การเจริญเติบโตและผลผลิตของเห็ดขอนขาว (*Lentinus squarrosulus* Mont.) ที่เพาะเลี้ยงในวัสดุก้อนเชื้อต่างชนิดกัน

Growth and Yield of Khon-Khao Mushroom (*Lentinus squarrosulus* Mont.) Cultured in Different Spawn Materials

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บทคัดย่อ

ในอนาคตข้างหน้าเป็นที่คาดกันว่าเมล็ดข้าวฟ่างจะขาดแคลน และมีราคาแพง เนื่องจากการลดปริมาณการผลิต และความต้องการที่เพิ่มขึ้นเพื่อใช้เป็นอาหารสัตว์ และผลิตเอทานอล ดังนั้นการหาวัสดุอื่นเพื่อมาแทนที่เมล็ดข้าวฟ่างในการเพาะเชื้อเห็ดจึงมีความสำคัญ การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการเจริญเติบโตและผลผลิตของเห็ดขอนขาว (*Lentinus squarrosulus* Mont.) ที่เพาะเลี้ยงในวัสดุก้อนเชื้อต่างชนิดกัน โดยใช้เห็ดขอนขาวสายพันธุ์ K2 จากฟาร์มเวียงหงส์ อำเภอแม่เมาะ จังหวัดลำปางในการศึกษา การทดลองวางแผนแบบสุ่มอย่างสมบูรณ์ (Completely randomized design CRD) โดยใช้การทดลองแบบซ้ำสี่ตัวอย่างด้วยวัสดุก้อนเชื้อ 3 ชนิด ได้แก่ เมล็ดข้าวฟ่าง ไม้ไผ่ และไม้กระถิน การเจริญเติบโตของเห็ดวัดจากจำนวนวันที่ไมซีเลียมเติบโตเต็มก้อนเชื้อและก้อนเห็ด ผลผลิตเห็ดวัดจากด้วยจำนวนเห็ดต่อถุง น้ำหนักเห็ดสดต่อถุง และความกว้างและความสูงของเห็ด ผลลัพธ์มีดังนี้ 1) จำนวนวันที่ไมซีเลียมเติบโตเต็มก้อนเชื้อที่ผลิตจากเมล็ดข้าวฟ่าง ไม้ไผ่ และไม้กระถิน เท่ากับ 10.28, 12.70 และ 17.75 วัน ตามลำดับ; 2) จำนวนวันที่ไมซีเลียมเติบโตเต็มก้อนเห็ดสำหรับเห็ดที่มาจากก้อนเชื้อที่ผลิตจากเมล็ดข้าวฟ่าง ไม้ไผ่ และไม้กระถิน คือ 23.16, 18.12, และ 18.02 วัน ตามลำดับ; 3) เห็ดที่เพาะเลี้ยงในก้อนเชื้อที่ผลิตจากเมล็ดข้าวฟ่างมีผลผลิตสูงสุดในทุกด้าน ได้แก่ จำนวนเห็ดต่อถุง เท่ากับ 17.95 น้ำหนักเห็ดสดต่อถุง เท่ากับ 61.13 กรัม และความกว้างและความสูงของเห็ด เท่ากับ 4.88 และ 6.32 เซนติเมตร ตามลำดับ ผลลัพธ์เหล่านี้แสดงให้เห็นถึงความเป็นไปได้ของการใช้ไม้ในการเพาะเชื้อเห็ดแทนเมล็ดข้าวฟ่าง เนื่องจากการใช้ก้อนเชื้อที่ผลิตจากไม้ไผ่และไม้กระถินสามารถลดระยะเวลาที่เส้นใยเจริญเต็มก้อนเห็ดได้ถึง 5 วัน แม้ว่าผลผลิตจะน้อยกว่าการใช้ก้อนหัวเชื้อที่ผลิตจากเมล็ดข้าวฟ่าง ในอนาคตหากมีการวิจัยเพิ่มเติมเพื่อหาสภาวะที่เหมาะสมต่อการผลิตเห็ดโดยใช้ก้อนเชื้อที่ผลิตจากไม้ ก็จะทำให้การผลิตเห็ดดังกล่าวมีประสิทธิภาพมากขึ้นทั้งในแง่ของการเจริญ และผลผลิตของเห็ด

