Mycelium growth, yielding, and fruiting body production of Yanangi mushroom (*Agrocybe cylindracea*) on different substrate packing under with evaporation-cooling system

Thidaporn Theunpao, MSc, KMUTT, Thailand
Supawadee Punyadee, BSc, KMUTT, Thailand
Sermsiri Mayteeworakoon, MSc, BIOTECH, Thailand
Todsaporn Thongthieng, PhD, KMUTT, Thailand

Abstract

Yanangi mushroom (Agrocybe cylindracea) is one type of widely edible mushroom that present high value in Thailand. High yield cultivation and quality improvement of Yanangi mushroom were rarely studies. Therefore, the aim of this study was to investigate the suitable substrate packing for mycelium growth, yielding, and fruiting body production under greenhouse with evaporative-cooling system. This study used two different substrate packings, compacted substrate and loose substrate for 1 kg packing bag. Mycelium growth presented significantly highest with 1 kg of loose substrate for 3 weeks. In turn, their yield was significantly lower than compacted substrate for first, second, and third harvesting cycle. Results of yielding shown 18.05, 19.90, and 26.58 g for 1st 2nd and 3th harvesting cycle of compacted substrate, respectively and 24.95, 18.39, and 17.53 g for 1st 2nd and 3th harvesting cycle of loose substrate, respectively. Both results of number of fruiting body shown significantly decrease. Length of fruiting body stalk were not significantly difference between compact and loose substrate. From results of mycelium growth, yielding, and fruiting body production, compacted substrate was selected for used to large scale cultivation under greenhouse with evaporation cooling system. It shown significantly highest percentage of yielding in the second harvesting cycle. Percentage of flowering and number of fruiting body were significantly decrease in second and third harvesting cycle after that it regrow and presented dramatically yielding increase in fourth and fifth cycle.

Introduction

Yanangi mushroom (*Agrocybe cylindracea*) is an eatable mushroom that belongs to Strophariacaae family in Agrocybe genus. It is widely economic and high value in Thailand agricultural market. Yanangi mushroom was clustered in group of white rod fungi that usually found on dead plants or base break of old wood. It habits with many perennial plants such as Populus and Salix including Quercus, Ulmus, Acer, Melia, Robinia, Broussonetia, Allophylus, Cupania, Phebe and Araucaria angustifolia genus [1]. Morphologically, Its Pileus generally brown or dark brown in the center and almost white in the margin. Pileus surface not hygrophanous and usually smooth; convex to flat, 8-200 mm broad. Its strip present white to vary pale brown and have specific smell when fruiting [2].

Cultivation of A. cylindracea in plastic bag usually used lignocellulosic based substrates i.e. sawdust, wheat straw, cotton waste, maize, and cotton seed and addition some nutrient supplement that essential for mycelium growth and fruiting body production such as organic nitrogen, phosphorus, and calcium. Lignocellulosic based substrates and some nutrient supplements is an enhancer for yielding and increase quality of mushroom that depend on ratio of substrate and cultivated condition [2].

Mushroom production process is generally begun with preparation of pure culture from selected fruiting body that is grown on Potato Dextrose Agar (PDA) with aseptic technique [3].

Fully mycelium on synthetic agar media (i.e. PDA) is transferred to grain seeds substrate such as sorghum, bean, rice, and corn. Then, it is packed in glass bottle or plastic bag and left to incubation room (25 °C – 30 °C) for incubation. Rubber tree sawdust or wood dust were usually used as mushroom substrate based in Thailand. It promotes the growing of Yanangi mushroom better than corn cob [3,4]. Rubber tree sawdust is generally used in Thailand.

Greenhouse with evaporation cooling system is a climate protection and cultivated gentle plants. Generally, greenhouse was used for cultivation of tender plants that are cultivated and sheltered from the weather. It has two main composition, roof shading and venilation for prevent petal burns and prevent ventilation of tender plants. Now a day, green house was improved and developed to control many conditions such as decrease temperature, increase humidity. System was usually controlled with spraying water or forcing fresh air through wet evaporative cooling pads, which make a temperature drop and controlled humidity. In this experiment used greenhouse with Evaporation cooling system for mention climate in greenhouse a 25 °C and 90 % relative humidity that was reported suitable condition for Yanangi Mushoom cultivation.

