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Research Article

The Effects of Temperature and Nutritional Conditions on Mycelium Growth of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*)

Ha Thi Hoa[1] and Chun-Li Wang[2,*]

[1]*Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*
[2]*Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*

Abstract The influences of temperature and nutritional conditions on the mycelium growth of oyster mushroom *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC) were investigated in laboratory experiment during the summer season of 2014. The results of the experiment indicated that potato dextrose agar (PDA) and yam dextrose agar (YDA) were the most suitable media for the mycelium growth of oyster mushroom PO while four media (PDA, YDA, sweet potato dextrose agar, and malt extract agar medium) were not significantly different in supporting mycelium growth of oyster mushroom PC. The optimal temperature for mycelium growth of both oyster mushroom species was obtained at 28°C. Mycelium growth of oyster mushroom PO was improved by carbon sources such as glucose, molasses, and at 1~5% sucrose concentration, mycelium colony diameter of mushroom PO was achieved the highest value. Whereas glucose, dextrose, and sucrose as carbon sources gave the good mycelium growth of oyster mushroom PC, and at 1~3% sucrose concentration, mycelium colony diameter of PC was achieved the maximum value. Ammonium chloride concentrations at 0.03~0.09% and 0.03~0.05% also gave the greatest values in mycelium colony diameter of mushroom PO and PC. Brown rice was found to be the most favourable for mycelium growth of two oyster mushroom species. In addition, sugarcane residue, acasia sawdust and corn cob were selected as favourable lignocellulosic substrate sources for mycelium growth of both oyster mushrooms.

Keywords Mycelium growth, Nutritional condition, Oyster mushroom, Temperature

Oyster mushroom (*Pleurotus* species) belongs to the family of Tricholomataceae and is usually found clustering naturally on dead trees at spring season [1]. Among all species of mushroom, the oyster mushroom is the second widely cultivated mushroom worldwide following the *Agaricus bisporus* [2]. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency [3]. *Pleurotus* species are efficient lignin degraders which can grow on a wide variety of agricultural wastes and grown at a wide range of temperatures [4]. They have high economical, ecological, and medicinal values. Moreover, they are able to colonize and degrade a large variety of lignocellulosic materials and other wastes which are produced in agricultural, forest and food-processing industries [5].

In general, mushrooms contain 90% of water and 10% of dry mater. Their nutritional value can be compared to that of eggs, milk and meat [6]. *Pleurotus* species are a rich source of proteins, minerals (P, Ca, Fe, K, and Na) and vitamin (thiamine, riboflavin, folic acid, and niacin) [7]. Mushroom protein is intermediate between that of animals and vegetables and it is of superior quality because of the presence of all the essential amino acids [8]. *Pleurotus* species contain high rate of potassium to sodium, which makes mushrooms an ideal food for patients suffering from hypertension and heart diseases [9].

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*Corresponding author
E-mail: lelia@mail.npust.edu.tw

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Several species of oyster mushrooms are very important in the field of medicine. *Pleurotus cystidiosus* (PC) is a strong antioxidant [10]. *Pleurotus ostreatus* (PO) also possesses antitumor activity and it has hypoglycaemic effects in experimentally induced diabetics [11]. These medicinal values of *Pleurotus* species are due to the chemical composition or nutrition of these mushrooms.

The process of cultivating oyster mushrooms has 3 main steps: isolating mushroom from fruiting bodies, preparing primary and secondary spawn, and cultivating mushrooms from these spawns to harvest fruiting bodies [12]. The main factors that affected mycelium growth for processing of spawn production include cultural media, temperature, carbon and nitrogen sources, grain sources, and lignocellulosic substrate sources. The aims of this study are to evaluate and find optimal temperature and nutritional conditions for mycelium growth of two oyster mushroom species including PO and PC for the mushroom spawn production.

MATERIALS AND METHODS

Experimental design. The experiments were conducted in Plant Physiology and Value Added Microorganisms Laboratory, Department of Plant Industry, National Pingtung University of science and Technology (NPUST) in Taiwan during summer 2014 (April to July). The experiments were arranged in a randomized complete design with five replications per treatment.

Sources of materials. Two species of oyster mushroom PC (strain AG 2041) and PO (train AG 2042) were obtained from Plant Physiology and Value Added Microorganisms Laboratory, Department of Plant Industry, NPUST in Taiwan and maintained in potato dextrose agar medium (PDA). The different grains (brown rice, millet, yellow corn, wheat grain), materials to make culture media (yam, sweet potato, potato), lignocellulose substrates (sugarcane residue, acasia sawdust, mixed sawdust (sawdust made from mango and wax apple), corn straw, corn cob, cow manure, wild grass) were obtained from the different sources such as local farmers, local market, the campus of NPUST, bought from merchant in Pingtung county, except the coconut shell fiber was imported from Vietnam. The different chemical and instruments used were obtained from different companies in Taiwan and some were imported from Japan, United States, and Germany.

Temperature and nutritional conditions affecting the growth of mycelium. The effects of culture media, temperature, carbon sources and concentration, nitrogen sources and concentration, grain sources, lignocellulosic substrate on the mycelium growth of two species of oyster mushroom PO and PC for spawn production were studied.

Several trials were designed to evaluate factors affecting mycelium growth of two oyster mushroom species. For each test, mycelium discs with similar diameter (1 cm) were used. The discs were obtained by using the sterilized punch-hole tool to cut the mycelium and place into a new sterilized medium plate by using a transplant needle. The diameter of the mycelium extension was measured every 2 days with the help of a transparent ruler at the same time (between 9:00 AM and 12:00 PM) and surface mycelium density was observed.

Effect of culture media: Four agar media were used including PDA, sweet potato dextrose agar medium (SPDA), yam dextrose agar medium (YDA), and malt extract agar medium (MEA). The ingredients for those culture media were as follows: PDA: 200 g potato, 20 g dextrose, 15 g agar powder, and 1,000-mL distilled water; SPDA: 200 g sweet potato, 20 g dextrose, 15 g agar powder, and 1,000-mL distilled water; YDA: 200 g white yam, 20 g dextrose, 15 g agar powder, and 1,000-mL distilled water; MEA: 20 g malt extract, 20 g dextrose, 15 g agar powder, peptone (1 g), and 1,000-mL distilled water. Media and petri dishes (10-cm diameter) were autoclaved at 121°C (at a pressure of 1.3 kg/cm^2) for 20 min. The mycelium discs (1-cm diameter) of each oyster mushroom were placed in petri dishes containing each culture medium (20 mL) under aseptic condition and incubated at 28°C temperature in the darkness. The diameter of the mycelium expansion was measured every 2 days for 8 days.