คำสำคัญ: เห็ดขอนขาว เมล็ดข้าวฟ่าง ไม้ไผ่ ไม้กระถิน

Abstract

In the future, it is expected that sorghum grain will be needy and high priced due to the reduction of its production and the increased demand for use as animal feeds and ethanol production. Therefore, it is important to find other materials to replace sorghum grain in mushroom spawn production. The research aims to study the growth and yield of Khon-Khao mushroom (*Lentinus squarrosulus* Mont.) cultured in different spawn materials. Khon-Khao mushroom strain K2 from Wiang Hong farm, Mae Mo district, Lampang province was used in this study. Completely randomized design (CRD) experiments were planned using four replications with three types of spawn materials including sorghum grain, bamboo wood and Leucaena wood. The mushroom growth was measured by the number of days for mycelium to grow entirely in spawns and composts. The mushroom yield was measured by the mushrooms per bag, fresh mushroom weight per bag and mushroom width and height. The results were as follows: 1) the number of days for mycelium to grow entirely in spawns produced from sorghum grain, bamboo wood and Leucaena wood were 10.28, 12.70, and 17.75 days, respectively; 2) the number of days for mycelium to grow entirely in composts for mushroom from sorghum grain, bamboo wood, and Leucaena wood spawns were 23.16, 18.12, and 18.02 days, respectively; 3) mushroom cultured in the spawn produced from sorghum grain spawn had the highest yield in all aspects namely the number of mushrooms per bag of 17.95, fresh mushroom weight per bag of 61.13 g and mushroom width and height of 4.88 and 6.32 cm, respectively. These results demonstrate the possibility of using wood in mushroom spawn cultivation instead of sorghum grain. This is because the use of spawns produced from bamboo wood and Leucaena wood could shorten spawn running time by 5 days, although the yield is lower than that of the spawn produced from sorghum grain. In the future, if further research works are conducted to determine the optimal conditions for mushroom production using wood spawns, the mushroom production will be effective in terms of mushroom growth and yield.

Keywords: *Lentinus squarrosulus* Mont., Sorghum grain, Bamboo wood, Leucaena wood

1. Introduction

Mushrooms are food that Thai people have known for a long time. They are available in fresh, dried, and canned forms. It is trendy to eat nowadays, especially those who do not like to eat meat, and the popularity of eating has become increasing because mushrooms can be used to cook a variety of foods and have unique flavors. Some mushrooms can also be used as medicinal or preventive herbs [1]. Thailand has many different types of mushrooms, either wild mushrooms or cultivated mushrooms. It is a country where the climate is suitable for growing almost all types of mushrooms and has many agricultural wastes that can be alternatively used as mushrooms' cultivation materials [2], [3].

Nowadays, mushroom cultivation has become another prevalent occupation both as the primary and supplementary career because its cultivation process is not complicated and does not require expertise and experience to succeed [4]. Currently, there are three types of mushroom cultivation in Thailand namely 1) cultivation on plastic bottles in closed greenhouses and controlled environments that is primarily used for Japanese mushrooms such as Shimeji mushrooms and Eringi mushrooms; 2) cultivation on the ground or in a semi-closed tiered greenhouse that is commonly used for straw mushrooms and termite mushrooms; 3) cultivation on plastic bags and subsequent mushroom opening in a non-closed greenhouse that is suitable for oyster mushrooms, fairy mushrooms and white log mushrooms. The last type of mushroom cultivation is the most popular one as it does not require modern equipment and is inexpensive.