Materials and Methods

The initial pure culture of Yanangi mushroom (*A. cylindracea* strain no.1) was received from collection of Biotechnology Research and Development Office, Department of Agriculture, Thailand. The regrowth granular mycelium was prepared in the Center of biological laboratory of King Mongkut's University of Technology Thonburi (Ratchaburi), Thailand. Initial pure culture was transferred on Potato Dextrose Agar (PDA). The Petri-plates were inoculated at 25 ± 2 °C in incubator for 2-3 weeks. It was used as initial pure culture for mother spawn preparation [3].

Sorghum was used as mycelium medium. Grains were clean washed with tap water about 30 minutes. Then it was boiled with Reverse Osmosis (RO) water for 10-15 minutes. Filled 350 g of grains in 325 ml glass bottle or upto 2/3 volume and plugged with non-absorbent cotton that were covered with aluminum foil. Glass bottles were autoclaved at 15 lb p.s.i. pressure at 121 °C for 15 min. Next, these autoclaved bottles were kept in the clean room for cooling. Then, a piece of mycelium (pure culture) was transferred to autoclaved bottles and inoculated at room temperature (25 °C) for 30-45 days. Fully colonized mother spawn bottles can be used for inoculating.

Substrate bag for grown Yanangi mushroom was prepared in polypropylene bags (PP) (35 cm x 17.5 cm x 40cm x 20 cm). This experiment used one kg substrate, which compost of rubber tree sawdust, rough rice bran, fine rice bran, $NaSO_4$, $CaCO_3$, $MgSO_4$. All compositions were homogenous mixed and packed into PP bags that were autoclaved at 15 lb p.s.i. pressure at 121 °C for 1-2 hours. These bags were left in clean room. Next, 45-50 mycelium grains from mother spawn bottle were transferred to substrate bag. The inoculated substrate bags were incubated at room temperature for 30-45 days. Fully colonized mycelium bags were used for fruiting body production.

This experiment was comparison of two different substrate packing bags. These were compacted substrate and loose substrate for 1 kg packing bag. Compacted substrate was prepared with 1 kg homogenous mixing substrate and packed it by a masher. Loose substrate similar was prepared with 1 kg homogenous mixing substrate but without mashed them. This experiment was measured length of mycelium on

substrate for 1st, 2nd, and 3rd week. After that, fully colonized mycelium bags were transferred to greenhouse with evaporative-cooling system. It was controlled at 25 °C, 90% of relative humidity. Fruiting bodies formation was induced by water spray on the surface of substrate twice a day for five days. Next, water spraying into the air twice a day for increase humidity under greenhouse until fruiting bodies were appeared. After fruiting, substrates were paused spraying of water for 14 days, then, produce the second crop cycle at day of 15th. Harvesting and Measurement of results for 3 cycles of fruiting bodies production (crop cycle). Percentage of yielding, weight of fruiting bodies per substrate bag, and number of fruiting bodies per substrate bags were measured. This experiment was designed as Completely Randomized Design with five replication (n=5) and five substrate packing bags per replication. Data were evaluated by Analysis of variance (ANOVA). Each parameter was computed by SPSS software. The mean values of results were compared by Tukey's HSD test with SPSS software (SPSS for Window version 17.0, SPSS Inc., USA).

