Effects of Natural Carbon Sources and Temperature on Mycelium Cultivations of *Lentinus squarrosulus* (Mont.), *Lentinus polychrous* Lev., *Pleurotus ostreatus* (Jacq.ex Fr.) P. Kumm. and *Volvariella volvacea* (Bull.) Singer

Chanida Kupradit*, Araya Ranok, Seksan Mangkalanan,

Chompoonuch Khongla and Sumalee Musika

Department of Applied Biology, Faculty of Sciences and Liberal Arts,

Rajamangala University of Technology Isan, Suranarai Road, Muang, Nakhon Ratchasima 30000

Received: December 6, 2019; Accepted: January 24, 2020

Abstract

Mycelial biomass can be used as an inoculum source for mushroom production and is a suitable source to produce useful substances. Temperature and carbon sources play a role in the production of biomass and bioactive compounds of mycelium cultivation. Thus, natural carbon sources including potato, sweet potato, and taro were used in this mycelium cultivation study. The purposes of this study were to investigate the effects of carbon sources and temperature on mycelium cultivation for four edible mushrooms. The growth of *Lentinus squarrosulus* (Mont.), *Lentinus polychrous* Lev., *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. and *Volvariella volvacea* (Bull.) Singer in culture media with various carbon sources at different temperatures were examined for their growth based on biomass and diameter. The results indicated that potato dextrose broth (PDB) was a suitable medium for growing *P. ostreatus*, *V. volvacea* and *L. squarrosulus*. The optimum temperature for the mycelial growth of *V. volvacea*, *L. squarrosulus* and *P. ostreatus* with maximum yields of 8.47±0.26 g/L, 6.44±0.30 g/L and 3.76±0.18 g/L, respectively, was 30±2°C after 7 days of incubation using a submerged cultivation. For *L. polychrous*, a sweet potato dextrose broth (SPDB) was a suitable medium for mycelial production (6.16±0.69 g/L) when cultivated at 35±2°C for 7 days. For mycelia cultivation on an agar medium, the maximum mycelium colony diameter of four edible mushrooms (6-9 cm) were obtained from the taro dextrose agar (TDA) medium at 3-8 days of incubation. Therefore, TDA was suitable and efficient mediums for cultivation of the four edible mushrooms.

Keywords: mushroom; mycelium cultivation; potato; sweet potato; taro

**Corresponding author: lego7823@hotmail.com* DOI: 10.14456/tjst.2020.57

1. Introduction

Mushrooms are highly nutritious and have an increasing demand worldwide. High protein content has been detected in the fruit bodies and mycelium of *Pleurotus ostreatus*, *Lentinus edodes*, *Volvariella esculenta* and *Termitomyces clypeatus*. Their mycelia contain amino acids, including glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine (Nwokoye *et al*., 2010). Mushrooms are also considered as the effective folk medicines. Their adaptations to extreme living conditions in liquid media have been exploited to produce useful substances such as antibacterial agents, antifungals and enzymes (Vahidi *et al*., 2006).

Mushrooms can be cultivated on a large scale using solid state and submerged culture techniques. However, commercial cultivation of the fruiting body of the mushrooms using a solid state limits their availability for use as foods and medicines (Anike *et al*., 2015). Submerged culture is an alternative for the efficient production of mycelium biomass. This process has a number of advantages, such as better control of the physicochemical parameters, as well as the potential for high biomass production in a compact space, shorter growth time, and independence of seasonality. The mycelial biomass produced by submerged fermentation can be used as an inoculum source for mushroom production in semi- solid fermentations, in food additives, and for the extraction of antimicrobial compounds, flavorings, polysaccharides, antioxidants, etc. (de Souza Kirsch *et al*., 2016). Therefore, mycelia from submerged fermentation are a suitable alternative source to produce useful substances from several mushrooms.

The fungal *Pleurotus*, *Lentinus* and *Volvariella* genus have been intensely studied and cultivated in many different parts of the world. *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. (oyster mushroom) is an edible mushroom with excellent flavor and taste (Ibekwe *et al*., 2008) while research on *L. squarrosulus* (Mont.) has shown antimicrobial activity (Giri *et al*., 2012). In our research, four edible mushrooms, including *L. squarrosulus* (Mont.) (Hed Khon Khaw), *L. polychrous* Lev. (Hed Kra dang), *P. ostreatus* (Jacq. ex Fr.) P. Kumm. (oyster mushroom) and *V. volvacea* (Bull.) Singer (straw mushroom) were investigated for the optimal conditions to produce mycelia biomass. These mushrooms are well suited for cultivation in the tropics because of their requirements for higher temperatures for rapid growth.

For mushroom mycelium cultivations, temperature and carbon sources influence the biomass and bioactive compound production. Various compounds can be used as a source of carbon and energy (Vahidi *et al*., 2006) for the cultivation of mushroom mycelium. A natural medium containing starchy root and tuber crops could be an alternative source for the production of food and cosmetic additives from mushroom mycelium. Starchy root and tuber crops such as *Solanum tuberosum* (potatoes), yams, and *Colocasia esculenta* (taro) are important global

sources of carbohydrates (Chandrasekara and Josheph, 2016). The allergen agents found in the synthetic medium should be reduced by using this natural medium for mycelium mushroom production. Thus, the mycelium biomass for other applications in food and cosmetics should be safe from the synthetic chemicals in the synthetic medium.

In most developing countries, potato is generally more expensive than other starchy root and tuber crops. In Thailand, *Ipomoea batatas* (sweet potato) and taro are widely cultivated and are available year round in contrast with potatoes. These cash crops not only have high carbohydrate content but are also rich in proteins, vitamins and mineral elements (Wongjiratthiti and Yottakot, 2017).

In this study, the effects of temperature and natural carbon sources on the mycelium cultivation for four different edible mushrooms were investigated. The growth of the four edible mushrooms, *L. squarrosulus*, *L. polychrous*, *P. ostreatus* and *V. volvacea*, in broth and on agar media of the tuber crops; sweet potato, potato, and taro, at different temperatures was examined and the optimal conditions for mycelia production were evaluated.

2. Materials and methods

2.1 Preparation of mushroom mycelium pure culture

The fresh fruiting bodies of mushroom were purchased from local mushroom farms, Nakhon Ratchasima, Thailand. Pure cultures of *L. squarrosulus*, *L. polychrous*, *P. ostreatus* and *V. volvacea* were prepared from a piece of a specific fruiting body mushroom. A small piece of each specific mushroom from each fresh fruiting body was aseptically transferred to potato dextrose agar (PDA) (Himedia, Mumbai, India) and incubated at 30±2 ºC for 5 days. The pure culture of each mushroom mycelium was maintained on a PDA slant and stored at 4 ˚C for long term use. For inoculum preparation, the PDA slant of each mushroom mycelium was aseptically placed in the center of a PDA plate and cultivated at 30±2 ºC for 5 days.