There are essential steps for cultivating mushrooms in plastic bags, including purification in agar, spawn making, compost making, and mushroom opening. Making mushroom spawn in Thailand mainly uses sorghum grain. However the current sorghum grain production has decreased due to the reduction of sorghum planting area and their utilization for other purposes such as animal feeds and ethanol production [5]. As a result, the demand for sorghum grain is much higher than their production volume leading to the high price of sorghum grain.

Therefore, the research was to study the growth and yield of Khon-Khao mushroom (*Lentinus squarrosulus* Mont.) cultured in different spawn materials including sorghum grain, bamboo wood, and Leucaena wood based on the speculation that using wooden sticks as mushroom spawn materials may facilitate inoculation process and mycelium growth faster than using sorghum grain as mushroom spawn material [6]. This research was conducted to compare the growth of mycelium during the inoculation process in spawn and compost. The findings may lead to the feasibility of using wood for mushroom inoculation instead of sorghum grain.

2. Materials and methods

Khon-Khao mushroom strain K2 from Wiang Hong farm, Mae Mo district, Lampang province was used in this study. The experiment was planned and conducted using a Completely Randomized Design (CRD) with three treatments and four replications. Each of which was as follows: Treatment 1, spawns with sorghum grain; Treatment 2, spawns with a bamboo wood; Treatment 3, spawns with Leucaena wood.

2.1. Preparation of mushroom spawns

2.1.1. Preparation of sorghum grain spawn

Sorghum grains were washed with clean water to discard undesired contaminants. They were soaked in water for 12-18 h and boiled until soft or ripe without breaking them. The water was drained and the ripe grains were left until they were dry. The dried grains were poured into a 250 ml bottle, about three-quarters of it. The bottle mouth was cleaned and covered with a cotton plug covered with paper fasten with a rubber band. The filled bottles were autoclaved for 20 min at 121°C, cooled, and then inoculated with mycelial plugs from the PDA agar. The spawn was incubated at room temperature until the substrate was completely colonized by *L. squarrosulus* Mont. mycelium.

2.1.2. Preparation of wood spawns

The sticks, which were from bamboo wood and Leucaena wood, were 12 cm long and 0.5 cm thick (Figure 1). The sticks were soaked in tap water for 24 h, boiled for 15 min and cooled. In the case of Leucaena wood, the bark was peeled off until only the wood core remained. Each of the woods was packed into a 250 ml bottle (about 40 sticks per bottle). The bottle mouth was cleaned and covered with a cotton plug covered with paper fasten with a rubber band. The filled bottles were autoclaved for 20 min at 121°C, cooled, and then inoculated with mycelial plugs from the PDA agar. The spawns were incubated at room temperature until the sticks were completely colonized by *L. squarrosulus* Mont. mycelium.

Figure 1 Characteristics of wooden sticks

Figure 2 Sealed bottles packed with wooden sticks

Figure 3 Mycelium growth on wooden sticks

2.2. Preparation of mushroom compost

The compost was made from sawdust mixed with various ingredients according to the formula: 100 kg of sawdust, 6 kg of fine bran, 3 kg of dolomite and approximately 65-70% moisture. The method of making it started from 1) stacking 100 kg of sawdust on the cement floor, removing all the wood chips and stones; 2) adding 6 kg of fine bran by crushing the fine bran clumping into lumps to break them apart; 3) adding 3 kg of dolomite and mixing well; 4) adding water until the moisture content was at the level of 65-70% (indicated by the clumping of sawdust when it was squeezed by hands); 5) putting the sawdust mixture about 800-900 g into a mushroom cultivation bag, 15-17 cm tall and 8-9 cm wide; 6) placing and tightening a bottle's neck at the top of the bag and covering it with paper fasten with a rubber band; and 7) steaming the compost in an autoclave for 3 h.

