# Mycelial Cultivation of 4 Edible Mushrooms from Khao Kra-Dong Volcano Forest Park, Thailand

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**Abstract**

The edible mushrooms become one of the world's most expensive foods and have a global market measured in Thailand. In Thailand, the fruiting body of all occurs once a year during rainy season in June - August. So, the objective of this research was to study the optimal mycelial conditions of 4 edible mushrooms collected from Khao Kra-Dong Volcano Forest Park in Thailand: *Russula cyanoxantha, Heimiell retispora, Russula virescens* and *Boletus colossus*. The highest mycelial growth of *R. virescens* and *B. colossus* were with potato dextrose agar (PDA), followed by potato dextrose agar with 2% volcanic soil (PDA+2% S). The best structures of *R. cyanoxantha* and *H. retispora* used for culturing on medium were cap, stalk and spore, respectively. For *R. Virescens* and *B. colossus*, the best structures used for culturing on medium were stalk, cap and spore, respectively. The highest colony diameter of *R. Cyanoxantha* on PDA+2% S with cap was 68.00±1.00 mm. For *H. retispora*, the highest colony diameters on PDA+2%S with cap and PDA with stalk were 87.00±1.00 mm. and 67.33±1.53 mm., respectively. For *R. virescens* and *B. colossus*, the highest colony diameters on PDA with stalk were 91.33±2.08 mm. and 87.00±1.00 mm., respectively. The optimal temperature and pH value for mycelial growth were at 30 °C with pH 7 after 7 days of incubation.

**Keywords:** mushroom cultivation, edible mushrooms, volcanic soil

## Introduction

Food stuff of plant origins such as cereals, vegetables, potatoes and pulses constitute an important dietary source of protein for many segments of world's population, particularly where animal protein is not only in short supply but are beyond the reach of middle and poor classes. Among unconventional sources of protein, higher fungi, particularly mushrooms, stand out as a distinct class. The protein contents of these food stuffs, i.e. vegetables, cereals, etc. is low as compared to mushrooms.

Fungi inhabit every possible environment including many unlink ones, utilizing the organic materials from plants and animals and even other fungi for their nutrition and energy source. The cultivation of edible mushrooms is a worldwide important commercial activity (Chang, 2000). Several aspects have been contributed to the development of this activity: (1) the raw materials used are waste from agribusiness that have little commercial value and are easy to acquire (Rajarathnam & Bano, 1991), (2) the product obtained has a visually attractive appearance, pleasant taste and a high quality protein which provides much of the essential amino acids to the diet and (3) some species can be grown with relatively simple technology and low investment. Mushrooms can play an important role contributing to the livelihoods of rural and peri-urban dwellers through food security and income generation, make a valuable dietary addition through protein and various micronutrients, and are coupled with their medicinal properties. Mushroom cultivation can represent a valuable small-scale enterprise option. There are 1200 species of fungi that are considered mushrooms, with at least 200 species showing various degree of edibility

(Chang, 2000). The commercial market was dominated by white button mushroom (*Agaricus Bisporus)*, Oyster mushroom (*Pleurotus* Spp.) and tropical paddy straw mushroom (*Volvariella* Spp.). Recently, cultivation of milky mushroom (*Calocybe Indica*) has started.

The edible mushrooms become one of the world's most expensive foods and have a global market measured in Thailand. In Thailand, the fruiting body of all occurs only once a year during rainy season in June – August. Despite this, a few have been cultivated with any degree of success, and certainly not in volumes that are likely to reverse the catastrophic declines in production that have occurred over the past 100 years. Wild edible mushrooms are not only collected for consumption but also recognized as a good source of digestible proteins, carbohydrates, fiber and vitamins (Barros, Ferreira, & Queiros, 2007; Heleno, Barros, Sousa, Martins, & Ferreira, 2009; Kalac, 2009; Ouzouni, Petridis, Koller, & Riganakos, 2009). The three main steps of the process for culturing mushrooms are: isolating the mushrooms from the fruiting bodies, preparing primary and secondary spawn, and culturing the mushroom from spawn to harvest fruiting bodies. The purpose of this research was to provide the growth parameters such as optimal culture conditions for domestication of 4 wild tropical edible mushroom species: *Russula cyanoxantha, Heimiell retispora, Russula virescens* and *Boletus colossus,* with the goal of promoting mycelial growth.