Results

First experiment to compared effect of two different substrate packing bags (compacted and loose substrate bag) on growth of Yanangi mycelium. Result, average mycelium length of compacted substrate bags were 3.73 cm, 7.26 cm, and 9.55 cm for 1st, 2nd, and 3th week, respectively (figure 1). Loose substrate bag's results were 4.47 cm, 8.27 cm, and 10.58 cm for 1st, 2nd, and 3rd week, respectively (figure 1). Length of mycelium significantly increased both compacted and loose substrate bag for 1st, 2nd, and 3th week. However, results of mycelium length of loose substrate bag shown significantly higher than compacted substrate bag for all the week of experiment. Second experiment was measurement of Yanangi mushroom yielding production on different substrate packing under greenhouse with evaporative-cooling system. Weight of fruiting bodies per substrate bag were measured. Results presented 18.05 g, 19.90 g, and 26.58 g for 1st, 2nd, and 3rd production cycle of compacted substrate bag, respectively (figure 2).

Total weight of three production cycle is 64.53 g. Results of loose substrate bag were 24.95 g, 18.39 g, and 17.53 g for 1st, 2nd, and 3rd production cycle (figure 2). Total weight of three production cycle is 60.87 g. Number of fruiting bodies were significantly decreased when repeated a cycle of production. It shown 9.28, 3.84, and 2.56 for 1st, 2nd, and 3rd production cycle of compacted substrate bag, respectively (figure 3). In contrast, loose substrate bag shown number of fruiting bodies of 3rd production cycle was significantly higher than 2nd production cycle. Results were 4.80, 2.00, and 2.92 for 1st, 2nd, and 3rd production cycle, respectively (figure 3). Length of stem of fruiting bodies were significantly increased for compacted substrate bag. Results were 5.60 cm, 8.25 cm, and 10.37 cm for 1st, 2nd, and 3rd production cycle, respectively. Results of loose substrate bag shown 7.22 cm, 11.09 cm, and 8.45 cm for 1st, 2nd, and 3rd production cycle, respectively (figure 4).

Last experiment was study about effect of large scaling Yanangi mushroom production for 10,000 compacted substrate bags under greenhouse with evaporative-cooling system. Percentage of yielding production for the second cycle was increased, then it was dramatically decreased for third and fourth cycle and reproduced high yield for the fifth cycle. Weight of fruiting bodies were decreased for second and third production cycle then it was increased for fourth and fifth cycle (figure 5).

Discussion

The first experiment is comparison of mycelium growth of two different substrate packings, compacted and loosed substrate packing. Result, average mycelium length of loose substrate packing at the third weeks were higher than compacted substrate packing. In turns, it shown lower yielding and fruiting bodies production than compacted substrate packing. This experiment, exposure time of mycelium growth spend 3-4 weeks or 21-28 days for full colonization of 1 kg of substrate. Previous study of mycelium growth of *Agrocybe aegerita* reported that fastest mycelium growth rate presented on beech sawdust substrate. It shown 7.1 cm per 18 days and the slowest mycelium growth rate reported 4.0 cm per 18 days [6]. Thus, our experiment shown lower mycelium growth rate of mushroom that the previous experiment. The result of mycelium growth may be depending on type of sawdust, strain and physiology of mycelium, and step of substrate preparation method.

Mycelium growth of this experiment did not relate with yielding and fruiting bodies production. Loose substrate packing gave highest mycelium growth, but it did not present the highest yielding and fruiting bodies formation. In turns, compacted substrate packing shown significantly higher yielding on second and third production cycle. Trend of this study similar WenJie Yang *et al*'s research on another mushroom, oyster mushroom [7]. Therefore, the trend of mycelium growth cannot use to indicate mushroom yield and fruiting bodies production that maybe depend on efficiency of substrate utilization and physiology of mushroom. Moreover, the air flow rate transfer may be a main factor that effected to mycelium growth rate and yielding of mushroom because loose substrate packing has more pore between sawdust particles. Sawdust pores were filled with air that consist of oxygen gas, nitrogen gas, and carbon dioxide etc. [8,9], which necessary for hyphae extension. Yielding and fruiting bodies production maybe due to physical nature and nutrient of substrate. Moreover, quantity and quality of lignocellulosic base is also affecting to yield production.