Effect of temperature: The petri dishes containing sterilized PDA medium (20 mL) were inoculated with the mycelium discs (1-cm diameter) of each oyster mushroom type under aseptic condition and incubated in the darkness at six levels of temperature (16°C, 20°C, 24°C, 28°C, 32°C, and 36°C). The diameter of the mycelium expansion was measured every 2 days for 8 days.

Effect of different carbon sources and concentrations: Potato agar (PA) medium containing different carbon sources (glucose, dextrose, fructose, maltose, sucrose, and molasses) at 2% concentration was used for the experiment of carbon sources. Influences of different concentrations of sucrose on mycelium growth were investigated at 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%. PA containing 200 g potato, 15 g agar powder, and 1,000-mL distilled water was used as control treatment. The media and petri dishes were autoclaved at 121°C (at a pressure of 1.3 kg/cm^2) for 20 min. The mycelium discs (1-cm diameter) of each oyster mushroom were placed in medium plates (20-mL medium) under aseptic condition and incubated at 28°C in the darkness. The diameter of the mycelium expansion was measured every 2 days for 6 days and the colony morphology was noted.

Effect of different nitrogen sources and concentrations: Potato sucrose agar (PSA) of 2% sucrose concentration containing different nitrogen sources such as 0.05% ammonium chloride (NH_4Cl), 0.05% ammonium sulfate ($N_2H_8SO_4$), 0.05% urea (NH_2CONH_2), 1% peptone, 1% malt extract, 1% soybean powder, and 1% yeast extract were tested. Influences of different ammonium chloride concentrations on mycelium growth were investigated at 0.01%, 0.03%,

0.05%, 0.07%, 0.09%, 0.11%, 0.13%, 0.15%, 0.17%, 0.19%, 0.21%, and 1%. After media and petri dishes were sterilized, the mycelium discs of 1-cm diameter of each oyster mushroom were placed in medium plates (20-mL medium) under aseptic condition and incubated at 28°C in the darkness. The diameter of the mycelium expansion was measured every 2 days for 6 days and the colony morphology was noted

Effect of different grain sources: Four grains were used for this experiment including yellow corn, millet, brown rice, and wheat grain. The grains were washed in clean water three times to remove chaff, dust, and other particles. The grains were then soaked in water for 6 hr for maximum absorption of water. Soaked grains were again washed and cooked by using autoclave for 10 min. After cooling down to room temperature, each grain was supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.036% monopotassium phosphate. Supplemented grains were filled into spawn petri dishes with 60 g grain/petri dish and autoclaved at 121°C for 5 hr. After cooling, each petri dish was inoculated with 1 cm mycelium disc of each oyster mushroom species under aseptic condition and was incubated at 28°C under dark condition. The diameter of the mycelium expansion was measured every 2 days for 10 days.

Effect of different lignocellulosic substrate sources: Eight lignocellulosic materials including sugarcane residue, coconut shell fiber, corn cob, corn straw, acasia sawdust, wild grass, mixed sawdust, and cow manure were investigated. Each lignocellulosic substrate was supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.036% monopotassium phosphate. Each lignocellulosic substrate after supplementing nutrient and distilled water was filled into petri dishes with 20 g lignocellulosic/petri dish and sterilized in an autoclave at 121°C for 5 hr. After cooling, each petri dish was inoculated with a 1-cm mycelium disc of each oyster mushroom (five replications) under aseptic condition and was incubated at 28°C under dark condition. The diameter of the mycelium expansion was measured every 2 days during 10 days.

Statistical analysis. One-way analysis of variance (ANOVA) was conducted with Duncan's multiple range tests to compare the mean significant differences ($p < 0.05$) among treatments by using computer software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA). Each value is expressed as mean ± SE (n = 5).

RESULTS AND DISCUSSION

Effect of different culture media on the mycelium growth. Every living being requires food for its growth and reproduction and oyster mushrooms are not an exception to it. In order to culture the oyster mushroom in the laboratory, it is necessary to furnish those compounds in the media which are requires for its growth and other life process. In the present study, two oyster mushrooms PO and PC were cultured successfully in the laboratory environment. During the present investigation, different media, i.e., PDA, MEA, SPDA, YDA were used to identify their effect on mycelium growth. Data (Table 1) showed that the observed mycelium colony diameter of mushroom PO on four different media significantly differed at ($p < 0.05$) while the mycelium colony diameter of mushroom PC on four media did not significantly differ. Mycelium densities of both oyster mushroom PO and PC were compact in all media. The average mycelium diameter of two oyster mushroom species ranged from 3.92 ± 0.05 to 9.00 ± 0.00 cm at 8 days after inoculation (DAI). The mycelium growth of mushroom PO on PDA, YDA media was better than those on MEA, SPDA media at 2, 4, and 6 DAI; however, they were not significantly different at 8 DAI. The result indicated that these four cultural media are suitable for mycelium growth of mushroom PC while PDA and YDA media are more suitable for the mycelium growth of mushroom PO. This may be due to availability of required nutrients for mushroom PO in PDA and YDA media.

