Mixture of Parawood Sawdust and Dried Napier Grass as a Substrate on *Lentinus squarrusulus* Mont. Cultivation

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Abstract:

Lentinus squarrosulus Mont., or Hed Khon Khao, is a commercial mushroom in Thailand. Parawood sawdust is a main substrate for bag mushroom cultivation but it is quite expensive. Napier grass is a fast growing grass which can be used as an alternative source for mushroom cultivation. This study aims to investigate the effect of parawood sawdust and Napier grass mixture on mycelial growth and fruiting body production of *L. squarrosulus*. Parawood sawdust and Napier grass mixtures in the ratio of 100:0(T1), 90:10(T2), 80:20(T3), 70:30(T4) and 50:50(T5) were used as a main substrate in bag cultivation. C/N ratio, substrate pH, mycelial growth, fruiting body and biological efficiency (BE) were determined. The results showed that all substrates had pH value and C/N ratio in the range of 6.23-6.80 and 107.56-160.57, respectively. Mycelial running on T1 to T4 substrates was faster than T5 substrate. T2 substrate gave higher fresh fruiting body weight (59.42±16.14 g/bag) than T1 (54.53 ± 10.67 g/bag), T3 (27.17 ± 11.98 g/bag), T4 (22.33 ± 13.70 g/bag) and T5 (4.14 ± 4.05 g/bag), respectively. In addition, there was no significant difference between BE of T1 (12.36±2.42%) and T2 (13.47±3.66%). These results suggested that 10% dried Napier grass mixture (T2) could be used to culture *L. squarrosulus* the same as parawood sawdust (T1) whereas higher amount of dried Napier grass resulted in significant decreasing in mushroom production. In conclusion, dried Napier grass can be used to mix with parawood sawdust for *L. squarrosulus* cultivation leading to the decrease of parawood sawdust usage.

Keywords: *Lentinus squarrosulus*, Parawood sawdust, Napier grass, C/N, Biological efficiency

Introduction

Edible mushroom is widely consumed in Thailand. Fast growing of mushroom farming business results in highly demand in raw materials for mushroom cultivation. Parawood sawdust is a main composition in bag mushroom cultivation. However, parawood sawdust is quite expensive because of delivery cost. The farmers have to buy this parawood sawdust in bulk to lower the cost and have to have a big area to keep the sawdust. In this study Napier grass was selected as an alternative source to mix with parawood sawdust in order to lower the parawood sawdust usage in the substrate bag. *Pennisetum purpureum*, also known as Napier grass or elephant grass is a rhizomatous perennial grass in Poaceae family. This grass is tall, fast growing and can be harvested several times a year [1]. Napier grass needs low water and nutrient inputs [2,3], therefore, this grass is widely grown in Thailand. Napier grass stem and leaf contain high carbon (44-48%), oxygen (44-49%), cellulose (29-38%), hemicellulose (15-20%), lignin (24-30%) and several elements especially potassium (49-64%) [3]. Carbon/Nitrogen (C/N) ratio of substrate is important for mushroom cultivation. High C/N ratio substrate is needed during mycelial growth period whereas low C/N ratio is needed during fruiting body formation [4,5]. Therefore, mycelial growth can be faster in high C/N ratio substrate than those of low C/N ratio substrate. Fruiting bodies need nitrogen for protein synthesis, thus, low C/N ratio substrate supports the formation of fruiting bodies better than high C/N ratio.

Lentinus squarrosulus (Mont.) or Hed Khon Khao is an edible mushroom commonly found in Thailand. This mushroom is in Polyporaceae family. Both fruiting bodies and mycelium of *L. squarrosulus* have high nutritional and pharmaceutical properties [6-8]. For commercial cultivation of *L. squarrosulus*, parawood sawdust is used as a main composition in a substrate bag. However, parawood sawdust price is expensive. In Thailand, parawood sawdust prices vary between 1,500-2,700 baht/ton depending on distance and purchase amount. And the farmers have to purchase at least 10 ton of sawdust in order to reduce the production cost. Finding an alternative source of substrate may lead to the reducing of mushroom production cost. Therefore, the objective of this study was to determine the effect of parawood sawdust and dried Napier grass mixture on mycelial growth and fruiting body production of *L. squarrosulus*.

