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ARTICLE

Optimal conditions of mycelial growth of three wild edible mushrooms from northern Thailand

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ABSTRACT In this study, three wild mushrooms namely *Lentinus connatus*, *L. roseus*, and *Pleurotus giganteus* were selected to study if they could be domesticated. Initially, the fruiting bodies of the three mushrooms were collected from forests in northern Thailand and morphologically characterized. In this paper we report the optimal *in vitro* culture conditions of three wild mushrooms. Among seven culture media tested for the optimal mycelial growth of three wild mushrooms, black bean agar, red bean and soy bean agar were the best for the mycelial growth of *L. connatus*, *L. roseus* and *Pleurotus giganteus*, respectively. The mushroom mycelia were able to grow at temperatures ranging from 20-30 °C, with optimal growth temperatures of 30 °C and 25 °C for *Lentinus* and *Pleurotus* species, respectively. The optimum pH range observed for mycelial growth was 5.0 - 7.0. **Acta Biol Szeged 58(1):39-43 (2014)**

KEY WORDS

Lentinus connatus
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Generally, mushrooms are regarded as 'functional food' due to their nutritive value and medicinal properties (Barros et al. 2008). Mushrooms are rich in protein and dietary fiber; and they also contain some vitamins and minerals such as vitamin B, vitamin D, potassium and magnesium (Sanmee et al. 2003; Chang and Miles 2004). Several bioactive compounds are also found in mushrooms; for example, eritadenine (known as hypocholesterolemic agent) is found in *Lentinus edodes* (Enman et al. 2007) and bioactive compounds responsible for neurite stimulation can be found in *P. giganteus* (Phan et al. 2012). Glucosylceramide exhibiting antimicrobial activity is present in *Pleurotus citrinopileatus* (Meng et al. 2012). At present, 650-700 mushroom species belonging to 200 genera are known as edible but only about 130 mushroom species can be cultivated (Vargas-Isla and Ishikawa 2008; Mortimer et al. 2012). Many edible mushrooms are wild collected and only available for limited period (*i.e.*, in the raining season), whereas some edible mushrooms are ectomycorrhizal and impossible to domesticate (Sanmee et al. 2003). It is therefore interesting if the new saprobic edible wild mushrooms could be cultivated for commercial purpose as then they would be available all year round.

In 2011, three wild mushrooms namely *Pleurotus giganteus*, *Lentinus roseus*, and *L. connatus*, were collected from Chiang Mai, Thailand (Karunarathna et al. 2010; Karunarathna et al. 2011). The cultivation of *P. giganteus* has recently been reported in China and Malaysia (Phan et al. 2012), whereas cultivation in northern Thailand has been partially successful (Klomklung et al. 2012). In contrast, there are no reports on the cultivation of the two *Lentinus* species (*L. roseus* and *L. connatus*). The discovery of *L. roseus* is quite recent and thus there is not much information except its morphological and phylogenetic data (Karunarathna et al. 2010). *L. connatus* also occurs in the wild and only two reports describe its active compound (connatusin A) exhibiting antimalarial and cytotoxic activities (Rukachaisirikul et al. 2005). Due to limited data of growing *P. giganteus*, *L. roseus*, and *L. connatus* and their potential use as food and medicine, our aim was to further investigate optimal culture conditions for growing these three mushrooms. In this paper, an initial step was performed to determine favorable culture conditions for mycelial growth of *P. giganteus*, *L. connatus* and *L. roseus*.

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Materials and Methods

Isolation of mushroom samples

Mushroom samples used in this study are shown in Table 1. For isolating the fungal mycelia, the sterile internal fungal tissues of their fruiting bodies were isolated and placed on potato dextrose agar (PDA). The mycelial culture was then sub-cultured on PDA supplemented with 0.5% yeast extract and incubated at 30 °C until the agar surface was fully covered with the fungal white mycelium.

Effect of culture media

Several raw materials including *Phaseolus vulgaris* (red bean and black bean), *Phaseolus aureus* (mung bean), *Glycine max*

Table 1. Mushroom samples used in this study.