Highest quality of fruiting bodies (weight and stem length) presented at the 3rd production cycle of compacted substrate. It may be cause of mature mycelium age that already to fruiting at the 3rd production cycle (about 56-60 days from fruiting induction). In contrast, loose substrate shown significantly highest weight at the first cycle, after that it significantly decrease at 2nd and 3rd of repeated cycle. The reason maybe the 1st crop need numerous nutrients for fruiting bodies formation that cause of substrate lost almost of nutrient. Thus, mushroom production at 2nd and 3rd cycles were decreased. However, length of stem of loose substrate packing shown highest result at the 2nd cycle. This trend did not relate with weigh of fruiting bodies so both quality and quantity of *A.cylindracea* maybe depending on exposure time of fruiting bodies induction, nutrient quantity of substrate, and substrate utilization.

Trend of number of fruiting bodies shown inversely trend with weight of fruiting bodies for compacted substrate. Moreover, both trend of number of fruiting bodies of two different substrate packings presented highest at the 1st production cycle. After that, the results were decreased at the 2nd and 3rd cycle. Thus, if Yanangi mushroom presented small size of fruiting bodies, high weight mushroom was presented.

Last experiment was large scaling Yanangi mushroom production under greenhouse with evaporation-cooling system. Result shown highest percentage of yielding production at 2nd cycle (81 %) and decrease at 3rd (49 %) and 4th cycle (34 %), after that, it regrown to give higher yielding at the 5th cycle (62 %). Previously studies, yield of Agrocybe sp. presented about 7.9-11.4 % per production cycle [6,10], which were lower production yield than this experiment. However, yielding results of this study did not relate with weight so it cannot use for indicated Yanangi mushroom weight and quality in each cycle.