Materials and methods

Raw material preparation

Napier grass was collected from Burapha University Sakaeo Campus farm. Fresh grass was cut and dried at 60ºC for 3 days by using hot air oven. The dried grass was mashed by using wood grinder and the approximate size of mashed grass was 1-5 mm. Dried parawood sawdust was bought from a farmer in Sa Kaeo Province. Rice bran was bought from a rice mill in Wattana Nakorn District, Sa Kaeo Province, Thailand.

Experimental design

This study was performed at Burapha University Sakaeo Campus, Thailand, since September to December 2016. Completely Randomized Design (CRD) was used in this experiment. The treatments were divided into 5 treatments including parawood sawdust and Napier grass mixtures in the ratio of 100:0(T1), 90:10(T2), 80:20(T3), 70:30(T4) and 50:50(T5). The experiments were performed in triplicate and each treatment consisted of 12 mushroom bags. Substrate ingredients of each treatment were shown in Table 1. The ingredients were mixed and then packed into a mushroom plastic bag (1 kg/bag). The bags were streamed for 5 h. The bags were sampling for pH measuring and C/N ratio analysis. C/N ratio was analyzed by iLab, Chiang Mai Province, Thailand. Carbon was analyzed as described by Allison [9] and nitrogen was analyzed by using AOAC [10].

Table 1 Substrate ingredients of each treatment

Substrate ingredients	Treatments (kg)				
	T1	T2	T3	T4	T5
Parawood sawdust	12.00	10.80	9.60	8.40	6.00
Dried and mashed Napier grass	0.00	1.20	2.40	3.60	6.00
Sugar	0.12	0.12	0.12	0.12	0.12
Lime	0.12	0.12	0.12	0.12	0.12
Pumice powder	0.12	0.12	0.12	0.12	0.12
Gypsum powder	0.12	0.12	0.12	0.12	0.12
Magnesium sulfate	0.06	0.06	0.06	0.06	0.06
Water	7.00	7.00	7.00	7.00	7.00

Mushroom spawn, mycelial and fruiting body cultivation

L. squarrosulus mycelia were isolated from fresh fruiting body and cultured in potato dextrose agar for 5 days. The mycelia were transferred to grow on sterile sorghum seeds. After incubation at room temperature for 5 days, the mushroom spawn completely grew on sorghum seeds. The mycelial spawn was inoculated into all substrate bags. The bags were incubated at room temperature until the mycelium completely grew (6 weeks). The bags were transferred into mushroom house and the upper part of bags was cut. During housing, water was sprayed twice a day to control the humidity (70-80%) and temperature (25-32ºC). Three or four days after primordium appearing, mature fruiting bodies were ready for picking up based on commercial size of this mushroom. In each day, fresh fruiting bodies were collected and weighed. After 48 days, accumulated weight of fresh fruiting body in each treatment was calculated for fresh fruiting body weight per bag and then calculated for biological efficiency (BE) as following equation.

$$BE = (\text{fresh fruiting body weight per bag} \div \text{substrate weight per bag}) \times 100$$

Statistical analysis

The results were expressed in mean ± S.D. One-Way ANOVA and Post-Hoc test (LSD) were analyzed by using SPSS version 16.0. Significant difference was p value < 0.05.

Results and discussion

The substrate pH and C/N ratio of each treatment were shown in Table 2. Substrate pH was in range of 6.23 – 6.80 and there was no significant difference between substrate pH of each treatment indicating that increased Napier grass mixing did not affect the whole substrate pH.

The C/N ratio is important for mushroom production. During mycelial growth, the mycelia adsorb carbon to produce an energy for their growth, therefore, mycelia rapidly grow in high C/N ratio substrate [11, 12]. Whereas low C/N ratio is necessary for fruiting body formation because high nitrogen needs for enzyme and protein production in fruiting bodies [5]. The C/N ratio of substrate treatments in this study were between 107.56 to 160.57. In Figure 1, the mycelial growth of T1, T2, T3 and T4 was in the same rate and faster than those of T5. However, all bags were ready to be transferred into mushroom house at 6th week. These results indicated that variation of C/N ratio of substrate might not directly affect to mycelial growth. The mycelium of this mushroom may possibly grow in wide range of C/N ratio. Chang and Miles (2009) explained C/N ratio that the most appropriate for *Pleurotus* spp. production is vary between 32 to 150 [13].