The results of the present research showed that the mycelium of PO took shorter time, but in the case of PC, the mycelium took longer time to complete growth in all media (data not shown). These findings were similar to the results reported by Mshandete and Mgonja [13]; Mansur *et*

Table 1. Effect of different culture media on the mycelium growth of oyster mushroom PO and PC

Culture media	Mycelium colony diameter (cm)								Mycelium density	
	2 DAI		4 DAI		6 DAI		8 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC
SPDA	1.64 ± 0.05^{c}	1.40 ± 0.06^{a}	4.52 ± 0.04^{b}	2.36 ± 0.06^{a}	7.54 ± 0.12^{b}	3.10 ± 0.04^{a}	9.00 ± 0.00^{a}	3.96 ± 0.04^{a}	C	C
PDA	1.96 ± 0.05^{ab}	1.36 ± 0.02^{a}	4.80 ± 0.07^{ab}	1.92 ± 0.04^{b}	8.20 ± 0.14^{a}	3.02 ± 0.03^{a}	9.00 ± 0.00^{a}	3.92 ± 0.05^{a}	C	C
MEA	1.80 ± 0.03^{bc}	1.46 ± 0.04^{a}	3.96 ± 0.11^{c}	2.34 ± 0.07^{a}	7.12 ± 0.05^{b}	3.06 ± 0.07^{a}	9.00 ± 0.00^{a}	3.94 ± 0.10^{a}	C	C
YDA	2.12 ± 0.07^{a}	1.44 ± 0.02^{a}	4.98 ± 0.04^{a}	2.24 ± 0.15^{ab}	8.46 ± 0.12^{a}	3.24 ± 0.18^{a}	9.00 ± 0.00^{a}	3.98 ± 0.09^{a}	C	C

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; DAI, day after inoculation; SPDA, sweet potato dextrose agar medium; PDA, potato dextrose agar medium; MEA, malt extract agar medium; YDA, yam dextrose agar medium; C, compact.

al. [14]. They indicated that PDA and YDA were suitable media for culture of *Pleurotus* species to achieve high level mycelium biomass, exopolysacharides and mycelium protein. In Taiwan, potato is so much cheaper than yam, sweet potato, so PDA medium was more suitable and efficient to use as alternatives for culture of mushroom PO and PC. From those results, we chose local materials as cheaper materials to prepare media for culture of mushroom PO and PC.

Effect of temperature on mycelium growth. Temperature is a very important environment factor for mycelium growth of fungi. To determine optimal temperature for mycelium growth, two species of oyster mushroom were cultivated in PDA medium at various temperatures (16°C, 20°C, 24°C, 28°C, 32°C, and 36°C). The trend of mycelium growth in response to temperature was very similar for the two oyster mushroom species, where the optimum temperature for both oyster mushroom species was found to be 28°C followed by 32°C and 24°C (Table 2, Fig. 1). The mycelium growth of mushroom PO was significantly faster than that of mushroom PC at each temperature tested and PC did not seem to grow after 8 days inoculation at 36°C. The mycelium density of mushroom PC was very thin at 16°C and 36°C. Neelam *et al.* [15] indicated that the optimum temperature for mycelium growth of oyster mushroom *P. florida* was 25~30°C. This optimum temperature result indicated that the two species of oyster mushroom PO and PC were able to grow better in summer and autumn season in subtropical and tropical regions as potential opportunity to develop oyster mushroom production in poor and developing countries in Asia. The result is quite similar to the results reported by Kashangura [16], Choi *et al.* [17] when demonstrating that the mycelium growth and fruiting formation of oyster mushroom species were affected by temperature and they could grow at high temperature as summer season in tropical regions.

Effect of carbon sources and sucrose concentration on mycelium growth. Carbohydrates, which play key roles as structural and storage compounds in cell, are

Table 2. Effect of various temperatures on the mycelium growth of oyster mushroom PO and PC grown on PDA medium

Temperature (°C)	Mycelium colony diameter (cm)								Mycelium density	
	2 DAI		4 DAI		6 DAI		8 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC
16	1.12 ± 0.02^{d}	1.00 ± 0.00^{c}	1.70 ± 0.03^{d}	1.16 ± 0.02^{d}	2.64 ± 0.09^{e}	1.32 ± 0.05^{e}	4.00 ± 0.24^{c}	1.66 ± 0.12^{e}	SC	T
20	1.24 ± 0.01^{cd}	1.00 ± 0.00^{c}	2.56 ± 0.04^{c}	1.28 ± 0.05^{c}	4.08 ± 0.05^{d}	1.78 ± 0.07^{d}	6.76 ± 0.04^{b}	2.38 ± 0.08^{d}	SC	ST
24	1.40 ± 0.71^{c}	1.02 ± 0.02^{c}	4.10 ± 0.03^{b}	1.88 ± 0.05^{b}	6.60 ± 0.10^{c}	2.68 ± 0.16^{c}	9.00 ± 0.00^{a}	3.46 ± 0.07^{c}	C	C
28	2.12 ± 0.05^{a}	1.48 ± 0.02^{a}	4.8 ± 0.09^{a}	2.34 ± 0.06^{a}	8.08 ± 0.14^{a}	3.34 ± 0.06^{a}	9.00 ± 0.00^{a}	4.58 ± 0.09^{a}	C	C
32	1.66 ± 0.07^{b}	1.20 ± 0.00^{b}	4.24 ± 0.07^{b}	2.24 ± 0.02^{a}	7.04 ± 0.24^{b}	2.98 ± 0.05^{b}	9.00 ± 0.00^{a}	4.08 ± 0.06^{b}	SC	C
36	1.68 ± 0.14^{b}	1.00 ± 0.00^{c}	2.70 ± 0.09^{c}	1.00 ± 0.00^{c}	2.96 ± 0.07^{e}	1.02 ± 0.02^{f}	3.12 ± 0.05^{d}	1.26 ± 0.06^{f}	C	T

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; PDA, potato dextrose agar; DAI, day after inoculation; SC, somewhat compact; T, thin; ST, somewhat thin; C, compact.