## Methods and Materials

Mushroom Strains

Mushroom samples were collected from Khao Kra-dong Volcano Forest Park in Samed Sub District, Muang District, Buriram Province, Thailand, during January – August 2015. The macro-fungi were collected for studying morphology and were identified by their scientific names at the generic level. This was achieved by comparing species with descriptions and photographs in the references and keys. The mushroom species selected were *Russula cyanoxantha, Heimiell retispora, Russula virescens* and *Boletus colossus.*

Effects of media on mycelium growth

Three different culture media for mycelium growth: potato dextrose agar (PDA), potato dextrose modified agar with cassava (PDA+C) and potato dextrose modified agar with 2% volcanic soil (PDA+2%), were used to determine suitable media for promoting mycelium growth. Fresh mushrooms were thoroughly washed with clean water, cut into pieces and air-dried. The parts of edible mushrooms such as spore, stalk and cap were placed in the center of agar plate containing optimized media. The petri dishes containing cultures were incubated at 30 °C for 7 days. The mycelial growth was evaluated by observing mycelial diameter for 7 days. All experiments mentioned in this paper were carried out in triplicates.

Effects of different temperatures on mycelium growth

The selected medium was used for the evaluation at different temperatures: 25°C, 30°C and 37°C, to find out the best mycelium growth. The fungal cultures were incubated in different incubator with different temperatures for 7 days. The colony diameter was measured and compared to find out the optimal temperature for mycelia growth of 4 edible mushrooms.

Effects of different pH values on mycelial growth

The selected medium and the optimum temperature were used to evaluate the optimal pH for mycelial growth. The pH were adjusted to 4, 7, 9, and 12 using 1N NaOH or 1N HCl. The pH ranges were measured using a digital pH meter before autoclave. The best pH optimum for promoting

mycelial growth was determined by measuring the colony diameter.

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 three replicates. The mycelial growth of 4 edible mushrooms was also determined for comparative purpose. The results were expressed as means and variance. Means were compared using Duncan's multiple range tests by using SPSS-22 program (Brosius, 2008).

**Results and Discussions**

To study the usefulness of macro-fungi, a survey on the diversity of macro-fungi was conducted during January – August 2015. The macro-fungi were collected for studying morphology and were identified to 25 species 10 families by their scientific names at the generic level. This was achieved by comparing species with descriptions and photograph in the references and keys. The mushroom species and morphology were shown in table 1 and figure 1.

*Russula cyanoxantha* *Boletus colossus* *Russula virescens* *Heimiell retispora*