After housing for 4 days, the primordium was formed and then subsequently developed to form mature fruiting bodies (Figure 2). T1 provided fruiting bodies faster and higher in yield than those of T2, T3, T4 and T5, respectively (Figure 3). However, at day 46 to 48 the cumulative weight of T2 became higher than those of T1. The results might indicate that T2 tended to give higher yield than T1 when housing longer. Interestingly, there was no significant difference between average weight of fresh fruiting bodies (59.42 ± 16.14 g/bag) and BE (13.47 ± 3.66 %) of T2 and those of T1 (54.53 ± 10.67 g/bag and 12.36 ± 2.42 %, respectively) (Table 2). Whereas T3, T4 and T5 showed lower yield, average weight of fresh fruiting bodies per bag and BE than T1 and T2 (Figure 3 and Table 2). These results indicated that Napier grass mixing higher than 10% might decrease the yield of this mushroom.

Table 2 pH, C/N, fruiting body weight and BE of each treatment

Treatments	Parawood : Napier grass	Substrate pH	Substrate C/N ratio	Average weight of fruiting bodies (g/bag)	BE (%)
T1	100 : 0	6.28±0.47	125.75	54.53 ± 10.67^{a}	12.36 ± 2.42^{A}
T2	90 : 10	6.23±0.42	107.56	59.42 ± 16.14^{a}	13.47 ± 3.66^{A}
T3	80 : 20	6.80±0.53	160.57	27.17 ± 11.98^{b}	6.16 ± 2.72^{B}
T4	70 : 30	6.27±0.15	119.71	22.33 ± 13.70^{b,c}	5.06 ± 3.11^{B,C}
T5	50 : 50	6.27±0.12	122.42	4.14 ± 4.05^{c}	0.94 ± 0.92^{C}

Note: a-c represents significant difference between average weight of fruiting bodies (p < 0.05)

A-C represents significant difference between BE (p < 0.05)

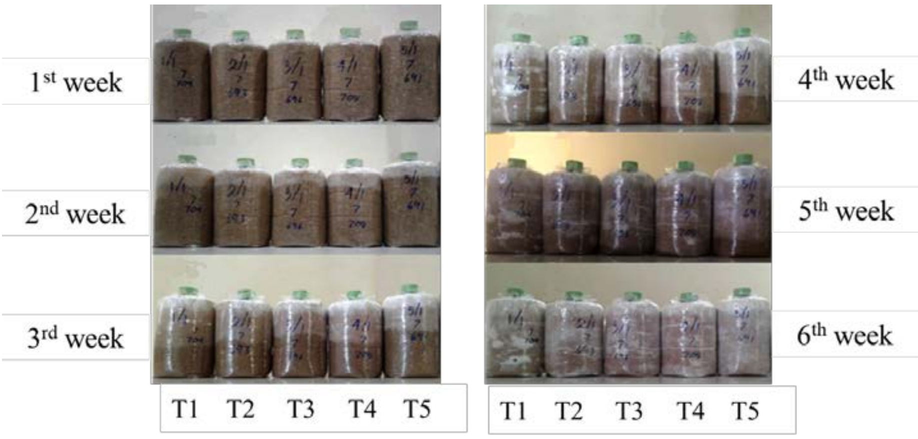

Figure 1 Mycelial growth of each treatment during incubation period.

Figure 2 Development of *L. squarrosulus* fruiting bodies. A white arrow indicates a primordium.

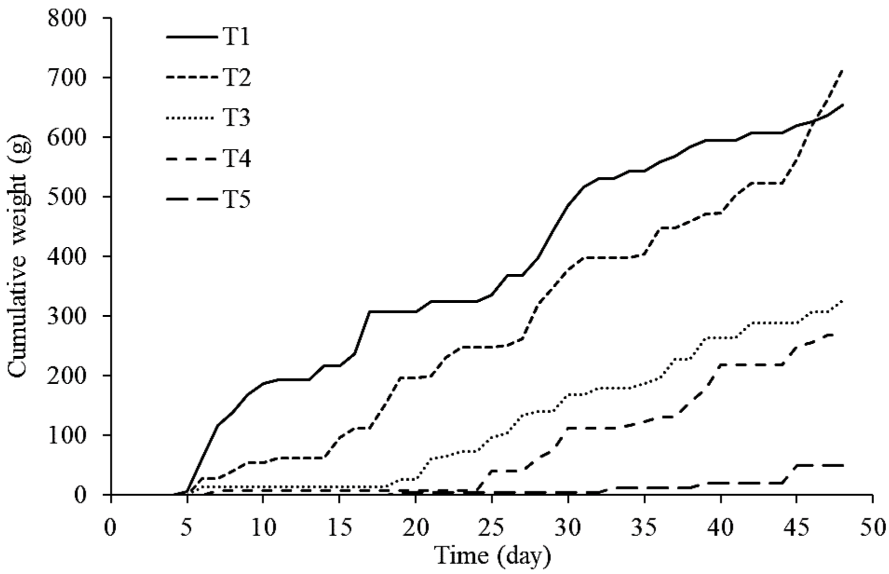

Figure 3 **C**umulative weight of fruiting bodies from each treatment within 48 days.

