pH for P. giganteus to produce big colony size as well as high mycelial fresh and dry weights. The use of broth containing corn flour, corn flour dextrose, coconut, coconut dextrose, ricebran or rice bran dextrose can be used as alternative media in growing mycelium. The use of the product “nano-KS1” produced mushroom fruiting bodies which were moreheavy than in the control and supplemented with amino and *Rhodopsuedomonas.*

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