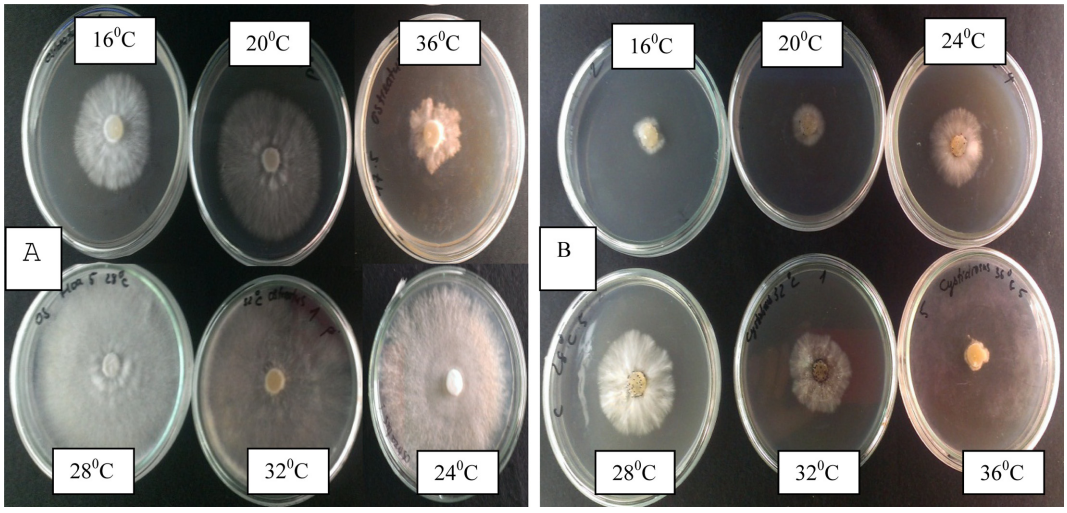

Fig. 1. Mycelium growth of oyster mushroom *Pleurotus ostreatus* (A) and *Pleurotus cystidiosus* (B) grown on potato dextrose agar medium for 8 days after inoculation at different temperatures.

Table 3. Effect of carbon sources of potato agar medium on the mycelium growth of oyster mushroom PO and PC

Carbon sources	Mycelium colony diameter (cm)						Mycelium density	
	2 DAI		4 DAI		6 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC
PA (control)	1.66 ± 0.02^{d}	1.10 ± 0.00^{c}	4.54 ± 0.02^{d}	1.68 ± 0.04^{c}	6.88 ± 0.10^{c}	2.50 ± 0.04^{c}	ST	ST
Glucose	1.94 ± 0.71^{a}	1.24 ± 0.02^{a}	5.26 ± 0.07^{a}	1.96 ± 0.08^{b}	8.60 ± 0.10^{a}	3.50 ± 0.09^{a}	C	C
Lactose	1.44 ± 0.02^{f}	1.18 ± 0.02^{ab}	1.94 ± 0.07^{f}	1.72 ± 0.05^{c}	3.36 ± 0.10^{d}	2.50 ± 0.07^{c}	SC	SC
Dextrose	1.70 ± 0.03^{cd}	1.18 ± 0.71^{ab}	4.52 ± 0.04^{d}	1.98 ± 0.04^{b}	7.96 ± 0.19^{b}	3.44 ± 0.05^{a}	C	C
Fructose	1.52 ± 0.02^{ef}	1.12 ± 0.02^{bc}	4.76 ± 0.06^{bc}	1.48 ± 0.02^{d}	7.98 ± 0.09^{b}	2.52 ± 0.07^{c}	SC	SC
Maltose	1.62 ± 0.04^{de}	1.18 ± 0.02^{ab}	4.26 ± 0.08^{e}	1.80 ± 0.33^{c}	7.06 ± 0.09^{c}	3.18 ± 0.07^{b}	SC	SC
Sucrose	1.82 ± 0.07^{b}	1.20 ± 0.00^{a}	4.86 ± 0.10^{b}	2.24 ± 0.02^{a}	8.54 ± 0.12^{a}	3.54 ± 0.02^{a}	C	C
Molasses	1.78 ± 0.05^{bc}	1.22 ± 0.04^{a}	4.62 ± 0.11^{cd}	1.80 ± 0.04^{c}	8.34 ± 0.09^{a}	3.06 ± 0.04^{b}	C	SC

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; DAI, day after inoculation; PA, potato agar; ST, somewhat thin; C, compact; SC, somewhat compact.

distinguished as monosacharides, disacharides, and polysacharides. Mycelium of many mushrooms will grow to some extent over a wide range of carbon sources. In order to find the optimal carbon, different kinds of carbon sources (2% concentration) including glucose, dextrose, fructose, maltose, sucrose, and molasses were added into the basal medium (PA medium). The result shown in Table 3 reveals the suitability of various carbon sources for mycelium growth of oyster mushroom PO and PC. The result indicated that there was a significant difference in mycelium growth of mushroom PO and PC grown on various carbon sources. Among seven carbon sources tested, glucose, sucrose, and molasses were favorable to the mycelium growth (mycelium colony diameter and mycelium density) of oyster mushroom PO, while glucose, sucrose, and dextrose also gave good mycelium growth of oyster mushroom PC (Table 3). Six DAI, the highest mycelium colony diameters of oyster mushroom PO were obtained from the media containing glucose (8.60 ± 0.10 cm), sucrose (8.54 ± 0.12 cm), and molasses (8.34 ± 0.09 cm), while the highest mycelium colony diameters of mushroom PC were recorded from the media containing glucose (3.50 ± 0.09 cm), sucrose (3.54 ± 0.02 cm), and dextrose (3.44 ± 0.05 cm). On the contrary, six DAI, the slowest mycelium growth obtained in the medium containing lactose as carbon source for both oyster mushroom PO and PC was 3.36 ± 0.10 cm and 2.50 ± 0.07 cm. Fu *et al.* [18], reported that sucrose was the best carbon source for mycelium growth of fungus *Villosiclava virens*. Neelam *et al.* [15] also indicated dextrose was the best carbon source for mycelium growth of *P. florida*. Mao *et al.* [19] demonstrated that glucose was an optimal carbon source for cordycepin production of Chinese traditional medicinal mushroom *Cordyceps militaris*. Moreover, glucose was also identified as the best carbon source for exopolysaccharides production of edible mushrooms such as *Phellinus* [20]. The preference of glucose, sucrose, and molasses by oyster mushroom PO and glucose, sucrose, and dextrose by oyster mushroom PC were may be due to the ease with which it was metabolized to produce cellular energy for the growth of organism. Kurtzman and Zadražil [21] mentioned that the ability of *Pleurotus* species to use different carbon sources may be an expression of the physiological differences in the species or of the isolates, since other isolates of the same species might give different results.

Among good carbon sources (glucose, dextrose, sucrose, and molasses) for PO and PC, sucrose and molasses are the cheapest sources. Sucrose gave significantly highest mycelium colony diameter for both oyster mushrooms PO and PC, while molasses only gave significantly highest mycelium growth of oyster mushroom PO. Sucrose also gave the compact mycelium density of both oyster mushroom PO and PC. Hence, sucrose was more suitable and effective than others for large scale production of oyster mushroom PO and PC. It was chosen as carbon source in the following test.