**Figure 1** Morphology of 4 edible mushrooms collected from Khao Kra-Dong Volcano Forest Park, Thailand

**Table 1** Morphology of 4 edible mushrooms collected from Khao Kra-Dong Volcano Forest Park, Thailand

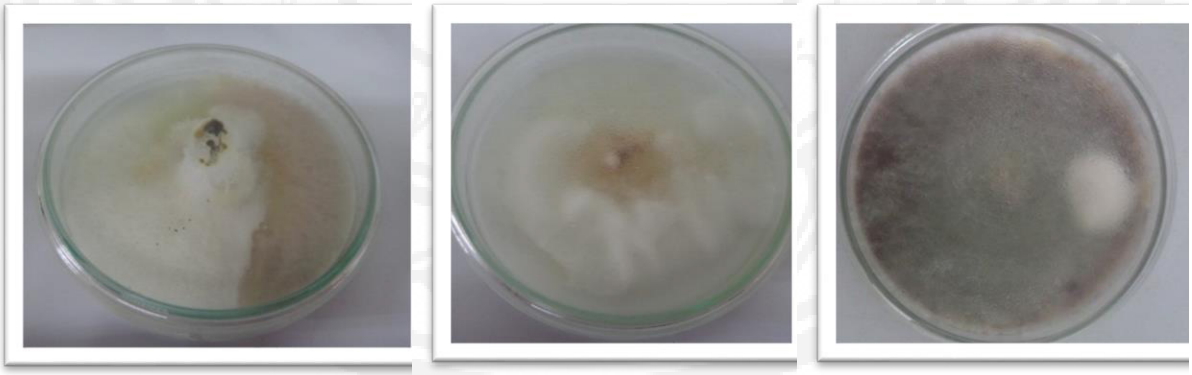
| No. | Scientific name | Morphology |
|---|---|---|
| 1. | *Russula cyanoxantha* | The most salient characteristic is the weak gills, which feel greasy to the touch and are flexible without breaking. The cap is 4-15 cm. wide, convex at first and later flattened, and greenish to bright brown (they vary considerably in colors). The stipe is pure white, slightly convex underneath, up to 10 cm. in height and 1.5-2.5 cm. in diameter. |
| 2. | *Heimiell retispora* | The pileus is 3.0 – 10.0 cm across, convex to plane at maturity, dull red when young, then pink, surface dry, coarsely. Fresh thick, yellow, unchanging when wounded or bruised. The tube is small and firm when it is young, then spongy, yellow green. Stipe is 5.0 – 15.0 cm. long, 1.5 – 2.0 cm. across, dull red, yellow at the apex, cylindric to ventricose, tapered and fusiform toward the base, surface with red longitudinal fibrils on cream colored background, firm fleshed. |
| 3. | *Russula virescens* | The cap is at first dome or barrel-shaped, becoming convex and flattened with age with a diameter of up to 15 cm. The cap center is often depressed. The cuticle of the cap is green, most profoundly in the center, with patches of the same color dispersed radially around the center in an areolate pattern. The color of the cuticle is often of variable shade, ranging from gray to verdigris to grass-green. |
| 4. | *Boletus colossus* | Pileus is 8.0 -20.0 cm. across, convex, thick and firm, black, smooth. The surface is lubricous and viscous when moist. Fresh yellow, unchanging when bruised or cut. Tube pore is small and firm, yellow to greenish at maturity. Stipe is 4.0-6.0 cm long, 3.0-4.0 cm across, thick, stipe attachment at central, cylindric to slightly clavate, with a black on a cream background. |

The present study pointed at the importance of 3 different ingredients: potato, cassava and volcanic soil on mycelial growth for commercial applications. There were 3 successful culturing media for culturing mushrooms in the laboratory environment. In this study, the effect of 3 different culture medium on mycelial growth of 4 edible mushrooms collected from Khao Kra-Dong Volcano Forest Park, Thailand were showed in table 2 and figure 2. After 7 days of incubation, *R. cyanoxantha* and *H. retispora* grew well on potato dextrose agar with 2% volcanic soil (PDA+S), followed by potato dextrose agar (PDA). The highest mycelial growth of *R. virescens* and *B. colossus* were on potato dextrose agar (PDA), followed by potato dextrose agar with 2% volcanic soil (PDA+2% S). The best structures of *R. cyanoxantha* and *H. retispora* used for culturing on medium were cap, stalk and spore, respectively. For *R. virescens* and *B. colossus*, the best structures were stalk, cap and spore, respectively. The highest colony diameter of *R. cyanoxantha* on PDA+2% S with cap was 68.00±1.00 mm. For *H. retispora*, the highest colony diameters on PDA+2%S with cap and PDA with stalk were 87.00±1.00 mm. and 67.33±1.53 mm. respectively. For *R. virescens* and *B. colossus*, the highest colony diameters on PDA with stalk were 91.33±2.08 mm. and 87.00±1.00 mm., respectively.

