Utilization of *Vetiveria zizaniodes* (L.) Nash Leaves in *Ganoderma lucidum* Cultivated

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Abstract

The aim of this research were investigate the substitution of sawdust with *Vetiveria zizaniodes* (L.) Nash, Sri Langka cultivar on the media in mycelia growth of *Ganoderma lucidum* in plastic bag. The suitable three media from eleven formulas with different weight ratio of sawdust and *V. zizaniodes* leaves at 100:0, 0:100 and 20:80 are selected in this research and gave the biological efficiency with 42.72, 00.00 and 40.71% respectively. This results show the opportunity for substitution of sawdust with *V. zizaniodes* leaves which can reduce the production cost of *G. lucidum*. Nutrition analysis of fruiting body of mushroom are cultivated on different type of the substrates, particularly sawdust 100:0 in plastic bag, found more protein than cultivated in sawdust 20:80 with 10.29 and 8.45 mg/100 g dry weight in *G. lucidum* respectively. In amount of essential amino acid and non-essential amino acid of *G. lucidum* cultivated sawdust 100:0 gave more amino acid than 20:80.

1. Introduction

His Majesty King Bhumibol Adulyadej, Rama IX had graciously provided initiative in utilization of vetiver on June 22, 1991 to Dr. Sumeth Tantivechakul, the general secretary of Royal Projects Development Board of Thailand. The substance concerned with utilization of vetiver for soil and water conservation with a wide range of cultivation patterns. In addition to its importance, the vetiver is advantageous in many patterns. All parts of vetiver, no matter what roots,
trunk and leave could be beneficial e.g. forage, mushroom substrate, green manure, cover crop, toxic substance absorption, waste water treatment, pest and weed control, fragrance, herbs, and handicrafts. Vetiver at the age of 3-4 months is required leave pruning to stimulate and maintain hedgerows and fertility or prolific and efficient root system. Mushroom is high protein food with mineral and high vitamin than some vegetable, in addition mushroom is good taste and smell. Due to its strong savory taste, mushroom consumption has been increasing in the present. Furthermore, its therapeutic property is disease healing. In the ancient time, Roman people believed that mushroom is food of gods. According to Chinese people’s belief, the mushroom was elixir of life while *Lentinula edodes* was usually consumed by Japanese people so as to be aphrodisiac. Actually, mushroom cultivation in plastic bags has been creating vital income for several entrepreneurs, i.e. *L. edodes*, *S. commune* and *G. lucidum* with main substrate in plastic bags, Parawood sawdust. Shortage of sawdust in the future. Furthermore, increasing of fuel price and long distance of transportation from sawdust sources would certainly make sawdust in short supply and high price.

2. Material and methods

2.1. Suitable substitution ratio of sawdust with *V. zizaniodes* leaves towards *G. lucidum* cultivation

2.1.1. Preparation of mushroom starter of *G. lucidum*

1) Transplant stock culture of *G. lucidum* based on Thailand Mushroom Culture Collection on PDA Petri dish incubated in room temperature for 5 days 2) Apply an apparatus for punching cork for 0.5 cm diameter of outer area of colony 3) Soak sorghum seeds for 12 hours and sieve for draining and dry in the air 4) Boil in water for 30 minutes then percolate with filter and expose in the room temperature till cool 5) Contain in 250 ml flask with only 2/3 part of the bottle then sterilize in autoclave at 121°C with 15 psi. for 30 minutes and 6) Stir substrate in item 2) upon the bottle of sorghum cool down, to incubate in the room temperature for 5 days resulting in the sorghum spawn

2.1.2. Preparation of mushroom substrates for *G. lucidum* cultivation

Main substrates of mushroom namely Para wood sawdust and *V. zizaniodes* leaves based on Education Development Center Soil and Water Conservation, Office of Land Management Research and Development, Land Development Department, Ministry of Agriculture and Cooperatives, Nakhonratchasima are detailed as following.

**Parawood sawdust:** 1) Sieve sawdust to separate unwanted materials 2) Bring sawdust to mixed with supplementary nutrients formula 1 for *G. lucidum* incubated in petri dish (Table 1) 3) Sterilize in autoclave at 121°C for 30 minutes and 4) Transplant sorghum spawn for 1 sorghum seed, then incubate in the room temperature for 7 days

**V. zizaniodes** leaves; Cut *V. zizaniodes* leaves from the parent plant and dry in the sun for 3 days. then cut the leaves with tool and soaking in the water for 30 minutes and draining. The preparation of *V. zizaniodes* leaves is taken for following necessary steps.