Among sucrose concentrations 1~5% (10~50 g/L), there was no significant difference in mycelium colony diameter

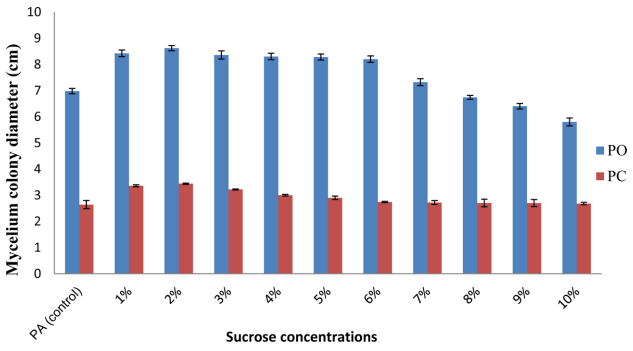

Fig. 2. Effect of sucrose concentrations of potato agar on the mycelium growth of oyster mushroom PO and PC grown for 6 days after inoculation at 28°C. Each value is expressed as mean ± standard error (SE) (n = 5). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; PA, potato agar.

of oyster mushroom PO (Fig. 2). However, six DAI, sucrose concentrations from 1% to 5% gave the highest mycelium colony diameter (8.28~8.62 cm) and significantly higher than those of PA media (control) and other sucrose concentrations. The highest mycelium colony diameter of mushroom PC (3.22~3.44 cm) was also achieved at 1~3% concentration levels of sucrose. However, high sucrose concentrations (more than 3% for PC and more than 5% for PO) were unable to increase mycelium colony diameter. This was explained that the osmotic pressure caused by a high sucrose concentration may be detrimental to the metabolite biosynthesis. On the other hand, a too high carbon concentration also made too high carbon/nitrogen ratio, which will inhibit the mycelium growth. Bai *et al.* [22] indicated that the low carbon concentration (30 g glucose/L) gave the highest mycelium growth and yield of betulin of *Inonotus obliquus* when compared to 40 and 50 g glucose/L. A high initial glucose concentration (55 or 70 g/L) was unfavourable to cordycepin biosynthesis of medicinal mushroom *Cordyceps militaris* [19].

Effect of nitrogen sources and concentration on mycelium growth. Nitrogen is an essential element required by all fungi for the synthesis of nitrogen containing compounds such as purines, pyrimidines, protein and for the cell wall component chitin, which is composed of β(1-4)-linked unit of N-acetylglucosamine [23]. The chemical composition of culture medium, especially the nitrogen concentration, is a mean of physiological control and regulation of microorganism metabolism [24]. In this research, both organic nitrogen and inorganic nitrogen sources were used to evaluate their effects on mycelium growth of oyster mushroom PO and PC.

There was a significant difference in mycelium growth of PO and PC among nitrogen sources (Table 4, Fig. 3). Six DAI, the significantly highest mycelium colony diameters of PO and PC (8.84 ± 0.07 cm and 3.70 ± 0.05 cm, respectively) were obtained in media containing ammonium chloride (NH_4Cl) as nitrogen source. Ammonium sulfate ($N_2H_8SO_4$) is the second best nitrogen source for mycelium growth of both PO and PC but the mycelium colony diameter of oyster mushroom PO was not significantly different as compared to control medium. Six DAI, the significantly lowest values of mycelium colony diameter of PO and PC (5.06 ± 0.06 cm and 3.16 ± 0.06 cm, respectively) were obtained in medium containing soybean (PO) and urea (PC) (Table 4, Fig. 3). There was an increase of mycelium density in all treatments added different nitrogen sources (except urea). However, mycelium colony diameters of both mushroom PO and PC were not improved in media containing some nitrogen sources such as malt extract, soybean, and yeast extract. Mycelium density occurred in the medium containing organic nitrogen was more compact than that in the medium using inorganic nitrogen except for the medium containing ammonium chloride (Table 4, Fig. 3). In another study, Cheng *et al.* [25] demonstrated that ammonium chloride was one of the nitrogen sources to get high dry weight of the fungus *Cryphonectria parasitica*. Neelam *et al.* [15] also reported that ammonium chloride supported growth of mycelium of *P. florida* and *P. ostreatus* better than sodium nitrate and calcium nitrate because nitrate ions have been implicated in the inhibitory effect of some basidiomycetes, which may be difficult to transport across the fungal membrane where it can promote growth. The result is in agreement with Fu *et al.* [18] who showed that ammonium chloride and ammonium sulfate were the most suitable nitrogen sources for mycelium growth of *Villosiclava virens* and in comparison with nitrate nitrogen sources, the ammonium nitrogen sources were favorable for mycelium growth.

Based on the above results, ammonium chloride was selected as a suitable nitrogen source for further studies. Fig. 4 summarized the effects of initial ammonium chloride concentrations on the mycelium growth of oyster mushroom PO and PC. The data presented in Fig. 4 indicate that the mycelium growth of oyster mushroom PO increased with the increasing of ammonium chloride concentration from 0.01% to 0.09% in PSA medium. The mycelium growth of

Table 4. Effect of nitrogen sources of potato sucrose agar medium on the mycelium growth of oyster mushroom PO and PC