Image

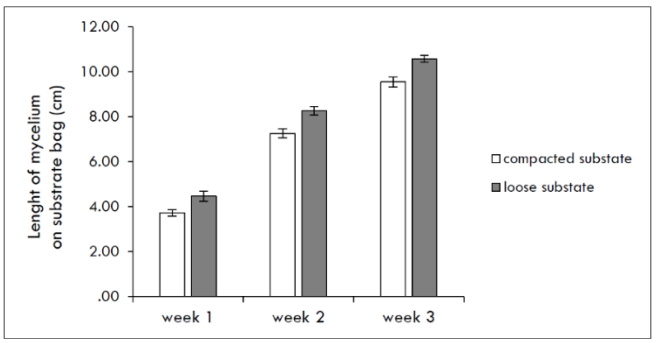


Figure 1 Comparison of length of mycelium on different substrate bags

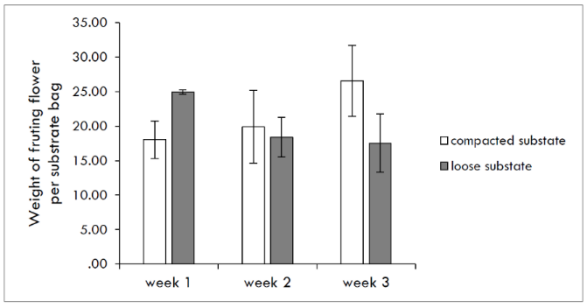


Figure 2 Comparison of weight of fruiting flower per substrate bag

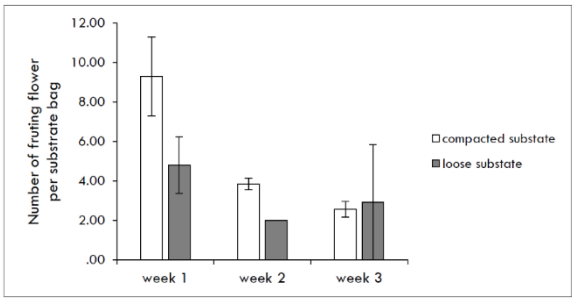


Figure 3 Comparison of number of fruiting flower per substrate bag

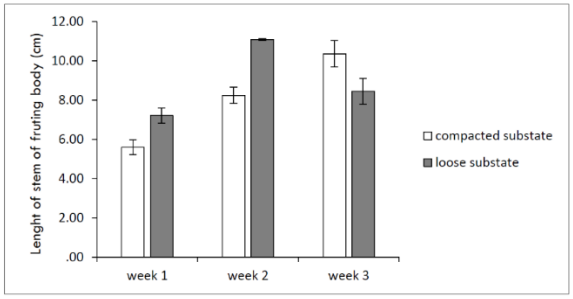


Figure 4 Comparison of length of stem of fruiting body

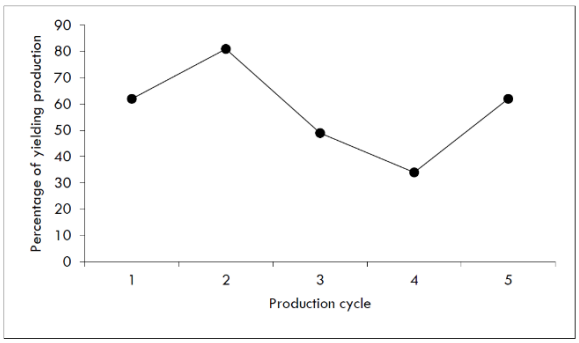


Figure 5 Percentage of yielding production

Recent Publications

1. Wright JE, Alberto E (2002) Guia de Hongos la region Pampena. I. Hongos con laminillas. Buenos Aries.
2. Morina U, Edgardo A (2007) Morphologic characterization of Agrocybe cylindracea (Basidiomycetes, Agaricales) from America, Europe and Asia. Revista Mexicana de micologia 24: 9-18.
3. Sharma V, Kumar S (2011) Spawn production technology. In: Mushrooms cultivation, Marketing and Consumption, Directorate of Mushroom Research (ICAR), Chambaghat, Solan, 35-42.
4. Biswas MK, Mrinmoy L (2014) Techniques for Increasing the Biological Efficiency of Paddy Straw Mushroom (Volvariella volvacea) in Eastern India. Food Science and Technology 2(4): 52-57.
5. Both AJ (2007) Evaporative cooling. Greenhouse management.
6. Agnieszka J, Marek S, Krzysztof S (2012) Mycelium Growth and Yielding of Black Popular Mushroom-Agrocybe aegerita (Brig.) Sing. on Different Substrates. Journal of Agricultural Science and Technology A 2: 1040-1047.
7. WenJie Y, FengLing G, ZhengJie W (2013) Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi Journal of Biological Sciences 20: 333-338.
8. Omoanghe S, Isikhuemhen, Nona A, Mikiashvii, Vinaya K (2009) Application of solid waste from anaerobic digestion of poultry litter in Agrocybe aegerita cultivation: Mushroom production, linocellulytic enzymes activity and substrate utilization Biodegradation 20: 351-361.
9. Noorlidah Abdullah, Rozila Ismail, Norjuliza Mohd Khir Johari, Annuar MSM (2013) Production of liquid spawn of an edible grey oyster mushroom, Pleurotus pulmonnarius (Fr.) Quel by submerged fermentation and sporophore on rubber wood sawdust. Scientia Horticulturae 161: 65-69.
10. Agnieszka J, Marek S, Krzysztof S (2011) Mycelium Growth and Yielding of *Agrocybe aegerita* (Brig.) Sing. on Different Substrates. The 7th International Conference on Mushroom Biology and Mushroom Products (ICMP7).



Biography

Thidaporn Theunpao was graduated Bachelor of Science (Biotechnology) and Master of Science (Plant Science) from Mahidol university, Thailand. She has been studied about development of *in-vitro* screening method for selection of stress tolerant plants in the Northeast of Thailand. Currently, she is a research assistance of King Mongkut's University of Technology Thonburi (KMUTT), Ratchaburi, Thailand. Now a day, she is studying a new strategy for improvement of Thai farmer life quality through the topic of economic mushroom production in Thailand.

Email: thidaporn.the@kmutt.ac.th

Notes/Comments: