
Research and Development Project of Monkey's Head Mushroom (*Hericium erinaceus*) Cultivation in East of Thailand

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Monkey's head mushrooms is an edible and medicinal mushroom, native to North America, Europe and Asia. The objective of this project was selection monkey's head mushrooms strains which had properties suitable for growing and gave high yield in east of Thailand, 6 different strains of monkey's head mushrooms from mushroom farms which located around of Thailand were selected, 1. strain from Anonbiotec Farm (AF), 2. strain from Department Of Agriculture #1 (DA1), 3. strain from Department Of Agriculture #2 (DA2), 4. strain from Aranyik Mushroom Center (AMC), 5. strain from Doi Pui Research Station (DPRS), 6. strain from Kasetsart University, Kampaeng Saen campus (KKU). The experiments were conducted at the faculty of agricultural technology, Rambhai Barni Rajabhat University, Chanthaburi Province, during September 2015 to February 2017, average temperature was 28 °C, relative humidity was 85%. The trials were laid out in a Completely Randomized Design (CRD). The results revealed that all of monkey's head mushrooms strains in this research could give fruit bodies if temperature during mycelium grown (incubation time) and induced fruiting time were 26 – 30 °C, obtained higher temperature affect mycelium grown slowly and did not gave fruit bodies and growing media was highly contaminated. Strains from AF, DA1 and DA2 could give fruit bodies 4 time, 80 – 100% of bag number gave 2 time, 50% and 10% gave 3 and 4 time respectively. While strains from AMC and DPRS could give fruit bodies 3 time, 50 – 60% of bag number gave 2 time, 10% - 20% gave 3 time and, could not give fourth fruiting. Strain from KKU could give fruiting only 1 time. Strains from AF and DA2 had adaptability in east of Thailand especially at Chanthaburi higher than the other strains, the yield per bag was 211.42 and 180.6 g respectively. While DA1, DPRS, KKU and AMC had the yield per bag 146.98, 111.94, 95.31 and 84.77 g respectively. Growing monkey's head mushrooms on rice straw substrates gave the yield higher than saw dust, increasing rice bran ratio in growing media also increased protein content in fruit bodies.

Keywords: monkey's head mushrooms, mushroom cultivation, medicinal mushroom

Introduction

There is a renaissance of interest in natural remedies in many parts of the world at present days. Natural products are the chemical compounds or

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substances produced by living organisms, found in nature that usually have pharmacological or biological activities. The preventive and therapeutic effects of copious natural products against life-threatening diseases have been well documented (Newman *et al.* 2007). Medicines from the natural sources are usually thought to have fewer side effects. From the ancient times, mushrooms have been recognized as nutritionally functional foods and source of physiologically beneficial medicines. Several species of mushrooms under the genus of *Pleurotus*, *Ganoderma*, *Cordyceps*, *Lentinus*, *Hericium* and *Grifola* have been reported widely to have anti-cancer, anti-diabetic, anti-hypertensive, anti-microbial, cardio-protective, hepato-protective and other health beneficial roles (Khan *et al.* 2013).

Monkey's head mushroom (*Hericium erinaceus*) have a long history of applications in traditional oriental therapies. *Hericium erinaceus* is a mushroom belonging to the family *Hericiaceae*, it is the tooth fungus that growing on hardwood. The body attached to the hidden "base", with size 8 – 40cm across and the spines measures at 1 – 6 cm long. The color of this mushroom is white and turns brownish or yellowish with age and has been known as Chinese medicine or food in China and Japan without harmful effects. *H. erinaceus* grows on old or dead broadleaf trees and has been used as a medicine for treatment of gastricism in traditional Chinese medicine for more than 1000 years (Mizuno *et al.* 1999). Recently, the chemical constituents of *H. erinaceus* have been investigated for its interesting and significant bioactivities. Hericenones and erinacines were isolated from the fruiting body and mycelium of *H. erinaceus*, respectively, and most of the compounds promote NGF biosynthesis in rodent cultured astrocytes. These results suggest the value of *H. erinaceus* for the treatment and prevention of dementia.

In east of Thailand, fruits is a major component of agriculture in the area, therefore during June to August which is the time that in season fruits go to the markets, the supply of fruits is highest. The heightened supply leads to lower prices, the farmers suffer and have this problem every year. The solution of the problem is promote monkey's head mushrooms cultivation as a second source of income. Monkey's head mushroom is very expensive in Thailand because it is temperate mushroom but Thailand is tropical zone, it can be cultivate only in Northern Thailand, in the east it can be cultivate only in the winter.

Objectives of this project was selection monkey's head mushrooms strains which had properties suitable for growing and gave high yield in east of Thailand around the year.