Nitrogen sources	Mycelium colony diameter (cm)						Mycelium density	
	2 DAI		4 DAI		6 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC
PSA (control)	1.60 ± 0.04^{cd}	1.24 ± 0.02^{c}	4.68 ± 0.07^{bc}	2.36 ± 0.02^{bc}	8.40 ± 0.10^{b}	3.36 ± 0.02^{de}	SC	SC
Malt extract	1.82 ± 0.08^{b}	1.20 ± 0.00^{c}	4.44 ± 0.04^{cd}	1.54 ± 0.04^{f}	7.60 ± 0.06^{c}	2.74 ± 0.07^{g}	C	C
Ammonium chloride (NH_4Cl)	2.12 ± 0.04^{a}	1.50 ± 0.03^{a}	5.04 ± 0.04^{a}	2.52 ± 0.02^{a}	8.84 ± 0.07^{a}	3.70 ± 0.05^{a}	C	C
Ammonium sulfate ($N_2H_8SO_4$)	2.08 ± 0.04^{a}	1.50 ± 0.00^{a}	4.88 ± 0.07^{ab}	2.40 ± 0.03^{b}	8.52 ± 0.07^{b}	3.56 ± 0.02^{b}	C	SC
Peptone	1.64 ± 0.04^{c}	1.22 ± 0.02^{c}	4.40 ± 0.06^{d}	2.28 ± 0.04^{cd}	7.02 ± 0.16^{d}	3.50 ± 0.03^{bc}	C	C
Urea	1.90 ± 0.71^{b}	1.32 ± 0.71^{b}	4.58 ± 0.05^{cd}	2.10 ± 0.04^{e}	8.32 ± 0.06^{b}	3.16 ± 0.06^{f}	SC	SC
Soybean	1.50 ± 0.00^{d}	1.50 ± 0.00^{a}	3.16 ± 0.02^{f}	2.26 ± 0.02^{d}	5.06 ± 0.06^{e}	3.28 ± 0.02^{ef}	C	C
Yeast extract	1.56 ± 0.04^{cd}	1.46 ± 0.04^{a}	3.78 ± 0.01^{e}	2.44 ± 0.02^{ab}	6.78 ± 0.11^{d}	3.42 ± 0.04^{cd}	C	C

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; DAI, day after inoculation; PSA, potato sucrose agar; SC, somewhat compact; C, compact.

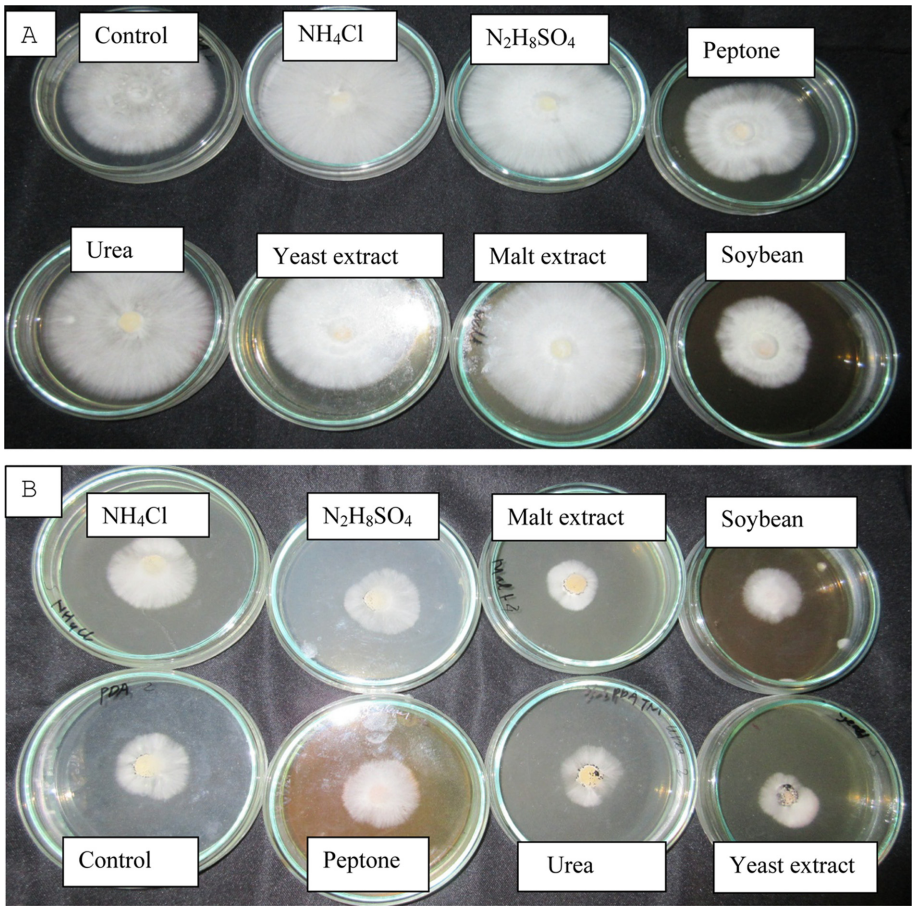

Fig. 3. Mycelium growth of oyster mushroom *Pleurotus ostreatus* (A) and *Pleurotus cystidiosus* (B) grown on different nitrogen sources for 6 days after inoculation at 28°C.

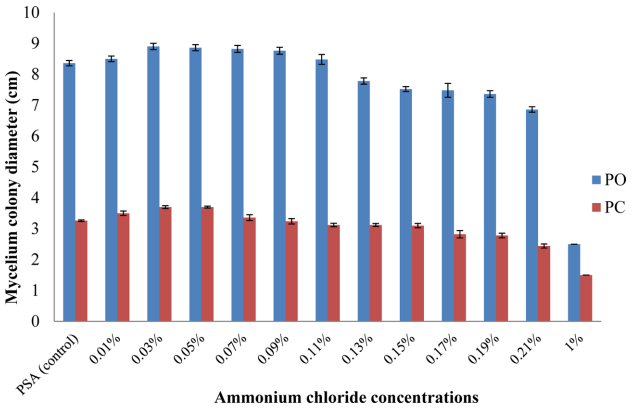

Fig. 4. Effect of ammonium chloride concentrations of potato sucrose agar on the mycelium growth of oyster mushroom PO and PC grown for 6 days after inoculation at 28°C. Each value is expressed as mean ± standard error (SE) (n = 5). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; PSA, potato sucrose agar.

oyster mushroom PC also increased with the concentration of ammonium chloride from 0.01% to 0.05%. The highest colony diameter of PO mycelium achieved with the ammonium chloride concentrations from 0.03% to 0.09%, while the PC mycelium achieved the highest colony diameter with the ammonium chloride concentrations from 0.03% to 0.05%. The mycelium growth of PO and PC decreased when the ammonium chloride concentrations are over 0.09% and 0.05%, respectively. It may be due to a high concentration of nitrogen source leading to a too low carbon/nitrogen ratio, which inhibited the growth of mycelium. In a study using different nitrogen rich supplements for *P. florida* cultivation on corn cob substrate, Naraian *et al.* [26] demonstrated that the low concentration (0.5%) of ammonium sulfate and urea is better for mycelium growth rate of *P. florida* than the higher concentration (1% and 1.5%). On the other hand, in another study, oyster mushroom yield decreases when the ammonia concentration is higher than 68 ppm [27].