2.2 Preparation of culture medium

All natural carbon sources including potato, sweet potato, and taro were purchased from a local market. For the preparation of the potato dextrose broth (PDB), sweet potato dextrose broth (SPDB) and taro dextrose broth (TDB), 200 g slices of the natural carbon sources (potato, sweet potato and taro) were boiled in 1,000 mL distilled water for 15 min. The boiling solutions were filtered through a cheesecloth. The filtrates were mixed with 20 g of dextrose. The medium broth volume was adjusted to 1,000 mL with distilled water. For the agar culture preparations, 15 g of agar were added to 1,000 mL of PDB, SPDB and TDB for potato dextrose agar (PDA), sweet potato dextrose agar (SPDA) and taro dextrose agar (TDA) preparations, respectively. All the culture media were adjusted to a final pH 5 and autoclaved at 121 ºC for 15 min.

2.2 Effects of carbon sources and temperature on mycelia cultivation in a submerged culture

One piece of a 10 mm mycelium disc from an inoculum culture (5 days of cultivation) of each mushroom mycelium on PDA was cut from the margin of an actively growing mycelium colony using a cork borer and transferred to 150 mL flasks containing 50 mL of PDB, SPDB and TDB. The cultures were incubated at 30±2 and 35±2 ºC in static conditions for 7 days. The mycelium dry weights were determined each day of the incubation for 7 days. The mycelium from 50 mL of each culture condition was filtered using Whatman No. 1 and dried at 65±2 ºC until achieving a constant weight. The dry weight of the mushroom mycelium was obtained using a digital balance.

2. 3 Effects of carbon sources on mycelia cultivation using agar medium

Three agar media, PDA, SPDA and TDA, were used. The mycelium disc 10 mm of each mushroom mycelium inoculums on PDA (5 days of cultivation) were placed in Petri dishes containing each culture medium under aseptic conditions and incubated at 30±2 ºC. The diameter of the mycelium expansion was measured every day for 5-8 days.

2.4 Statistical analysis

All experiments were performed in duplicates. The data were expressed as mean± standard deviations and shown as error bars. Statistical analysis was conducted using PASW Statistics 18 Release 18.0.0 software. Mycelium yield obtained from different carbon sources at the same cultivation temperature were determined to be significantly different ($p < 0.05$) by one- way analysis of variance (one- way ANOVA). The effect of cultivation temperature on the mycelium yield in the same medium were determined to be significantly different ($p < 0.05$) by student T- test.

3. Results and discussion

3.1 Effects of carbon sources and temperature on mycelia cultivation in a submerged culture

In the present study, the effects of three carbon sources, PDB, SPDB and TDB, on the mycelia growth of four edible mushrooms were investigated. The inoculated media were incubated at 30±2 and 35±2 ºC for 7 days. The mycelium morphology of four edible mushrooms were different and specific for each mushroom species but it was not different between the carbon sources and temperatures as shown in Figure 1. The results of the mycelium biomass yield from different media and incubation temperatures are shown in Figure 2, 3 and Table 1. The mycelium biomass yields of *P. ostreatus* harvested from PDB (1.33±0.60 g/L), SPDB (1.92±0.18 g/L) and TDB (0.89±0.23 g/L) media did not significantly increase after 5 days of cultivation at 35±2 ºC at $p > 0.05$ (Figure 2A, Table 1). However, the mycelium biomass yields of *V. volvacea* and *L. squarrosulus* from the cultivation on PDB was significantly higher than that on TDB and SPDB after 7 days of incubation at 35±2 ºC ($p < 0.05$, Figure 2B, 2D and Table 1). For the production of *L. polychrous*, the mycelium biomass yield on SPDB (6.16±0.69 g/L) was significantly higher than that on TDB (4.31±0.06 g/L) at $p < 0.05$, but it did not differ

significantly ($p > 0.05$) from cultivation on PDB (5.80±0.66 g/L) after 7 days of incubation at 35±2 ºC (Figure 2C, Table 1). The mycelium biomass yield of *P. ostreatus* harvested from PDB (3.76±0.18 g/L) was significantly higher than that from TDB (2.45±0.24 g/L) after 7 days of incubation at 30±2°C with $p < 0.05$ (Table 1). However, the mycelium biomass yields of *P. ostreatus* from cultivation on PDB (3.76±0.18 g/L) and on SPDB (2.95±0.46 g/L) were not significantly different after 7 days of cultivation at 30±2 ºC with $p > 0.05$ (Figure 3A, Table 1). Similar results were found for the *V. volvacea* and *L. squarrosulus* cultivations where the mycelium biomass production of *V. volvacea* and *L. squarrosulus* on