The results are similar to several other studies. The growth of mushrooms in pure culture was greatly influenced by changes to the culture media (Nasim, Malik, Bajwa, & Afzal, 2001; Gbolagade, Fasidi, Ajayi, & Sobowale, 2006; Wei, Yao, Wang, & Pegler, 2004) However, this research showed that PDA and PDA+2%S were the best media for mycelial growth of these mushrooms.

*Solanum tuberosum* *Manihot esculenta* Volcanic soil

**Figure 2** Different ingredients used in culture medium mixed with potato, cassava and volcanic soil.



**PDA medium** **PDA+Cassava medium** **PDA+2%Volcanic soil medium**

**Figure 3** Colony diameter of *R. cyanoxantha* on different culture medium at 30 ºC after 7 days of inoculation

All of 4 edible mushrooms were tested to find suitable temperature for promoting mycelial growth on the best medium. The different temperatures: 25 °C, 30 °C and 37 °C, were used for the mycelial growth (Table 3). Mycelia grew well between 25 ºC and 30 °C, and the optimum temperature for the highest mycelial growth was 30 °C. The colony diameter of *R. cyanoxantha*, *H. retispora*, *R.*

*virescens* and *B. colossus* on medium at 30 °C after 7 days of inoculations were 45.33±2.52, 69.00±2.65, 88.67±2.08, and 87.33±1.53 mm., respectively.

Effects of different pH values on mycelial growth of 4 edible mushrooms on the best medium at 30 ºC after 7 days of incubation were shown in table 4. The pH 4-12 was suitable for the mycelial growth of these mushrooms. The optimal pH on mycelial growth was pH 7 which was similar with the results. The optimal pH for *M. procera*, *Lignosus rhinoceros* and Thai oyster mushroom were 5-8 (Shim, Oh, Lee, & Lee, 2005; Lai, Siti Murni, Fauzi, Abas Mazni, & Saleh, 2011; Kumla, Danell, & Lumyong, 2014). The colony diameters of *R. cyanoxantha*, *H. retispora*, *R. virescens* and *B. colossus* on the best medium at 30 °C with pH 7 after 7 days of inoculations were 43.67±1.53,70.00±1.73, 87.67±1.53 and 88.00±1.00 mm., respectively.

**Table 2** Effect of different culture media on mycelial growth of 4 edible mushrooms at 30 ºC after 7 days of inoculation. Within a column, values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's test.

| Name | Medium | Mycelial growth (Colony diameter (cm)) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Spore | | Cap | | Stalk | |
| | | Aver | ±SD | Aver | ±SD | Aver | ±SD |
| *R. cyanoxantha* | PDA | 0.00 | ±0.00$^{a}$ | 32.67 | ±1.53$^{b}$ | 45.00 | ±1.00$^{c}$ |
| | PDA+C | 0.00 | ±0.00$^{a}$ | 21.33 | ±0.58$^{b}$ | 43.67 | ±0.58$^{c}$ |
| | PDA+S | 0.00 | ±0.00$^{a}$ | 68.00 | ±1.00$^{c}$ | 43.67 | ±0.58$^{b}$ |
| *H. retispora* | PDA | 0.00 | ±0.00$^{a}$ | 55.33 | ±0.58$^{b}$ | 67.33 | ±1.53$^{c}$ |
| | PDA+C | 0.00 | ±0.00$^{a}$ | 36.00 | ±1.00$^{c}$ | 23.67 | ±0.58$^{b}$ |
| | PDA+S | 0.00 | ±0.00$^{a}$ | 87.00 | ±1.00$^{c}$ | 23.67 | ±0.58$^{b}$ |
| *R. virescens* | PDA | 46.67 | ±0.58$^{b}$ | 24.00 | ±1.00$^{a}$ | 91.33 | ±2.08$^{c}$ |
| | PDA+C | 12.67 | ±0.58$^{a}$ | 25.00 | ±1.00$^{c}$ | 21.00 | ±1.00$^{b}$ |
| | PDA+S | 12.67 | ±0.58$^{a}$ | 25.00 | ±1.00$^{c}$ | 21.00 | ±1.00$^{b}$ |
| *B. colossus* | PDA | 0.00 | ±0.00$^{a}$ | 13.00 | ±1.00$^{b}$ | 87.00 | ±1.00$^{c}$ |
| | PDA+C | 0.00 | ±0.00$^{a}$ | 36.33 | ±1.53$^{c}$ | 22.00 | ±1.00$^{b}$ |
| | PDA+S | 0.00 | ±0.00$^{a}$ | 75.67 | ±0.58$^{c}$ | 22.00 | ±1.00$^{b}$ |