2.3. Inoculation of composts with sorghum grain and wood spawns

Inoculation of composts with sorghum grain spawn was done by shaking the spawn bottle to break apart the grains. The paper cover on the top of a compost bag was opened. About 7-10 of sorghum grains were poured into the opened compost bag that was then shaken to allow the sorghum grains to spread apart. The inoculated compost bag was sealed back by the same paper cover. The process was repeated until all of the assigned compost bags were inoculated.

Inoculation of composts with wood spawns was done by opening the paper covering the top of a compost and using a sterilized stick similar in size to the spawn stick to drill a 12-cm-deep hole at the center of the compost bag. A wooden stick was pinched from a wooden stick spawn by sterilized tweezers and placed it into the prepared hole in the compost. The inoculated compost bag was sealed back by the same paper cover. The process was repeated until all of the assigned compost bags were inoculated.

All steps of this procedure were performed using aseptic technique in a wind-free cabinet cleaned with 70% alcohol.

All of the inoculated composts were incubated at room temperature until the composts were completely colonized by *L. squarrosulus* Mont. mycelium.

For the fructification, mature composts were transferred into a mushroom greenhouse at 20°C and 80-90% relative humidity. When the primordial formed, the covers were removed from the compost bags. The mushroom was harvested only in first flush when the mushroom became mature.

2.4. Examination of mushroom growth and yield

The mushroom growth was measured by the number of days for mycelium to grow entirely in spawns and composts. The number of days for mycelium to grow entirely in composts is also call the spawn running time. The mushroom yield was measured by the mushrooms per bag, fresh mushroom weight per bag and mushroom width and height.

2.5. Data analysis

Data were analyzed by the statistical program IRRISTAT 4.3. One way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were used for analysis. Differences were considered significant at $p < 0.05$.

3. Results and discussion

The results on using different materials to cultivate mushrooms were as follows.

3.1. Mushroom growth

Table 1 shows the number of days for mycelium to grow entirely in spawns and composts.

The number of days for mycelium to grow entirely in the spawns produced from 3 different materials was significantly different. The mycelium cultured in mushroom spawn produced from sorghum grain used the least number of days to grow entirely in the spawn, 10.28 days, followed by 12.70 and 17.75 days for the spawns produced by bamboo wood and Leucaena wood, respectively (Table 1)

The number of days for mycelium to grow entirely in the composts (or spawn running time) produced from bamboo wood and Leucaena wood was not significantly different from each other but significantly different from the compost produced by sorghum grain. The mycelium cultured in mushroom compost produced from Leucaena wood used the least number of days to grow entirely in the compost, 18.02 days, followed by 18.12 and 23.16 days for the composts produced by bamboo wood and sorghum grain, respectively (Table 1).

Stick spawns or spawns made from wooden sticks have been emerging as a new type of mushroom cultivation inoculum with remarkable characteristics such as ease of inoculation and fast colonization [7]. In this study, the use of spawns produced from bamboo wood and Leucaena wood could shorten spawn running time which might infer that they may shorten the mushroom production time [8]. Similar result was reported by Zhang et al. [8] who found that the use of stick spawn for cultivation of *Pleurotus eryngii* had shorter spawn running time compared with the use of sawdust spawn.

Table 1 Number of days for mycelium to grow entirely in spawns and composts

Treatments	Number of days for mycelium to grow entirely in spawns	Number of days for mycelium to grow entirely in composts
Sorghum grain	10.28^{c}	23.16^{a}
Bamboo wood	12.70^{b}	18.12^{b}
Leucaena wood	17.75^{a}	18.02^{b}
CV	5.03	14.22

Means in the same column followed by different superscripts are significantly different at $p < 0.05$

CV = coefficient of variation

3.2. Mushroom yield

Table 2 shows the yield of mushrooms cultured in different spawn materials.

The average of mushrooms per bag for the mushrooms cultured in bamboo wood and Leucaena wood spawns were not significantly different from each other but significantly different from that cultured in sorghum grain spawn. The highest average of mushrooms per bag was found in the mushroom cultured in sorghum grain spawn, 17.95, followed by 10.16 and 9.71 for those cultured in bamboo wood and Leucaena wood spawns, respectively (Table 2).