Effect of different grain sources on the mycelium growth. Grains which are rich in protein and carbohydrate, usually used as media to produce spawn of edible mushrooms. In the present research, four different grains including brown rice, yellow corn, wheat, and millet were used to indicate their effects on spawn production of oyster mushroom PO and PC (Table 5). The result indicated that,

Table 5. Effect of grain sources on the mycelium growth of oyster mushroom PO and PC

Grain sources	Mycelium colony diameter (cm)										Mycelium density	
	2 DAI		4 DAI		6 DAI		8 DAI		10 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC
PSA (control)	1.72 ± 0.02^{a}	1.20 ± 0.00^{a}	4.82 ± 0.07^{a}	2.24 ± 0.02^{a}	8.56 ± 0.2^{a}	3.44 ± 0.02^{a}	9.00 ± 0.00^{a}	4.60 ± 0.03^{a}	900 ± 0.00^{a}	5.82 ± 0.03^{a}	C	C
Brown rice	1.72 ± 0.04^{a}	1.00 ± 0.00^{b}	2.74 ± 0.17^{bc}	1.20 ± 0.00^{cb}	4.22 ± 0.24^{b}	1.74 ± 0.06^{b}	6.14 ± 0.2^{b}	2.88 ± 0.11^{b}	8.88 ± 0.12^{a}	3.96 ± 0.17^{b}	C	C
Wheat	1.64 ± 0.01^{a}	1.00 ± 0.00^{b}	2.84 ± 0.10^{b}	1.24 ± 0.02^{b}	4.04 ± 0.17^{bc}	1.60 ± 0.04^{c}	5.50 ± 0.13^{c}	2.32 ± 0.15^{c}	8.10 ± 0.11^{b}	3.32 ± 0.15^{c}	SC	SC
Yellow corn	1.50 ± 0.00^{b}	1.00 ± 0.00^{b}	2.46 ± 0.06^{c}	1.12 ± 0.02^{d}	3.56 ± 0.10^{c}	1.54 ± 0.04^{c}	5.14 ± 0.16^{c}	2.32 ± 0.14^{c}	8.28 ± 0.12^{b}	3.28 ± 0.12^{c}	C	C
Millet	1.48 ± 0.02^{b}	1.00 ± 0.00^{b}	1.86 ± 0.02^{d}	1.14 ± 0.02^{cd}	2.40 ± 0.07^{d}	1.30 ± 0.00^{d}	3.58 ± 0.05^{d}	1.56 ± 0.04^{d}	5.06 ± 0.04^{c}	2.48 ± 0.02^{d}	C	SC

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).
PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; DAI, day after inoculation; PSA, potato sucrose agar; C, compact; SC, somewhat compact.

Table 6. Effect of lignocellulosic substrate sources on the mycelium growth of oyster mushroom PO and PC

Linocellosic sources	Mycelium colony diameter (cm)										Mycelium density	
	2 DAI		4 DAI		6 DAI		8 DAI		10 DAI			
	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC	PO	PC
PSA (control)	1.74 ± 0.06^{a}	1.24 ± 0.02^{a}	4.88 ± 0.07^{d}	2.36 ± 0.02^{a}	8.48 ± 0.12^{a}	3.36 ± 0.02^{a}	9.00 ± 0.00^{a}	4.40 ± 0.06^{a}	9.00 ± 0.00^{a}	5.42 ± 0.06^{a}	C	C
Sugarcane residue	1.58 ± 0.02^{bc}	1.00 ± 0.00^{b}	2.12 ± 0.04^{c}	1.28 ± 0.05^{b}	3.84 ± 0.07^{b}	1.82 ± 0.07^{b}	5.42 ± 0.06^{b}	2.90 ± 0.04^{b}	6.80 ± 0.09^{b}	3.78 ± 0.06^{b}	C	C
Acasia sawdust	1.62 ± 0.02^{b}	1.00 ± 0.00^{b}	2.30 ± 0.04^{b}	1.30 ± 0.00^{b}	3.44 ± 0.03^{c}	1.78 ± 0.05^{bc}	4.66 ± 0.09^{c}	2.72 ± 0.07^{b}	6.68 ± 0.05^{b}	3.66 ± 0.10^{b}	C	C
Mixed sawdust	1.52 ± 0.02^{cde}	1.00 ± 0.00^{b}	2.04 ± 0.04^{cd}	1.10 ± 0.00^{de}	2.80 ± 0.03^{e}	1.48 ± 0.02^{e}	3.76 ± 0.09^{e}	2.88 ± 0.02^{b}	4.74 ± 0.05^{d}	2.28 ± 0.02^{e}	SC	SC
Corn straw	1.54 ± 0.02^{bcd}	1.00 ± 0.00^{b}	2.14 ± 0.04^{c}	1.20 ± 0.00^{c}	3.24 ± 0.06^{d}	1.66 ± 0.02^{d}	4.22 ± 0.04^{d}	2.10 ± 0.13^{c}	6.54 ± 0.02^{d}	3.16 ± 0.09^{c}	SC	ST
Corn cob	1.46 ± 0.02^{de}	1.00 ± 0.00^{b}	1.92 ± 0.09^{d}	1.20 ± 0.00^{c}	3.20 ± 0.03^{d}	1.68 ± 0.02^{cd}	4.18 ± 0.02^{d}	2.20 ± 0.04^{c}	5.72 ± 0.02^{c}	3.24 ± 0.02^{c}	C	C
Cow manure	1.46 ± 0.02^{de}	1.00 ± 0.00^{b}	1.70 ± 0.04^{e}	1.04 ± 0.02^{ef}	2.60 ± 0.05^{f}	1.28 ± 0.05^{f}	3.54 ± 0.07^{e}	1.70 ± 0.05^{d}	4.16 ± 0.10^{e}	2.08 ± 0.04^{f}	ST	T
Wild grass	1.44 ± 0.02^{e}	1.00 ± 0.00^{b}	1.64 ± 0.02^{e}	1.16 ± 0.02^{cd}	2.36 ± 0.06^{g}	1.40 ± 0.03^{e}	3.50 ± 0.14^{e}	1.74 ± 0.07^{d}	4.06 ± 0.23^{e}	2.46 ± 0.04^{d}	ST	T
Coconut shell fiber	1.44 ± 0.02^{e}	1.00 ± 0.00^{b}	1.72 ± 0.04^{e}	1.00 ± 0.00^{f}	2.12 ± 0.09^{h}	1.10 ± 0.00^{g}	2.52 ± 0.12^{f}	1.32 ± 0.02^{e}	3.34 ± 0.10^{f}	1.48 ± 0.02^{g}	SC	ST

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean ± standard error (SE) (n = 5).
PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; DAI, day after inoculation; PSA, potato sucrose agar; C, compact; SC, somewhat compact; ST, somewhat thin; T, thin.

in all grain cases, mycelium extensions of mushroom PC and PO were significantly slower than that of control treatments (Table 5). When compared to mycelium extension of mushroom PC, mycelium extension of mushroom PO was higher. Mycelium densities of both oyster mushroom PO and PC on grain media were compact and somewhat compact. Brown rice was found to be the most favorable to the mycelium growth of mushroom PO and PC. On the 10th day of incubation, the highest mycelium colony diameters of PO and PC obtained in brown rice medium were 8.88 ± 0.12 cm and 3.96 ± 0.17 cm, respectively. Yellow corn and wheat were next to brown rice with mean values for mycelium colony diameter of PO being 8.28 ± 0.12 cm and 8.10 ± 0.11 cm, respectively. Those values of PC were 3.28 ± 0.12 cm and 3.32 ± 0.15 cm, respectively. All grains supported the mycelium growth of two studied *Pleurotus* species but in varying degrees. The least support for both species studied was shown by millet. This may be due to millet grain size being very small when compared to brown rice, wheat, yellow corn. Tinoco *et al.* [28] found that larger surface and pore of substrates were better supports for mycelium growth rate. This could account for the results recorded by brown rice, wheat and yellow corn. Sofi *et al.* [29] reported that mycelium colony diameter of *P. ostreatus* fungi MTCC-1801 in various grains was significantly affected by substrate style. The author also indicated that the larger surface area and spore of substrates are responsible for the more mycelium growth rate.

Effect of different lignocellulosic substrate sources. In this research, some agroforestry wastes such as sugarcane residue, acasia sawdust, mixed sawdust, corn straw, corn cob, cow manure, wild grass, and coconut shell fiber were used as substrates to evaluate their effect on the mycelium growth of oyster mushroom PO and PC. The data summarized in Table 6 indicated that the mycelium growth of oyster mushroom PO and PC in all cases was significantly lower than that in control media. The mycelium growth trend of two oyster mushroom species in response to lignocellulosic substrates was very similar. Ten DAI, the greatest values of mycelium colony diameter of oyster mushroom PO and PC were recorded at sugarcane residue substrate (6.68 ± 0.05 cm and 3.66 ± 0.10 cm, respectively). However, they were not significantly different when compared to those in acasia sawdust substrate. The lowest mycelium colony diameters were obtained in coconut shell fiber for both PO and PC mushroom. Table 6 showed that substrates made from sugarcane residue, acasia sawdust, and corn cob were more suitable for the mycelium growth (including mycelium colony diameter and density) of both mushroom PO and PC as compared to mixed sawdust, corn straw, cow manure, wild grass and coconut shell fiber. This may be due to rich carbon and nitrogen composition in these substrates which support good mycelium growth. Moreover, the saprophytic ability of those fungi also supported to produce extracellulase hydrolyzing enzymes (cellulose, laccase, and lignase) to hydrolyze cellulose, lignin and hemicelluloses present in these substrates to simple sugars for growth. On the other hand, Badu *et al.* [30] demonstrated that the nutrient content, the growth and yield of oyster mushroom cultivated on sawdust depend on the chemical constituents such as the cellulose content, the hemicelluloses content and the lignin content. Substrates made from *Triplochiton scleraxylon* containing high concentration of lignin (34.08%) gave the lower yield of mushroom than that in *Ceiba pentandra* sawdust substrates containing low rate of lignin (27.55%). Shah *et al.* [31] reported that sawdust gave maximum yield and the leaves gave minimum yield of PO. In another study, Kuforiji and Fasidi [32] also reported that substrates such as corn cob, rice straw, and sawdust of *Mansonia altissima* and *Boscia angustifolia* supported the growth of *Pleurotus tube-regiun.*

Based on the above results, sugarcane residue, acasia sawdust, and corn cob were selected as the most suitable lignicellulosic substrate sources to produce PO and PC mushroom spawn besides spawn source produced from grains. Moreover, these lignocellulosic substrates were also suggested to cultivate oyster mushroom PO and PC.

In conclusion, the mycelium growth of oyster mushroom PO and PC were affected by different temperature conditions and nutritional sources. It was concluded that PDA, SPDA, YDA, and MEA were the most favourable media for the mycelium growth of mushroom PC while PDA and YDA were the most suitable media for mycelium growth of oyster mushroom PO. Maximum mycelium growth of oyster mushroom PO and PC was achieved by cultivating them at the optimum temperature of 28°C. In regarding to carbon source and concentration, PO mushroom was identified to be the most suitable to PA medium with 1~5% sucrose concentration, while 1~3% sucrose concentration gave the good mycelium growth of oyster mushroom PC. This study also determined that ammonium chloride concentrations at 0.03~0.09% and 0.03~0.05% gave the greatest values in mycelium colony diameter of oyster mushroom PO and PC, respectively. Regarding to grain and lignicellulosic substrate sources to produce mushroom spawn, brown rice followed by yellow corn and wheat was found to be favourable grains, while sugarcane residue, acasia sawdust, and corn cob were suitable lignicellulosic substrate sources for the mycelium growth of oyster mushroom PO and PC. In all studied cases, mycelium growth of oyster mushroom PO was significantly better than that of oyster mushroom PC.

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