**Table 3** Mycelial growth of 4 edible mushrooms at different temperatures after 7 days of inoculation. Mean and standard deviation of three replicates.

| Name | Colony diameter at different temperatures (mm.) | | | | | |
|---|---|---|---|---|---|---|
| | 25 | | 30 | | 37 | |
| | Aver | ±SD | Aver | ±SD | Aver | ±SD |
| *R. cyanoxantha* | 40.33 | ±2.08$^{a}$ | 45.33 | ±2.52$^{c}$ | 44.33 | ±1.53$^{b}$ |
| *H. retispora* | 65.67 | ±2.31$^{b}$ | 69.00 | ±2.65$^{c}$ | 55.00 | ±1.00$^{a}$ |
| *R. virescens* | 65.00 | ±4.58$^{b}$ | 88.67 | ±2.08$^{c}$ | 58.00 | ±1.00$^{a}$ |
| *B. colossus* | 69.67 | ±1.53$^{b}$ | 87.33 | ±1.53$^{c}$ | 34.00 | ±1.00$^{a}$ |

Within a column, values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's test.

**Table 4** Mycelial growth of 4 edible mushrooms at different pH values on medium at 30 ºC after 7 days of inoculation

| | Colony diameter at different pH values (mm.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | | 7 | | 9 | | 12 | |
| Name | Aver | ±SD | Aver | ±SD | Aver | SD | Aver | SD |
| ***R. cyanoxantha*** | 25.33 | ±2.08 | 43.67 | ±1.53 | 27.33 | ±1.53 | 24.67 | ±1.53 |
| ***H. retispora*** | 41.00 | ±1.00 | 70.00 | ±1.73 | 44.67 | ±2.08 | 21.00 | ±1.00 |
| ***R. virescens*** | 48.67 | ±0.58 | 87.67 | ±1.53 | 44.33 | ±2.52 | 33.67 | ±2.08 |
| ***B. colossus*** | 57.67 | ±1.53 | 88.00 | ±1.00 | 44.33 | ±1.53 | 25.00 | ±2.00 |

Within a column, values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's test.

## Conclusion

Mushrooms are a good cash crop; they are rather easy to grow and are brimming with protein, vitamin B and minerals. They also have medicinal properties. The time for spawning and harvesting can be as short as three weeks. Furthermore, after the cultivation, you can still use the substrate as a good soil conditioner. In this study, the mycelium of the 4 edible mushrooms grew in PDA and PDA modified agar based culture media. The most suitable medium for the mycelial growth were PDA and PDA+2% volcanic soil medium. The mycelium grew very well at 25-30 °C. The growth sharply decreased at the temperature below 18 °C and no growth occurred when the temperatures were up to 39 °C. These optimal temperatures indicate that these 4 edible mushrooms grow well in summer and autumn in subtropical and tropical regions, and are full of protein. So, these mushrooms can be used for developing poor and developing countries in Asia. Fungi grow differently at different pH levels. Some tropical mushrooms produce fruiting bodies in a neutral or slightly acidic pH of 7.0 or 6.0 (Adebayo-Tayo, Jonathan, Popoola, & Egbomuche, 2011). Unlike other saprobic mushrooms, *Macrolepiota* species are difficult to collect and to study their ecology and diversity in details. This is because *Macrolepiota* species are found to grow singly at specific humidity and temperature during rainy season. However, this study suggests the culture of the 4 edible mushrooms by stalk based on mushroom cultivations. The results showed the colony diameter on PDA media and the mycelium optimal conditions to be applied to mushroom cultivation.

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