FINAL REPORT

Cultivation of Giant Mushroom (*Pleurotus giganteaus*)

Dr. Kasem Soytong

Department of Plant Production, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

2014

Summary:

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*. Giant cup mushroom (*Pleurotus giganteus*) was conducted to determine the effect of various media and pH levels on the colony growth of giant mushroom *in vitro*. Based on the results, pH 7 was shown to be the optimum pH for mycelial growth. Indigenus media such as rice bran and coconut water with or without addition of dextrose can be used as culture media for *Pleurotus gigantaeus*.

Key words: pH, culture media, giant mushroom

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 924 consuming mushroom either as food or medicine several hundred of years ago (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*, 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.

Giant cup mushroom scientifically know as *Pleurotus giganteus* or *Lentiuns giganteus*. It is the native to south of China more specification in Yunnan. Chinese call it "pig stomach" because of it's size and it's sticky when eaten. Some strain of this mushroom has been cultivated commercially in Malaysia and Thailand. The optimal condition for the growth of *Pleurotus giganteus* is the temperature ranging from 15-35°c with high humidity. The fruiting bodies of *P. giganteus* were found to get high carbohydrate, dietary fibre, potassium, phenolic compounds and triterpenoids. Both aqueous and ethanolic extracts induced neurite outgrowth of PC12 cells in a dose- and time-dependant manner with no detectable cytotoxic effect. At 3 days cultivation, 25 μg/ml of aqueous extract and 15 μg/ml of ethanolic extract showed the highest percentage of neurite-bearing cells, i.e. $31.7 \pm 1.1\%$ and $33.3 \pm 0.9\%$; respectively. Inhibition treatment results suggested that MEK/ERK and PI3K/Akt were responsible for neurite outgrowth of PC12 cells stimulated by *P. giganteus* extract. The high potassium content (1345.7 mg/100 g) may be responsible for promoting neurite extension

(Phan, *et al.*,2012). The pH and suitable media growth of this fungus is rarely found from lietratures. So, in this study was conducted to determine the effect of different media and pH levels on the colony growth of giant mushroom (*Pleurotus giganteus) in vitro*.

Materials and methods

Experimental 1

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. A piece of mushroom tissue of 1x1 MM. was 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 throw 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 potatoes was soft , taken out and the remaining liquid called 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. The reduced the PH 1 Molar lactic acid was added drop by drop until the desired PH was achieved. To increase the PH 1 Molar NaOH was used. After adjusting the pH agar for each concentration, 15g/L agar was added to each medium. The mixture was then boiled to dissolve the agar. The mixture was then sterilized by autoclaving at 121°c for 30 minutes.

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.

Experimental design

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 it measured 0.4 cm , then transferred to each pH level of PDA. All transferred plates were incubated at room temperature. Data was collected by measured colony diameter (cm) and statistically computed using analysis of variance (ANOVA).treatment means were compared using duncan's multiply range test (DMRT) at P=0.05 and p=0.01

Results and discussion

The colony size of *Pleurotus giganteus* in difference levels of pH is show in table 1. Analysis of variance revealed that was no significant difference among treatments on the first day. However, on 2^{nd} day of incubation, pH 7 showed the highest colony size and significantly higher when compare to another treatments and pH 3 showed least colony size among treatments. On 3^{th} day up to 5^{th} day of incubation, pH 7 still showed the highest colony size and significantly higher than all other treatments.

Table1. Colony size as affected by pH levels

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5
pH3	0.5[1]	0.5b	0.5c	0.5c	0.5d
pH5	0.5	0.5b	1.65b	2.9625b	4.0375b
pH7	0.5	1.7a	2.9375a	3.9875a	5.95a
pH9	0.5	0.5b	1.55b	2.4625b	3.2125c

[1]Mean of four replications. Mean followed by a common letters are not significantly different by DMRT at P=0.01.

The colony size of *P. giganteus* is shown in table 2. Analysis of variance revealed that there was no significant difference among treatment on the first day however, on the 2[rd] day of incubation in corn starch medium, coconut and coconut dextrose medium had the highest colony size as compared to another treatment. On 3[th]day of incubation corn starch dextrose medium, ricebran medium, ricebran dextrose medium, coconut medium, coconut dextrose medium and PDA media had the highest colony size as compared to another treatments. On 4[th] day of incubation all treatments except corn starch showed the same colony sizeaccoeding to statistically analysis. The corn starch medium had the least colony growth which significantly lower than the other treatments. On 5[th] day of incubation all kinds of media used except corn starch showed the same colony size according to statistically analysis and the least colony size was observed in corn starch which significantly lower than all other treatments.

Table2. Colony size as affected by media

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5
Corn strach	0.5	1.5375b	2.4250b	4.55b	5.5750b
Corn starch dextrose	0.5	2.1375a	3.3375a	4.8125ab	6.3375a
ricebran	0.5	1.8ab	3.0875a	5.0250a	6.5a
Ricebran dextrose	0.5	1.9250ab	3.3625a	4.55b	6.25a
coconut	0.5	2.0750a	3.3625a	4.9250a	6.3875a
Coconut dextrose	0.5	2.1875a	3.3625a	5.0250a	6.3875a
PDA	0.5	1.8375ab	2.8375ab	4.6375b	6.3750a

[1]Mean of four replications. Mean followed by a common letters are not significantly different by DMRT at P=0.01.

Experimental 2

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.

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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.

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	0.20d	0.012d
pH5	4.03b	8.06c	0.324c
pH7	5.95a	11.36a	0.802a
pH9	3.21c	8.68b	0.371b

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	11.495a	0.765a
Corn flour broth	6.3375a	3.776g	0.154e
Corn flour dextrose broth	6.5a	4.715f	0.178d
Coconut broth	6.25a	9.341c	0.193d
Coconut dextrose broth	6.3875a	9.571b	0.185d
Ricebran broth	6.3875a	7.894e	0.640c
Ricebran dextrose broth	6.3750a	9.207d	0.727b

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	62.2016c	6.3008c	21.9160b
Ordinary garden soil with 1% of nano-KS1	4.250	178.9710a	18.6695a	68.9388a
Ordinary garden soil with 0.5% of nano-KS1	3.500	128.7754ab	14.1154ab	46.2923ab
Ordinary garden soil with 1%of amino and *Rhodopseudomonas*	3.75	81.1039bc	11.4601bc	25.4360ab
Ordinary garden soil with 0.5% of amino and	2.500	75.1648bc	10.8906bc	29.5588ab

Rhodopseudomo nas

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

Based on the results, pH 7 was the optimum pH for mycelial growth. Indigenus media such as rice bran and coconut water with or without addition of dextrose can be used as culture media for *Pleurotus gigantaeus*. 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*.

Acknowledgements

The authors wish to acknowledge the support of Faculty of Agricultural Technology, KMITL, Bangkok, Thailand for funding this research.

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PUBLICATION OF RESERCH PROJECT

Kasem Soytong and Thao Asue (2014) Study on physiological and cultural requirements of *Pleurotus giganteus*. International Journal of Agricultural Technology 10(4):923-930. (TCI group 1).

International Journal of Agricultural Technology 2014 Vol. 10(4): 923-930
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Study on Physiological and Cultural Requirements of *Pleurotus giganteus*

Kasem Soytong* **and Thao Asue**

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* **Corresponding author:** Kasem Soytong; **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).

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Significance of the study

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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*.

Acknowledgements

The authors wish to acknowledge the support of Faculty of Agricultural Technology, KMITL, Bangkok, Thailand for funding this research.

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(Received 31 May 2014; accepted 30 June 2014)