The weight of fresh mushroom per bag for the mushrooms cultured in bamboo wood and Leucaena wood spawns were not significantly different from each other but significantly different from that cultured in sorghum grain spawn. The highest weight of fresh mushroom per bag was found in the mushroom cultured in sorghum grain spawn, 61.13 g, followed by 44.64 and 43.21 g for those cultured in bamboo wood and Leucaena wood spawns, respectively (Table 2).

The mushroom width of the mushrooms cultured in bamboo wood and Leucaena wood spawns were not significantly different from each other but significantly different from that cultured in sorghum grain spawn. The widest mushroom was the one cultured in sorghum grain spawn, 4.88 cm, followed by 3.17 and 3.09 cm for those cultured in bamboo wood and Leucaena wood spawns, respectively (Table 2).

The mushroom height of the mushrooms cultured in bamboo wood and Leucaena wood spawns were not significantly different from each other but significantly different from that cultured in sorghum grain spawn. The highest mushroom was the one cultured in sorghum grain spawn, 6.32 cm, followed by 5.84 and 5.75 cm for those cultured in bamboo wood and Leucaena wood spawns, respectively (Table 2).

Table 2 The yield of mushrooms cultured in different spawn materials

Treatments	Mushrooms per bag	Fresh mushroom weight (kg) per bag	Mushroom width (cm)	Mushroom height (cm)
Sorghum grain	17.95^{a}	61.13^{a}	4.88^{a}	6.32^{a}
Bamboo wood	10.16^{b}	44.64^{b}	3.17^{b}	5.84^{b}
Leucaena wood	9.71^{b}	43.21^{b}	3.09^{b}	5.75^{b}
CV	26.58	12.38	7.46	5.87

Means in the same column followed by different superscripts are significantly different at $p < 0.05$

CV = coefficient of variation

Several research works have been done to use different materials to cultivate mushroom. Zhang et al. [8] developed a new type of solid spawn (stick spawn) to cultivate *Pleurotus eryngii*. The stick was from broadleaf tree wood. It was found that the use of stick spawn to cultivate *P. eryngii* reduced spawn running time by 43.8% compared with the use of sawdust spawn, one of traditional spawns used for mushroom cultivation. In addition, the use of stick spawn increased the mushroom yield by 11.5% compared with the use of sawdust spawn. Senhtilnambi, Balabaskar and Eswaran [9] cultivated *Calocybe indica* by using spawns produced from different grains including sorghum grain, ragi grain, maize grain, cotton grain, cow pea grain, horse gram grain, and black gram grain. It was found that sorghum grain spawn was the most suitable spawn for *C. indica* cultivation in terms of mycelium growth rate and mushroom yield. In addition, several reports have shown the possibility of using woods and other agricultural by-products for mushroom cultivation [10], [11], [12], [13]. Although this study presents the possibility of using bamboo wood and Leucaena wood spawns to replace sorghum grain spawn, further development are required to improve the yields given by the wood spawns. This drawback can be overcome by adding supplements to cultivation substrates [14]. Supplements can be high-cost commercial products or low-cost agricultural by-products such as chicken manure, grape pomace and feather flour. By adding supplements in the optimal ratio, the highest yield in the shortest period of production can be achieved [14].

4. Conclusion

It is estimated that in the future, sorghum grain will be needy and high priced and this situation is going to get worse and worse. This has substantial impact on mushroom producers who still use sorghum grain as a cultivation material. This study provides preliminary data on the possibility of using spawns produced from bamboo wood and Leucaena wood to replace that produced from sorghum grain in *L. squarrosulus* Mont. cultivation. Although they can shorten spawn running time, low mushroom yield is still their drawback that is needed to be overcome. Therefore, further research should focus on determining the optimal conditions for mushroom production using wood spawns in order to obtain the effective mushroom production in terms of mushroom growth and yield.

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