Mushrooms	Isolate No.	Collection site
Pleurotus giganteus	MFLU 10-0154	Chiang Mai
Lentinus connatus	MFLU 08-1389	Chiang Mai
Lentinus roseus	MFLU 08-1376	Chiang Mai

Table 2. Raw materials used for preparation of culture media.

Raw materials	Sources
Red bean (*Phaseolus vulgaris*)	Tesco-Lotus, Chiang Rai
Black bean (*Phaseolus vulgaris*)	Tesco-Lotus, Chiang Rai
Mung bean (*Phaseolus aureus*)	Tesco-Lotus, Chiang Rai
Soy bean (*Glycine max* L.)	Tesco-Lotus, Chiang Rai
Sorghum (*Sorghum bicolor* (L.) Moench)	Chiang Rai local market

L. (soybean), and *Sorghum bicolor* (L.) Moench (sorghum) were used in this study (Table 2). The modified agar media containing such raw materials were prepared as follows: 50 g of each grain were separately soaked in 250 ml distilled water for 12 hours or overnight and boiled for 30 min. These grains were grounded using mortar and pestle, and then filtered through clean cheesecloth. Twenty grams of agar was added to each grain filtrate and the media volume was adjusted to 1 L by adding distilled water. The media were then autoclaved at 15 psi, 121 °C for 15 min; 15 ml of each medium was poured into Petri dishes. Malt extract agar (Difco) and PDA (Criterion) were also used for comparative study of the mycelial growth. Fungal mycelium discs (5 mm in diameter) were used as inocula and transferred onto the center surface of each grain media. After 10 days of incubation at 30 °C, the mycelial growth, density, and growth rate of the three mushrooms were measured.

Effect of pH

To screen an optimal pH for the mycelial growth, a mycelial agar disc (5 mm in diameter) was transferred to soy bean agar, red bean agar and black bean agar for *P. giganteus*, *L. roseus*, and *L. connatus*. The pH of the media was adjusted to a pH range of 5-8 with 1 M NaOH or HCl. The plates were incubated at 30 °C for 12 days. The mycelial growth, density, and growth rate of the three mushrooms were measured.

Effect of temperature

Four different temperatures (20, 25, 30, and 35 °C) were used to find the optimum temperature for mycelial growth of three wild mushrooms. Mycelia discs (5 mm in diameter) were taken from the Petri dishes which were grown under suitable culture media and pH and then placed on the centre of a culture medium plate. Samples were incubated at four different temperatures for 10 days. The mycelial growth, density, and growth rate of the three mushrooms were measured.

Data collection and statistical analysis

A completely randomized design was used in this study. The data obtained for mycelial growth under different conditions were from five replicates. The results were expressed as means and variance. Means were also compared using Duncan's multiple range test by using SPSS-16 program.

Results and Discussion

Effect of culture media

Seven different culture media were used to screen the optimal mycelial growth of three wild mushrooms (Table 3). After 10 days of incubation, *P. giganteus* was able to grow equally well on mung bean agar, black bean agar, red bean agar, sorghum agar, and soy bean agar. On these media, the mushrooms grew best on soy bean agar with the growth rate of 12.59 ± 0.34 mm/day (Table 3). In contrast, *P. giganteus* did not grow well when grown on PDA and MEA. This result, however, was different from the result of Kumla et al. (2013), who reported that the best mycelial growth of *P. giganteus* was observed on PDA.

After one week of incubation, *L. connatus* showed a very good equal growth on mung bean agar, black bean agar, red bean agar, sorghum agar, and soy bean agar (Table 3). The best mycelial growth and density of *L. connatus* was observed on black bean agar with the growth rate of 13.99 ± 0.33 mm/day (Table 3). PDA and MEA also appeared to support its mycelial growth. This result is related to Gbolagade et al. (2006), who reported food materials such as yellow corn agar could support the mycelial growth of *Lentinus subnudus*.

After 8 days of incubation, *L. roseus* grew equally well on mung bean agar, black bean agar, red bean agar, and soy bean agar. The best mycelial growth and density showed on red bean agar with the growth rate of 11.73± 0.25 mm/day (Table 3). The least mycelial growth rate and density were found on MEA. This result is related to Fasola et al. (2007), who reported food grains such as Ife brown beans, wheat, white corn and yellow corn agar could support mycelial growth of *Volvariella speciosa*.