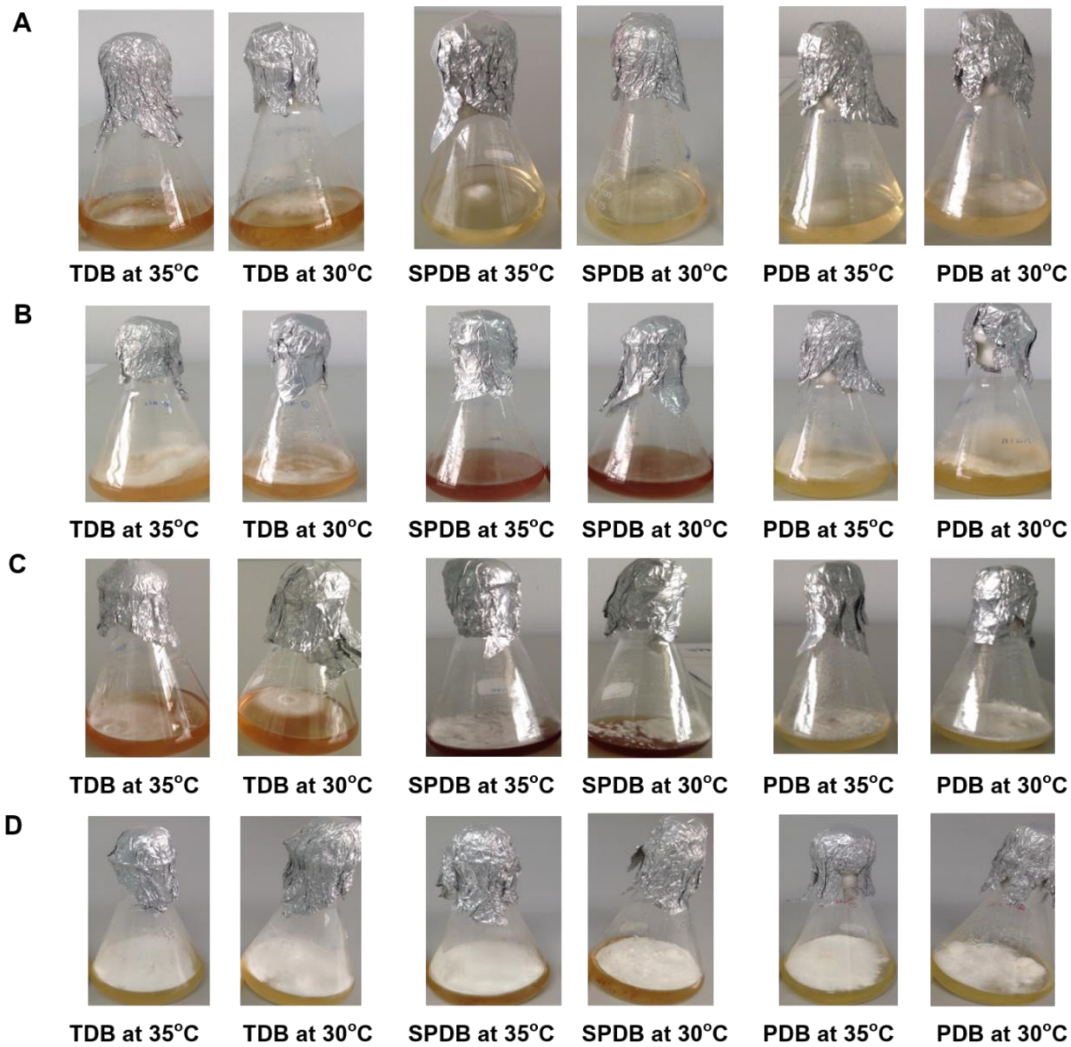

Figure 1 Mycelium morphology of: (A) *P. ostreatus*; (B) *V. volvacea*; (C) *L. polychrous*; and (D) *L. squarrosulus* cultivations in different carbon sources at 35±2 and 30±2 ºC using submerge cultivation for 7 days.

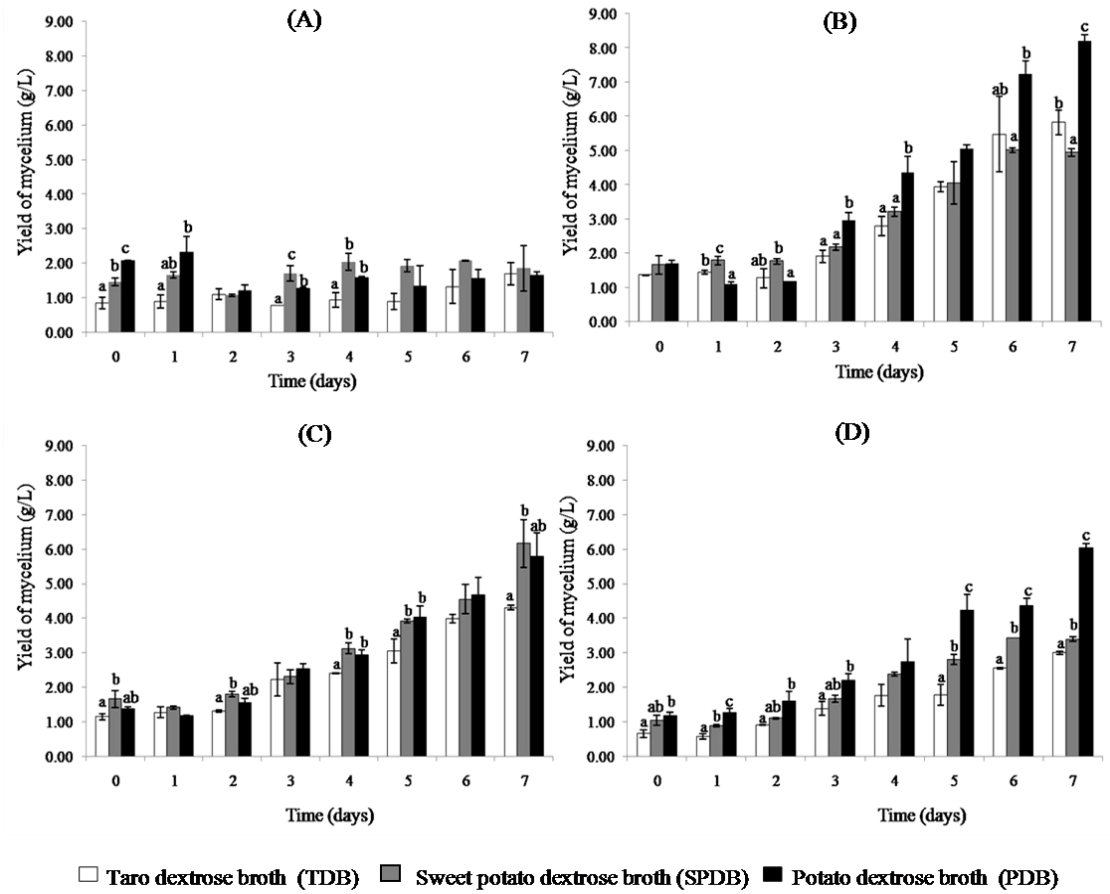

Figure 2 The effects of different natural carbon sources on the mycelium biomass yields of: (A) *P. ostreatus*; (B) *V. volvacea*; (C) *L. polychrous*; and (D) *L. squarrosulus* at 35±2 ºC using submerged cultivation. The error bars in the figure indicate standard deviation. Bars with different letters determined the significant difference between carbon sources for each day ($p < 0.05$).