Although low C/N ratio is necessary for fruiting body formation, low C/N ratio may affect negatively fruiting body productivity. According to Donini et al. (2009), lowering C/N ratio by adding 10%

(C/N ratio of 43) and 20% soybean bran (C/N ratio of 21) into Napier grass-based substrate did not increase *Pleurotus ostreatus* productivity and also lower in productivity than the grass without soybean bran supplement (C/N ratio of 162) [14]. Even though C/N ratio of 10% supplement was higher than 20% supplement, they found that there was no significant difference in productivity of *P. ostreatus* between using of 10% and 20% soybean, wheat, rice and corn brans. In addition, they also found the difference in productivity of different strains in the same species. They concluded that addition of 10% bran was superior and 20% supplement did not increase productivity nor BE [14]. According to Ruilova Cueva et al. (2017), they reported C/N ratio of the substrate mixture for *P. ostreatus* cultivation. The treatment M1 that contained corn stover (40%), rice husk (20%) and wheat straw (38%) had C/N of 104.63. Whereas M2 that contained stover lentil (30%), wheat straw (40%) and bagasse sugarcane (28%) had C/N of 72.40. Although C/N ratio of M1 and M2 were different, both treatments provided similar results in first harvest day, period of harvesting and also no significant difference between fresh fruiting body weight and BE [15]. According to de Leon and colleagues [16] reported productivity and BE of *L. squarrosulus* when supplementing rice straw and sawdust based substrate with 5% to 25% rice bran and rice hull for increasing nitrogen. They found that productivity and BE did not sequentially increase when increasing of nitrogen source enrichment [16]. Osibe and Chiejina (2015) reported C/N ratio of palm press fibre (33.54), mahogany sawdust (235.95), *Gmelina* saqdust (158.77) and wheat bran (17.76) that used in the substrate mixture for *L. squarrosulus* cultivation [17]. Although mahogany and *Gmelina* sawdust had very different in C/N ratio but both sawdust gave no difference in BE (8.96% and 8.83%, respectively) and yield (44.80 and 44.15 g/kg, respectively). And when supplementation with nitrogen source from wheat bran (3% to 18%) they found that addition of 3% and 8% wheat bran into mahogany sawdust gave higher BE (15.91% and 58.95%) than mahogany sawdust alone (8.96%). But addition of 13% and 18% wheat bran into this sawdust decreased BE to 45.47% and 13.39%, respectively [18]. These results suggest that nitrogen supplement may reduce the C/N ratio but not certainly increase the fruiting body production. There are several physical, chemical and biological factors that affect the growth of mycelium and fruiting body development including chemical composition, water activity, C/N ratio, minerals, surfactant, pH, moisture, source of nitrogen, particle size, light, CO_2, temperature, salinity, inoculum and microbial contamination. These factors may affect individually or have interactive effects among the factors that may influence to mushroom growth and production [18,19]. In the production of each mushroom, therefore, the substrate and culture condition have to be optimize.

By the way, T1 and T2 in our study provided higher BE than the previous reports that used the different substrate on *L. squarrosulus* cultivation. This mushroom was reported to grow on rice straw, parawood sawdust and their mixture with the BE of 2.78 – 7.83 % [16]. Substrate mixture of parawood sawdust and 20% oil palm fruit fiber could advance the time of primordial appearance, induce the fresh fruiting body weight with BE of 3.94 – 8.13 % and increase number of flushes of *L. squarrosulus* [20]. Therefore, agricultural residues or agro-industrial wastes that have high nitrogen content can be mixed with the main substrate for increasing of yield and/or decreasing the use of parawood sawdust.

Conclusions

Napier grass can be mixed with parawood sawdust for *L. squarrosulus* cultivation, especially ratio of 1:9, can produce fruiting bodies as well as parawood sawdust alone. Therefore, the amount of parawood sawdust usage in *L. squarrosulus* production can be reduced by adding of dried Napier grass. For further study, fermented Napier grass will be applied in the substrate development.

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