Carbon source, nitrogen source, minerals (such as phosphorus, potassium and magnesium) and vitamins (such as thiamin and biotin) are essential for mycelial growth of fungi (Chang and Miles 2004). Five raw materials were also containing carbon, nitrogen, mineral and vitamin for mycelial growth with different values (Berrios et al. 1999; Mubarak 2005; Habibullah et al. 2007; Rani et al. 2008; Sasanam et al. 2011; Liu et al. 2012). The result showed that the mushrooms could be grown in all the media at 30 °C. Thus, the effect of culture media on mycelial growth varies according to the mushroom species.

Table 3. Effect of culture media on mycelial growth rate (mm/day) of three wild mushrooms. Mycelial density was given in parentheses. Means followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

Mushrooms	Mung bean agar	Black bean agar	Red bean agar	Sorghum agar
Pleurotus giganteus	10.76 ± 0.25^{b} (+4)	11.96 ± 0.41^{c} (+3)	10.66 ± 0.28^{b} (+2)	12.59 ± 0.38^{d} (+)
Lentinus connatus	13.02 ± 0.27^{e} (+3)	13.99 ± 0.33^{f} (+4)	11.39 ± 0.54^{c} (+4)	12.49 ± 0.28^{d} (+)
Lentinus roseus	10.43 ± 0.25^{d} (+4)	10.89 ± 0.38^{e} (+4)	11.7 3± 0.25^{f} (+5)	7.99 ± 0.16^{b} (+)

Mushrooms	Soy bean agar	MEA	PDA
Pleurotus giganteus	12.59 ± 0.34^{d} (+4)	4.69 ± 0.37^{a} (+4)	4.26 ± 0.34^{a} (+)
Lentinus connatus	10.89 ± 0.22^{b} (+2)	10.26 ± 0.36^{a} (+3)	11.43 ± 0.38^{c} (+4)
Lentinus roseus	10.69 ± 0.39^{de} (+4)	5.93 ± 0.14^{a} (+3)	8.73 ± 0.27^{c} (+5)

MEA = malt extract agar, PDA = potato dextrose agar.

Table 4. Effect of pH on mycelial growth rate (mm/day) of three wild mushrooms. Mycelial density was given in parentheses. Means followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

Mushrooms	5.0	5.5	6.0	6.5
Pleurotus giganteus	10.20 ± 0.41^{c} (+4)	9.36 ± 0.24^{b} (+4)	9.96 ± 0.24^{c} (+4)	9.63 ± 0.31^{b} (+4)
Lentinus connatus	10.89 ± 0.25^{c} (+4)	12.76 ±0.19^{d} (+4)	10.86 ± 0.24^{c} (+4)	9.76 ±0.34^{b} (+4)
Lentinus roseus	7.13 ± 0.31^{a} (+5)	8.43 ± 0.14^{c} (+5)	7.96 ± 0.24^{b} (+5)	7.93 ±0.36^{b} (+5)

Mushrooms	7.0	7.5	8.0
Pleurotus giganteus	8.22 ± 0.36^{a} (+4)	8.29 ± 0.39^{a} (+4)	8.49 ± 0.33^{a} (+4)
Lentinus connatus	9.76 ± 0.38^{b} (+4)	9.26 ±0.41^{a} (+4)	9.26 ± 0.43^{a} (+4)
Lentinus roseus	8.36 ± 0.29^{c} (+5)	8.09 ± 0.32^{bc} (+5)	6.83 ± 0.23^{a} (+5)

Table 5. Effect of temperature on mycelial growth rate (mm/day) of three wild mushrooms. Mycelial density was given in parentheses. Means followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