Materials and methods

Selection Fungal Strains

Monkey's head mushroom (*Hericium erinaceus*) strains were selected from mushroom farms which located around of Thailand, the most of monkey's head mushroom were same strains, only 6 strains were different, they were maintained on Potato Dextrose Agar (PDA) medium in Petri dishes, incubated at room temperature (30 ± 2 C) and send to Rambhai Barni Rajabhat University for experiment, 6 strains were: 1. strain from Anonbiotec Farm (AF), 2. strain from Department Of Agriculture #1 (DA1), 3. strain from Department Of Agriculture #2 (DA2), 4. strain from Aranyik Mushroom Center (AMC), 5. strain from Doi Pui Research Station (DPRS), 6. strain from Kasetsart University, Kampaeng Saen campus (KKU). The experiments were conducted at the faculty of agricultural technology, Rambhai Barni Rajabhat University, Chanthaburi Province, during September 2015 to February 2017, average temperature was 28 °C, and relative humidity was 85%. The trials were laid out in a Completely Randomized Design (CRD) with arrangement of three replications.

Growing Media and Cultivation

The spawn of each strains were prepared on white sorghum grains. The grains were half boiled and filled in transparent glass bottles. The bottles (containing half boiled grains) were sterilized in an autoclave at 15 psi for 30 minutes. The inoculation was made on the following day under aseptic conditions and then were incubated at room temperature (32 ± 2 C), till the grains were covered with white mycelial growth.

The growing media was the combination of 100 kg para rubber sawdust + 4 kg rice bran + 1 kg gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) + 1 kg Dolomite ($\text{CaMg}(\text{CO}_3)_2$) + 1 kg brewer's yeast + 1 kg leucaena leaf meal + 0.1 magnesium sulphate (MgSO_4) + EM (Effective Microorganism) 200 ml. The moisture content of aforementioned media formular were adjusted to approximately 63 – 64%, after that the growing media was filled in the polyethylene bags (0.8 g each) and autoclaved at 121 °C for 1 hour, when the steriled media was cooled down, the bags were inoculated by the previously prepared grain spawn, then being incubated at room temperature for spawn run (mycelium growth). When the bags become full of mycelial growth and or pinheads started appearing on the mycelial surface, the bag mouths were opened to facilitate the development of fruiting bodies. As soon as the fruiting bodies developed with full size, these were harvested just above surface of the substrate with sharp knife or blade.

Temperature, humidity and light during spawn run and developed fruiting bodies depend on real environment at that time for study on adaptability of mushroom in east of Thailand. The growth and development of mushroom was observed daily.

Analysis

Data collection was investigated length of mycelium at the time pinheads started appearing, the time taken for pinhead formation from the date of spawning, the number of flush per bag, circumference, height, fresh weight, dry weight and protein content of each strains's fruiting bodies. The weight of fresh mushrooms and number of fruiting bodies per bag per each flush were recorded after harvesting, the dry weight of mushroom was recorded by keeping the fresh mushroom in hot air oven at 70 °C for 48 hours. Protein content was analyzed with Kjeldahl method.

Statistical analysis

Differences between the means of individual groups were assessed by one-way ANOVA with Duncan's multiple range tests.

Results

Adaptability of Different Strains of Monkey's Head Mushroom in East of Thailand

All strains could give fruit bodies if temperature during mycelium grown (incubation time) and induced fruiting time were 26 – 30 °C, obtained higher temperature affect mycelium grown slowly and did not gave fruit bodies and growing media was highly contaminated. Strains from AF, DA1 and DA2 could give fruit bodies (flush) 4 time, 80 – 100 % of bag number gave 2 time, 50 % and 10 % gave 3 and 4 time respectively. While strains from AMC and DPRS could give fruit bodies 3 time, 50 – 60 % of bag number gave 2 time, 10 % - 20 % gave 3 time and, could not give fourth fruiting. Strain from KKU could give fruiting only 1 time. (Table 1)

The mean number of days taken for pinhead formation of monkey 's head mushroom from the date of spawning (flush1) exhibited significant difference between 6 strains, AF strain taken shortest time followed by DA2, DA1, AMC, DPRS and KKU strains which had time taken 57.1, 66.33, 67, 70.7, 77.5 and 149.67 days respectively. (Table 1)

AF strain flush1 gave the highest yield of 211.42 g, followed by DA2, DA1, DPRS, K KU and the lowest yield was AMC strain which had total yield of 180.6, 146.98, 111.94, 95.31 and 84.77 g. respectively. (Table 1)

Table 1 Time taken (mean days) for flush 1 – flush 4, number of bags (%) give fruiting bodies and total yield of 6 different strains of monkey's head mushroom

Strains	flush1	flush2	flush3	flush4	Total yield (g)
AF	57.1 ^b	56.25 ^{ab} (80%)	58 (50%)	33 (10%)	211.42 ^a
DA1	67 ^{ab}	63 ^a (100%)	49 (50%)	39 (10%)	146.98 ^{ab}
DA2	66.33 ^{ab}	52.25 ^{ab} (80%)	47.29 (50%)	26 (10%)	180.6 ^a
AMC	70.7 ^{ab}	59.75 ^a (60%)	24.33 (20%)	-	84.77 ^b
DPRS	77.5 ^a	40 ^b (50%)	61 (10%)	-	111.94 ^b
K KU	149.67 ^c	-	-	-	95.31 ^b
F-test	*	*			*

Means within column followed by different superscripts, are significantly different at $P \leq 0.05$ by Duncan's test.