PDB was significantly higher than that on TDB and SPDB after 7 days of incubation at 30±2 ºC ($p < 0.05$, Figure 3B, 3D and Table 1). However, a different result was observed in the cultivation of *L. polychrous*. The mycelium biomass yield of *L. polychrous* obtained from cultivation on SPDB (5.24±0.06 g/L) was significantly higher than that on TDB (3.37±0.48 g/L) and on PDB (4.23±0.01 g/L) after 7 days of incubation at 30 ºC ($p < 0.05$, Figure 3C, Table 1). The suitable conditions for maximum mycelium yield of *P. ostreatus* cultivation were 30±2 ºC for 7 days using PDB as the culture medium. The maximum mycelium yield obtained was 3.76±0.18 g/L, which was significantly higher than that obtained using TDB (2.45±0.24 g/L) under the same conditions ($p < 0.05$). However, sweet potato could also be used as a carbon

Table 1 The effects of natural carbon source on mycelial yield of four edible mushroom using submerged cultivation

Mushrooms	Days	Yields of mycelium (g/L)					
		35±2 ºC			30±2 ºC		
		TDB	SPDB	PDB	TDB	SPDB	PDB
P. ostreatus	0	0.85±0.17^{a}	1.46±0.12^{b}	2.07±0.01^{c}	0.65±0.45^{a}	1.46±0.12^{ab}	2.07±0.01^{b}
	1	0.89±0.18^{a}	1.66±0.08^{ab}	2.31±0.47^{c}	0.69±0.40^{a}	1.55±0.09^{b}	2.51±0.16^{c}
	2	1.10±0.16	1.07±0.03	1.20±0.17	1.30±0.05	1.77±0.41	1.28±0.11
	3	0.79±0.00^{a}	1.70±0.23^{c}	1.28±0.03^{b}	0.79±0.02^{a}	1.32±0.02^{b}	1.77±0.01^{c}
	4	0.93±0.21^{a}	2.04±0.25^{b}	1.58±0.04^{b}	1.16±0.00^{a}	2.27±0.55^{b}	1.79±0.19^{ab}
	5	0.89±0.23	1.92±0.18	1.33±0.60	1.48±0.01^{a}	2.54±0.31^{b}	2.02±0.19^{ab}
	6	1.33±0.49	2.06±0.01	1.56±0.26	1.98±0.01	2.28±0.51	2.60±0.01
	7	1.69±0.32	1.85±0.65	1.65±0.10	2.45±0.24^{a}	2.95±0.46 ^{ab}	3.76±0.18^{b}
V. volvacea	0	1.37±0.01	1.66±0.27	1.70±0.10	1.37±0.01	1.66±0.27	1.69±0.10
	1	1.45±0.06^{b}	1.79±0.13^{c}	1.11±0.05^{a}	1.35±0.19^{a}	1.70±0.04^{b}	1.10±0.04^{a}
	2	1.27±0.28 ^{ab}	1.77±0.08^{b}	1.20±0.00^{a}	1.27±0.34	1.52±0.05	1.17±0.02
	3	1.91±0.19^{a}	2.17±0.09^{a}	2.97±0.22^{b}	1.33±0.03^{ab}	1.24±0.20^{a}	1.72±0.09^{b}
	4	2.80±0.27^{a}	3.21±0.14^{a}	4.38±0.47^{b}	2.41±0.06	2.69±0.20	2.88±0.16
	5	3.94±0.14	4.06±0.62	5.07±0.09	3.34±0.77	3.85±0.32	3.96±1.06
	6	5.48±1.10^{ab}	5.01±0.06^{a}	7.24±0.39^{b}	4.57±0.05^{ab}	4.29±0.27^{a}	5.53±0.46^{b}
	7	5.82±0.37^{b}	4.95±0.12^{a}	8.22±0.17^{c}	5.64±0.29^{a}	5.16±0.40^{a}	8.47±0.26^{b}
L. polychrous	0	1.15±0.09^{a}	1.66±0.24^{b}	1.37±0.07^{ab}	1.15±0.09^{a}	1.66±0.24^{b}	1.37±0.07^{a}
	1	1.28±0.15^{a}	1.41±0.05^{a}	1.17±0.02^{a}	1.12±0.00^{a}	1.46±0.13^{b}	1.27±0.12^{ab}
	2	1.31±0.03^{a}	1.80±0.08^{b}	1.56±0.12^{ab}	1.16±0.01^{a}	1.55±0.07^{c}	1.39±0.02^{b}
	3	2.22±0.48^{a}	2.30±0.20^{a}	2.54±0.15^{a}	1.53±0.09^{a}	2.04±0.05^{b}	2.01±0.00^{b}
	4	2.40±0.01^{a}	3.12±0.15^{b}	2.93±0.15^{b}	1.89±0.14	2.38±0.24	2.82±0.59
	5	3.05±0.34^{a}	3.92±0.06^{b}	4.03±0.32^{b}	2.64±0.15^{a}	3.62±0.31^{b}	2.80±0.31^{ab}
	6	3.98±0.13^{a}	4.55±0.42^{a}	4.67±0.50^{a}	3.24±0.06^{a}	3.75±0.11^{b}	3.82±0.02^{b}
	7	4.31±0.06^{a}	6.16±0.69^{b}	5.80±0.66^{ab}	3.37±0.48^{a}	5.24±0.06^{b}	4.23±0.01^{a}
L. squarrosulus	0	0.66±0.11^{a}	1.05±0.15^{ab}	1.19±0.10^{b}	0.66±0.11^{a}	1.05±0.15^{ab}	1.19±0.10^{b}
	1	0.58±0.07^{a}	0.89±0.03^{b}	1.26±0.13^{c}	0.69±0.07^{a}	0.97±0.05^{b}	1.27±0.08^{c}
	2	0.92±0.01^{a}	1.11±0.02^{ab}	1.59±0.29^{b}	0.93±0.06	1.13±0.05	2.41±1.47
	3	1.38±0.20^{a}	1.67±0.10^{ab}	2.20±0.18^{b}	1.52±0.15^{a}	1.79±0.04^{ab}	2.25±0.28^{b}
	4	1.77±0.31	2.38±0.06	2.73±0.67	2.01±0.11^{a}	2.88±0.01^{b}	3.28±0.24^{b}
	5	1.78±0.31^{a}	2.80±0.14^{b}	4.24±0.44^{c}	2.89±0.05^{a}	3.53±0.21^{b}	5.17±0.10^{c}
	6	2.56±0.02^{a}	3.44±0.00^{b}	4.37±0.21^{c}	3.41±0.28^{a}	4.73±0.34^{b}	5.02±0.45^{b}
	7	3.00±0.04^{a}	3.39±0.06^{b}	6.03±0.13^{c}	5.16±0.32^{a}	4.91±0.15^{a}	6.44±0.30^{b}

$^{a-c}$ Means ± standard deviations in the same row with different superscript lowercase letters indicate the significant difference between the carbon sources in the same cultivation temperature for each day by Duncan's multiple range test ($p < 0.05$)

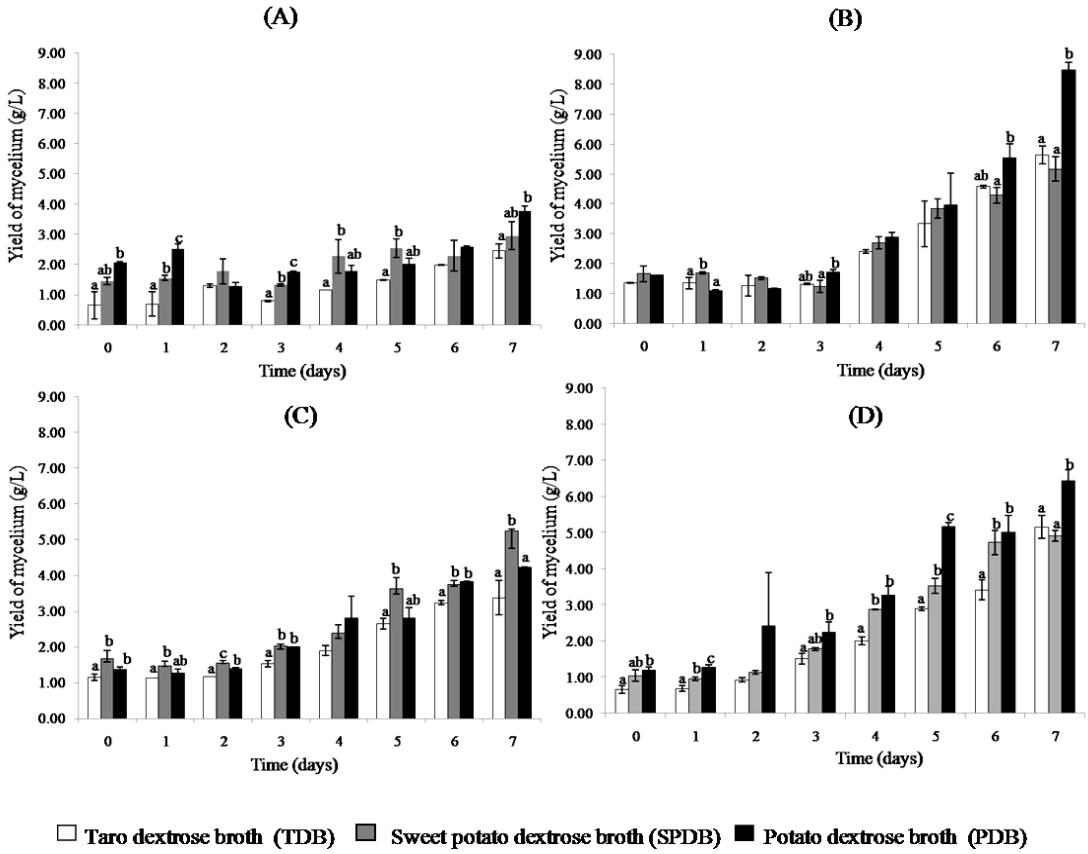

Figure 3 The effects of the different natural carbon sources on mycelium biomass yields for: (A) *P. ostreatus*; (B) *V. volvacea*; (C) *L. polychrous*; and (D) *L. squarrosulus* at 35±2 ºC using submerged cultivation. The error bars in the figure indicate standard deviation. Bars with different letters determined the significant difference between carbon sources for each day ($p < 0.05$).

source in the culture medium at 30±2 ºC for *P. ostreatus* mycelium production (2.95±0.46 g/L) (Figure 3A). For the mycelial production of *V. volvacea* and *L. squarrosulus*, the highest mycelial yields of 8.47±0.26 g/L and 6.44±0.30 g/L were obtained when PDB was used as the culture medium incubated at 30±2 ºC for 7 days, respectively (Table 1). The maximum mycelium biomass yield of *L. polychrous* (6.16±0.69 g/L) was obtained when SPDB was used as the culture medium after 7 days of cultivation at 35±2 ºC. However, PDB could also be used as a culture broth when the cultivation temperature was 35±2 ºC (5.80±0.66 g/L). All available data indicated that carbon sources were important for biomass production by mycelia mushrooms. The cultivation of mycelia mushrooms for bioactive compound production with different conditions have been reported by several researchers. Barakat and Sadik (2014) reported that *P.*

ostreatus grown in a medium containing starch as the carbon source produced the highest mycelium biomass after 10 and 15 days of incubation. Anike *et al*. (2015) determined that starch (30 g/L) and yeast extract (25 g/L) were both suitable for mycelium growth and crude exo-polysaccharide secretion in *L. squarrosulus*. Many kinds of mushrooms frequently require starch, glucose, sucrose, etc. for their growth in a submerged culture (Vahidi *et al.*, 2006). In addition to the carbon source, the nitrogen source, minerals (such as phosphorus, potassium and magnesium) and vitamins (such as thiamin and biotin) are also essential for the mycelial growth of fungi (Klomklung *et al*., 2014). Potatoes and yams contain high amounts of protein along with other tubers. Potatoes provide significant amounts of carbohydrates, potassium, and ascorbic acid to the diet (Chandrasekara and Josheph Kumar, 2016). In this research, potato, sweet potato, and taro that provided a natural carbon source, vitamin and other nutrients, could be a potential substrate for mycelia mushroom production. PDB was the most suitable medium for the cultivation of *L. squarrosulus*, *P. ostreatus*, and *V. volvacea* and SPDB was most suitable for the cultivation of *L. polychrous* using submerged cultivation. The results of this research indicate that the natural starchy root and tuber crops grown in the local area with their high carbohydrate content could be used as carbon sources for edible mushroom mycelium production. Thus, the allergens from synthetic media or chemicals could be reduced by using a natural carbon source as the cultivation medium. In the future, the mycelium and supernatant from submerged cultivation could be extracted for the production of crude exo-polysaccharide and the mushroom mycelia extracts. These extracts could be applied as bioactive compounds, food and cosmetic additives, or for other applications.

The effects of temperature on mycelia cultivation in a submerged culture are shown in Table 2. For *P. ostreatus* mycelium production, the temperature of cultivation had no effect on mycelial yield production when SPDB and TDB were used as the culture media (Table 2). On the other hand, when a PDB was used as the culture medium, the mycelium biomass yield of *P. ostreatus* obtained from 30±2 ºC cultivation was significantly higher than that at 35±2^{o}C after 7 days of incubation ($p < 0.05$) (Table 2). Similar findings were observed from cultivations of *V. volvacea* and *L. polychrous*. The cultivation temperature had no effect on the mycelial production for both mushrooms after incubation for 7 days (Table 2). No significant differences for the biomass yields of *L. squarrosulus* were observed between 35±2 ºC (6.03±0.13 g/L) and 30±2 ºC (6.44±0.30 g) ($p > 0.05$) when PDB was used as the culture medium with 7 days of incubation (Table 2). In contrast, the mycelium biomass yields of *L. squarrosulus* from cultivation using SPDB and TDB at 30±2^{o}C were significantly higher ($p < 0.05$) than cultivations at 35±2^{o}C in the same media (Table 2). The effects of temperature on mycelia growth depended on the mushroom species and carbon

Table 2 The effects of cultivation temperature on mycelial yields of four edible mushroom using submerged cultivation

Mushrooms	Days	Yields of mycelium (g/L)					
		TDB		SPDB		PDB	
		30±2 ºC	35±2 ºC	30±2 ºC	35±2 ºC	30±2 ºC	35±2 ºC
P. ostreatus	0	0.65±0.45	0.85±0.17	1.46±0.12	1.46±0.12	2.07±0.01	2.07±0.01
	1	0.69±0.40	0.89±0.18	1.55±0.09	1.66±0.08	2.51±0.16	2.31±0.47
	2	1.30±0.05	1.10±0.16	1.77±0.41	1.07±0.03	1.28±0.11	1.20±0.17
	3	0.79±0.02	0.79±0.00	1.32±0.02	1.70±0.23	1.77±0.01*	1.28±0.03*
	4	1.16±.00	0.93±0.21	2.27±0.55	2.04±0.25	1.79±0.19	1.58±0.04
	5	1.48±0.01	0.89±0.23	2.54±0.31	1.92±0.18	2.02±0.19	1.33±0.60
	6	1.98±0.01	1.33±0.49	2.28±0.51	2.06±0.01	2.60±0.01*	1.56±0.26*
	7	2.45±0.24	1.69±0.32	2.95±0.46	1.85±0.65	3.76±0.18*	1.65±0.10*
V. volvacea	0	1.37±0.01	1.37±0.01	1.66±0.27	1.66±0.27	1.69±0.10	1.70±0.10
	1	1.35±0.19	1.45±0.06	1.70±0.04	1.79±0.13	1.10±0.04	1.11±0.05
	2	1.27±0.34	1.27±0.28	1.52±0.05	1.77±0.08	1.17±0.02	1.20±0.00
	3	1.33±0.03*	1.91±0.19*	1.24±0.20*	2.17±0.09*	1.72±0.09*	2.97±0.22*
	4	2.41±0.06	2.80±0.27	2.69±0.20	3.21±0.14	2.88±0.16*	4.38±0.47*
	5	3.34±0.77	3.94±0.14	3.85±0.32	4.06±0.62	3.96±1.06	5.07±0.09
	6	4.57±0.05	5.48±1.10	4.29±0.27	5.01±0.06	5.53±0.46	7.24±0.39
	7	5.64±0.29	5.82±0.37	5.16±0.40	4.95±0.12	8.47±0.26	8.22±0.17
L. polychrous	0	1.15±0.09	1.15±0.09	1.66±0.24	1.66±0.24	1.37±0.07	1.37±0.07
	1	1.12±0.00	1.28±0.15	1.46±0.13	1.41±0.05	1.27±0.12	1.17±0.02
	2	1.16±0.01*	1.31±0.03*	1.55±0.07	1.80±0.08	1.39±0.02	1.56±0.12
	3	1.53±0.09	2.22±0.48	2.04±0.05	2.30±0.20	2.01±0.00*	2.54±0.15*
	4	1.89±0.14*	2.40±0.01*	2.38±0.24	3.12±0.15	2.82±0.59	2.93±0.15
	5	2.64±0.15	3.05±0.34	3.62±0.31	3.92±0.06	2.80±0.31	4.03±0.32
	6	3.24±0.06*	3.98±0.13*	3.75±0.11	4.55±0.42	3.82±0.02	4.67±0.50
	7	3.37±0.48	4.31±0.06	5.24±0.06	6.16±0.69	4.23±0.01	5.80±0.66
L. squarrosulus	0	0.66±0.11	0.66±0.11	1.05±0.15	1.05±0.15	1.19±0.01	1.19±0.10
	1	0.69±0.07	0.58±0.07	0.97±0.05	0.89±0.03	1.27±0.08	1.26±0.13
	2	0.93±0.06	0.92±0.01	1.13±0.05	1.11±0.02	2.41±1.47	1.59±0.29
	3	1.52±0.15	1.38±0.20	1.79±0.04	1.67±0.10	2.25±0.28	2.20±0.18
	4	2.01±0.11	1.77±0.31	2.88±0.01*	2.38±0.06*	3.28±0.24	2.73±0.67
	5	2.89±0.05*	1.78±0.31*	3.53±0.21	2.80±0.14	5.17±0.01	4.24±0.44
	6	3.41±0.28*	2.56±0.02*	4.73±0.34*	3.44±0.00*	5.02±0.45	4.37±0.21
	7	5.16±0.32*	3.00±0.04*	4.91±0.15*	3.39±0.06*	6.44±0.30	6.03±0.13

* Means ± standard deviations in the same row indicate the significant difference between the cultivation temperature in the same carbon source for each day by Student T test ($p < 0.05$)

sources in the cultural medium. Similar results have been reported by Klomklung *et al*. (2014). Their studies have shown that the *Pleurotus* and *Lentinus* species could be grown at 25 °C or higher temperatures (45 °C) (Klomklung *et al*., 2014). Temperature is one of the most important physical factors affecting mycelia growth in mushroom cultivation. The optimum temperature is very important for growth, the production of metabolic products and the sporulation of mushrooms. Increasing the temperature generally accelerates enzymatic activity but high temperatures can reduce enzyme activities, which affect the metabolism and growth of mushrooms (Chang and Miles, 2004). The results in this research showed that the mushroom could be grown in all the tested media at a temperature range of 30–35±2 ºC but the optimum temperature for biomass yield production varies according to the mushroom species and medium. All results from this study indicate that carbon sources and temperature are essential and can influence the growth of the fungi.

3. 2 Effects of carbon sources on mycelium growth with an agar medium

In this investigation, four edible mushrooms were cultured on different agar media, including PDA, SPDA, and TDA. All cultures were incubated at 30±2 ºC for 5-8 days. The results indicate that the maximum mycelium colony diameter of four edible mushrooms (6–9 cm) were obtained from the TDA medium at 3-8 days of incubation (Figure 4, Table 3). For *P. ostreatus* cultivation, the means for the mycelium colony diameter on 3 different media expanded significantly at $p < 0.05$ after 7 days of incubation. The mycelium diameter of *P. ostreatus* on TDA (6.30±0.07cm) was larger than those on the PDA (5.18±0.04 cm) and SPDA (5.73±0.04 cm) after 8 days of incubation (Figure 4A). These results indicate that TDA is the most suitable agar medium for the mycelium growth of *P. ostreatus*. The diameter of *V. volvacea* on TDA (5.80±0.21 cm) was significantly larger than those on the PDA (4.60±0.07 cm) and SPDA (5.20±0.07 cm) after 2 days of incubation ($p < 0.05$, Figure 4B, Table 3). However, the mycelium colony diameters of *V. volvacea* on 3 different media were not significantly different at $p > 0.05$ after 4 days of incubation (Figure 4B). In the case of the *L. polychrous* cultivation, the mycelium growth on SPDA (6.90±0.07 cm) was significantly larger ($p < 0.05$) than those on the PDA (6.00±0.07 cm) and TDA (6.48±0.04 cm) after 3 days of incubation (Figure 4C, Table 3). However, the mycelium diameters of *L. polychrous* on 3 different media were not significantly increased ($p > 0.05$) after 5 days of incubation (Figure 4C). The growth of *L. squarrosulus* mycelium on SPDA (6.78±0.32 cm) and TDA (6.83±0.04 cm) was significantly higher than that on the PDA (6.13±0.04 cm) after 3 days of incubation (Table 3). After 4 days of incubation, growth of the *L. squarrosulus* mycelium on 3 different media was not significantly different ($p > 0.05$, Figure 4D). However, TDA was most suitable for the mycelium growth of *L. squarrosulus* with the largest diameter of the colony (9.00 cm) after 4

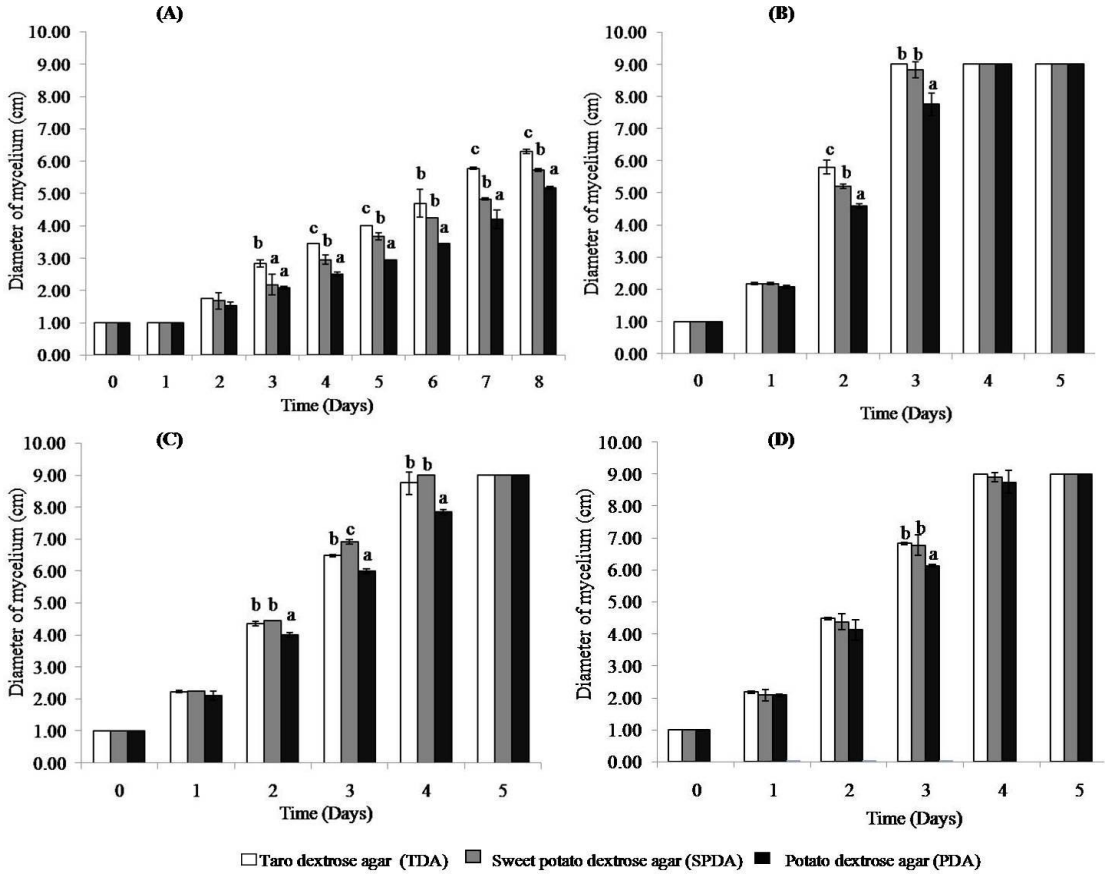

Figure 4 The effects of different natural carbon sources on the diameter expansion of: (A) *P. ostreatus*; (B) *V. volvacea*; (C) *L. polychrous*; and (D) *L. squarrosulus* using agar cultivation. The error bars in the figure indicate standard deviation. Bars with different letters determined the significant difference between carbon sources for each day ($p < 0.05$).

days of incubation. These results indicate that all 3 culture agar media are suitable for the mycelium growth of *V. volvacea*, *L. polychrous* and *L. squarrosulus* after 5 days of incubation. For *P. ostreatus*, TDA is the most suitable for mycelium growth.

For mushroom mycelium cultivation on an agar medium, taro and sweet potato were suitable and efficient for use as alternative sources for the cultivation of *P. ostreatus*, *V. volvacea*, *L. polychrous* and *L. squarrosulus* on an agar medium. Similar results were reported by Wongjiratthiti and Yottakot (2017). They reported that molds grew significantly higher growth on sweet potato dextrose agar (SPDA). However, both SPDA and TDA stimulated higher mycelial growth than PDA in all test molds (Wongjiratthiti and Yottakot, 2017). The results in this work show that sweet potato and potato were excellent in enhancing the growth of the mushroom mycelia, especially in a broth culture while taro was suitable for an agar medium. This

Table 3 The effects of natural carbon source on the diameter expansion of four edible mushrooms using different agar cultivation media

Mushrooms	Days	Diameters of mycelium (cm)		
		TDA	SPDA	PDA
P. ostreatus	0	1.00±0.00	1.00±0.00	1.00±0.00
	1	1.00±0.00	1.00±0.00	1.00±0.00
	2	1.75±0.00	1.68±0.25	1.53±0.11
	3	2.83±0.11^{b}	2.18±0.32^{a}	2.08±0.04^{a}
	4	3.45±0.00^{c}	2.95±0.14^{b}	2.50±0.07^{a}
	5	4.00±0.00^{c}	3.68±0.11^{b}	2.95±0.00^{a}
	6	4.70±0.42^{b}	4.25±0.00^{b}	3.45±0.00^{a}
	7	5.78±0.04^{c}	4.83±0.04^{b}	4.20±0.28^{a}
	8	6.30±0.07^{c}	5.73±0.04^{b}	5.18±0.04^{a}
V. volvacea	0	1.00±0.00	1.00±0.00	1.00±0.00
	1	2.18±0.04	2.18±0.04	2.08±0.04
	2	5.80±0.21^{c}	5.20±0.07^{b}	4.60±0.07^{a}
	3	9.00±0.00^{b}	8.83±0.25^{b}	7.75±0.35^{a}
	4	9.00±0.00	9.00±0.00	9.00±0.00
	5	9.00±0.00	9.00±0.00	9.00±0.00
L. polychrous	0	1.00±0.00	1.00±0.00	1.00±0.00
	1	2.23±0.04	2.25±0.00	2.10±0.14
	2	4.35±0.07^{b}	4.45±0.00^{b}	4.00±0.07^{a}
	3	6.48±0.04^{b}	6.90±0.07^{c}	6.00±0.07^{a}
	4	8.75±0.35^{b}	9.00±0.00^{b}	7.85±0.07^{a}
	5	9.00±0.00	9.00±0.00	9.00±0.00
L. squarrosulus	0	1.00±0.00	1.00±0.00	1.00±0.00
	1	2.18±0.04	2.08±0.18	2.08±0.04
	2	4.48±0.04	4.38±0.25	4.13±0.32
	3	6.83±0.04^{b}	6.78±0.32^{b}	6.13±0.04^{a}
	4	9.00±0.00	8.90±.14	8.75±0.35
	5	9.00±0.00	9.00±0.00	9.00±0.00

$^{a-c}$ Means ± standard deviations in the same row with different superscript lowercase letters indicate the significant difference between the carbon sources for each day by Duncan Test ($p < 0.05$)

might be because the sticky characteristic of the taro on fungal growth has an effect on the broth media, but does not affect the agar media (Wongjiratthiti and Yottakot, 2017).

4. Conclusion

PDB is a suitable medium for the mycelial production of *V. volvacea*, *L. squarrosulus* and *P. ostreatus* in submerged cultures at 30±2 ºC for a 7-day incubation while SPDB is a suitable medium for the cultivation of *L. polychrous* at 35±2 ºC for a 7-day incubation. For cultivation of mycelial on an agar medium, TDA and SPDA were suitable for the cultivation of *V. volvacea*, *L. polychrous* and *L. squarrosulus* after an incubation of 3-4 days. For *P. ostreatus* cultivation, the TDA was the most suitable medium after 7 days of incubation. The cultivation of mycelia mushrooms using these carbon sources not only enhanced mycelia biomass production, but also reduced the cost of the culture medium. Moreover, the mycelia biomass obtained from this natural medium should be safe from the contamination of allergen agents or chemicals associated with a synthetic medium. Thus, the mycelia extracted products shall be suitable to apply as food and cosmetic additives or for other health products.

5. Acknowledgements

This study was supported by a research grant from Rajamangala University of Technology Isan, contract no. NKR2561REV014. The authors thank the Faculty of Science and Liberal Arts, Rajamangala University of Technology Isan for providing some chemicals and instruments.

6. References

Anike, F.N., Isikhuemhen, O.S., Blum, D. and Neda, H., 2015, Nutrient requirements and fermentation conditions for mycelia and crude exo-polysaccharides production by *Lentinus squarrosulus*, Adv. Biosci. Biotechnol. 6: 526-536.

Barakat, O.S. and Sadik, M.W., 2014, Mycelial growth and bioactive substance production of *Pleurotus ostreatus* in submerged culture, Int. J. Curr. Microbiol. Appl. Sci. 3: 1073-1085.

Chandrasekara, A. and Josheph Kumar, T., 2016, Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health benefits, Int. J. Food Sci. 2016: 1-15.

Chang, S.T. and Miles, P.G., 2004, Overview of the Biology of Fungi, pp. 53-92, In Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, CRC Press, New York.

de Souza Kirsch, L., de Macedo, A.J.P. and Teixeira, M. F. S., 2016, Production of mycelial biomass by the Amazonian edible mushroom *Pleurotus albidus*, Braz. J. Microbiol. 47: 658-664.

Giri, S., Biswas, G., Pradhan, P., Mandal, S.C. and Acharya, K., 2012, Antimicrobial activities of basidiocarps of wild edible mushrooms of west Bengal, India, Int. J. Pharmtech. Res. 4: 1555-1560.

Ibekwe, V. I., Azubuike, P. I., Ezeji, E. U. and Chinakwe, E. C., 2008, Effects of nutrient sources and environmental factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*), Pak. J. Nutr. 7: 349-351.

Klomklung, N., Karunarathna, S. C., Hyde, K. D. and Chukeatirote, E. , 2014, Optimal conditions of mycelial growth of three wild edible mushrooms from northern Thailand, Acta Biol. Szeged. 58: 39-43.

Nwokoye, A.I., Kuforiji, O.O. and Oni, P.I., 2010, Studies on mycelial growth requirements of *Pleurotus ostreatus* (Fr.) Singer, J. Basic Appl. Sci. 10: 47-52.

Vahidi, H., Mojab, F. and Taghavi, N., 2006, Effects of carbon sources on growth and production of antifungal agents by *Gymnopilus spectabilis*, Iran J. Pharm. Res. 3: 219-222.

Wongjiratthiti, A. and Yottakot, S. , 2017, Utilization of local crops as alternative media for fungal growth, Pertanika J. Trop. Agric. Sci. 40: 295-304.