Mushrooms	Temperature (°C)			
	20	25	30	35
Pleurotus giganteus	5.93 ± 0.25^{b} (+3)	9.66 ± 0.23^{d} (+4)	6.33 ± 0.31^{c} (+5)	0.00 ±0.00^{a}
Lentinus connatus	2.69 ± 0.21^{b} (+4)	8.39 ± 0.40^{c} (+4)	10.89 ± 0.19^{d} (+4)	0.00 ± 0.00^{a}
Lentinus roseus	13.25 ± 0.46^{b} (+5)	13.50 ± 0.25^{b} (+4)	14.15 ± 0.28^{c} (+4)	0.00 ± 0.00^{a}

Effect of pH

The effects of pH on mycelial growth of three wild mushrooms are shown in Table 4. The results showed that, these mushrooms grew fairly well in acidic, neutral and alkaline environments (pH 5.0-8.0). The best mycelial growth and density of *P. giganteus* were observed in acidic media of pH 5.0-6.5. This result is related to the result of Kumla et al. (2013), who reported that the optimal pH for the mycelial growth of *P. giganteus* is at pH 7 but it normally grows well at pH range of 4-9.

The best mycelial growth and density of *L. connatus* and *L. roseus* were obtained in slightly acidic to neutral pH ranges from pH 5.0-7.0 (Table 4). These results are related to Gbolagade et al. (2006), who reported that pH range from 4.0-8.0 could support the mycelial growth of *Lentinus subnudus* and the acidic medium (pH 5.0-5.5) is the best for mycelial growth.

The effect of pH is very important when choosing the substrate for mushroom cultivation because the substrate could be a buffer to control the pH (Chang and Quimio 1982).

Generally, calcium carbonate is used in mushroom cultivation to control pH of the medium (Change and Quimio 1982).

Effect of temperature

The results were obtained when three wild mushrooms were under four different temperatures from 20-35 °C (Table 5). The result showed that the three mushrooms were able to grow at a temperature range of 20-30 °C, however, they did not grow at 35 °C. The statistical analysis of *P. giganteus* showed that 25 °C was the best temperature for mycelial growth with the growth rate of 9.66 ± 0.23 mm/day (Table 5). This result is related to Kumla et al. (2013), who reported that the best temperature for mycelial growth of mushrooms is 25 °C.

The best temperature for mycelial growth of *L. connatus* on black bean agar was observed at 30 °C with the growth rate of 10.89 ± 0.19 mm/day (Table 5). *L. roseus* was able to grow well at a temperature range of 20-30 °C. The best mycelial growth and density of *L. roseus* occurred at 30 °C with the growth rate of 14.15± 0.28 mm/day (Table 5). This result is related to Gbolagade et al. (2006) who reported that the most suitable temperature for mycelial growth of *Lentinus subnudus* is 30 °C.

Three wild mushrooms in this study were collected in the tropic region and the results showed that the temperature for mycelial growth of *P. giganteus* was 25 °C and those of the two *Lentinus* were 30°C. Temperature is one of the most important and critical physical factors affecting mycelial growth in mushroom cultivation (Chang and Miles 2004). The optimum temperature is very important for growth, production of metabolic products and sporulation of mushrooms (Chang and Miles 2004). Increasing temperature generally accelerates enzymatic activity but high temperatures can make enzymes inactive which affect the metabolism and growth of mushrooms (Chang and Miles 2004). Several studies have shown that *Pleurotus* and *Lentinus* species could be grown at 25 °C or higher temperatures (45 °C) (Chang and Quimio 1982; Gbolagade et al. 2006; Vargas-Isla and Ishikawa 2008).

Conclusion

In this study, attempts were made to investigate the effect of raw materials (*i.e.*, red bean, black bean, mung bean, soybean, and sorghum), pH, and temperature on the mycelial growth of *P. giganteus*, *L. roseus* and *L. connatus* species. Our results showed that the best mycelial growth can be obtained on black bean agar and red bean agar for *L. connatus* and *L. roseus*, respectively, and both species grew well within a pH range of 5.0-8.0 (optimum 5.5-6.0) and their optimal temperature was 30 °C. The optimal growth of *P. giganteus* was obtained on soybean agar within a pH range of 5.0-6.5 and the optimal temperature of 30 °C. Since almost no researches have been done on growing *L. connatus* and *L. roseus*, these results will be a base for the future domestication research.

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