Morphological Characteristics of Monkey's Head Mushroom

All strains in this experiment had mycelium growth at the time pinheads started appearing not fully the media bags. DA1 and DA2 strains trend to have length of mycelium at the time pinheads started appearing higher than AF and AMC strains but not significant, K KU strain had length of mycelium shortest but not significant with DPRS, AMC and AF strains. (Table 2)

As for the morphological characteristics of fruit bodies each strain are given in Table 2 and Figure 1

Table 2 Morphological characteristics, length of mycelium at the time pinheads started appearing and protein content of each strains's fruiting bodies at flush 1

Strains	length of mycelium at the time pinheads started appearing	circumference	Number of fruiting body /bag	Fresh weight/ bag (g)	dry weight/bag (g)	Hight of fruit body (cm)	Protein contain (%)
AF	16.33 ^{abc}	23.77	10 ^{ab}	47.25	8.25	5.6 ^{ab}	11.54 ^{ab}
DA1	17.69 ^a	26.27	5 ^b	62.47	11.12	7.03 ^a	10.81 ^b
DA2	17.24 ^{ab}	25.68	9 ^{ab}	54.18	8.50	7.03 ^a	11.29 ^{ab}
AMC	16.06 ^{abc}	25.18	10 ^{ab}	61.03	10.02	6.8 ^a	12.67 ^{ab}
DPRS	14.27 ^{bc}	23.25	14 ^a	38.6	9.77	3.96 ^b	11.44 ^{ab}
K KU	13.23 ^c	28.87	8 ^{ab}	95.31	12.06	7.4 ^a	13.46 ^a
F-test	*	ns	*	ns	ns	*	*

Means within column followed by different superscripts, are significantly different at $P \leq 0.05$ by Duncan's test.

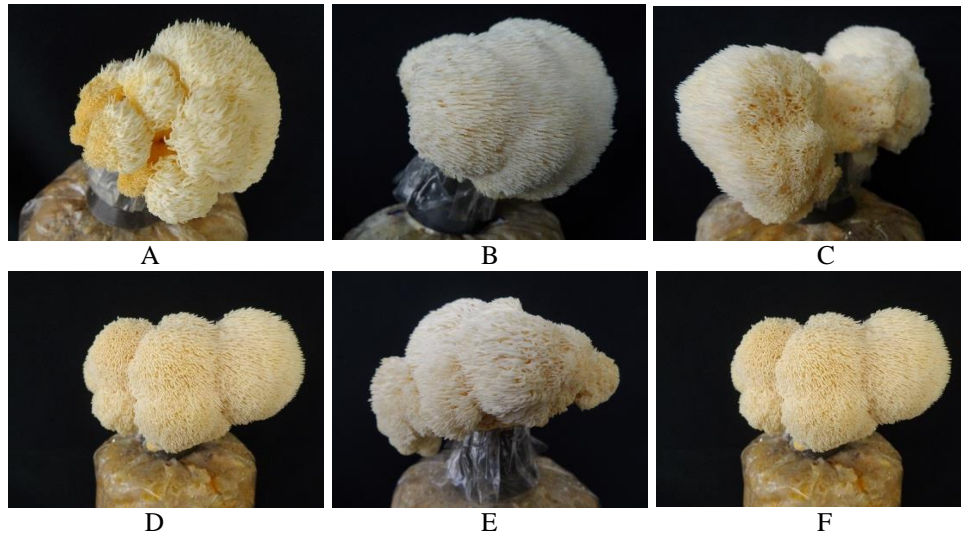


Figure 1. Show the fruit bodies of AF strain (A) , DA1 (B), DA2 strain(C), AMC strain (D), DPRS strain (E) and KKU strain (F)

Discussion

It could be observed that 6 strains of monkey's head mushroom started appearing pinheads when the mycelium never fully the media bags, for this reason it may be the effect of weather in east of Thailand which usually has high relative humidity especially in rainy season, this condition optimum for induce fruiting bodies, but it affect the yield decrease , according to reported of Yang, *et al.*,(2013) reported that the yield of the mushroom is directly related to the spread of mycelium into the substrate.

High contamination of the media bags occurred in our experiments, this may be due to the experiments conducted both in summer and rainy seasons of east of Thailand which had high temperature (28 – 32 °C), this is not optimal temperature conditions for monkey' s head mushrooms which had origin from China and Japan (growth conditions temperature: 24.0 °C). Thus, the monkey' s head mushroom mycelium growth in this study were very slow and had highly contaminated.

Finally, the result showed that 6 strains of monkey's head mushrooms was not gave the high yield and often to had high contamination in summer and rainy season in east of Thailand. Thus, further research on breeding of new strains of the monkey' s head mushrooms which has high-temperature tolerance is necessary.

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