1) *V. zizaniodes* leaves; 1.1) *V. zizaniodes* leaves was mixed with supplementary nutrients formula 2 for *G. lucidum*, then incubated in petri dish (Table 1) 1.2) *V. zizaniodes* leaves mixed with sawdust by the different proportion by weight ratio with formula 4-7 for *G. lucidum* incubated in petri dish (Table 1) 1.3) All are sterilized in autoclave at 121°C for 30 minutes and 1.4) Transplant sorghum spawn for 1 sorghum seed, then incubate in the room temperature for 7 days

2) *V. zizaniodes* fermented leaves; Bring *V. zizaniodes* leaves to mix with Urea, CaCO3 covering with plastic for 2 days then firstly turn over the spawn (layer of materials). In the day 5 of ferment, turn over the spawn mixing with MgSO4·7 H2O then keeping ferment for 7 days resulting in *V. zizaniodes* fermented leaves; 2.1) *V. zizaniodes* leaves proportion mixed with nutrient formula 3 for *G. lucidum* incubated in petri dish (Table 1) 2.2) *V. zizaniodes* fermented leaves mixed with sawdust by the different proportion weight ratio with formula 8-11 for *G. lucidum* incubated in petri dish (Table 1) and 2.3) Transplant sorghum spawn for 1 sorghum seed, then incubate in the room temperature for 7 days

**Laboratory Testing Record:** 1. Growth of mushroom mycelium with observation in terms of color, liquid
substance and density of mycelia and 2. Average diameter of colonies size **Data analysis:** 1. Design the test data and develop the test plan conformed to the requirement of CRD Method, 11 treatments each with 9 duplicated tests, totally 99 PDA petri dishes. and 2. Comparative study on average variation by DMRT

2.2. Biological efficiency for Cultivation of G. lucidum with V. zizaniodes leaves in plastic bags

2.2.1. Preparation of sorghum spawn
Repeat the same manner in the process of in item 2.1.1

2.2.2. Preparation of mushroom substrates in plastic bags
Prepared with an appropriate formula in item 2.1.2, all are contained in plastic bags for 500 g each, then sterilize at 121°C for 30 minutes.

2.2.3. Mushroom substrate transplant and incubation
Bring Sorghum spawn with 10 seeds to transplant into the spawn then incubate in the room temperature till mushroom mycelium grow up throughout container.

2.2.4. Fruiting bodies production
1) Substrates of G. lucidum is uncovered by the lid and kept in the particular fruiting bodies nursery horizontally layered watering 2 times daily at 8:00 A.M. and 5:00 P.M. and 2) Mushroom harvesting for 3 months

2.2.5. Nutritional analysis of mushroom from G. lucidum
Comparison of nutritional analysis of mushroom on sawdust bags and mushroom cultivated in suitable formula from essential amino acid and non-essential amino acid is taken with following steps: 1) Dried the mushroom in hot air oven at 60°C for 72 hours and 2) Grinding mushroom fruit for nutritional analysis on essential amino acid and non-essential amino acid in compliance with AOAC (2005) at Central Laboratory (Thailand) Co., Ltd.

**Laboratory Testing Record:** 1. % Biological Efficiency 2. Growth characteristics of mushroom mycelium and 3. Food nutrition from mushroom **Data analysis:** 1. Design the test data and develop the test plan according to the requirement of CRD by 3 treatment with 100 duplicated tests 2. Comparative study of average variation by DMRT and 3. Percent coefficient of variation

\[
\% \text{ B.E.} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrates (g)}} \times 100 \tag{1}
\]

Table 1. Formula for cultivation of *G. lucidum* in petri dish

<table>
<thead>
<tr>
<th>Formula</th>
<th>Main substrates for cultivation</th>
<th>Proportion by weight</th>
<th>Supplementary nutrients</th>
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<tbody>
<tr>
<td>1</td>
<td>Sawdust</td>
<td>100</td>
<td>CaCO₃ 0.5%</td>
</tr>
<tr>
<td>2</td>
<td><em>V. zizaniodes</em> leaves</td>
<td>100</td>
<td>MgSO₄·7 H₂O 0.2%</td>
</tr>
<tr>
<td>3</td>
<td><em>V. zizaniodes</em> fermented leaves</td>
<td>100</td>
<td>Fine rice barn 6%</td>
</tr>
<tr>
<td>4</td>
<td>Sawdust : <em>V. zizaniodes</em> leaves</td>
<td>80 : 20</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sawdust : <em>V. zizaniodes</em> leaves</td>
<td>60 : 40</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sawdust : <em>V. zizaniodes</em> leaves</td>
<td>40 : 60</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sawdust : <em>V. zizaniodes</em> leaves</td>
<td>20 : 80</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sawdust : <em>V. zizaniodes</em> fermented leaves</td>
<td>80 : 20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sawdust : <em>V. zizaniodes</em> fermented leaves</td>
<td>60 : 40</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sawdust : <em>V. zizaniodes</em> fermented leaves</td>
<td>40 : 60</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Sawdust : <em>V. zizaniodes</em> fermented leaves</td>
<td>20 : 80</td>
<td></td>
</tr>
</tbody>
</table>
3. Results and discussion

3.1. Suitable substitution ratio of sawdust with V. zizaniodes leaves towards G. lucidum cultivation

Growth of fungus mycelium of G. lucidum cultivated on substrates at formula 1-11 in petri dish with proportion by weight i.e. is found that at age of 2 days 2.58, 1.76, 1.54, 2.27, 1.78, 2.08, 1.48, 1.58, 1.47, 1.40, 1.40 cm respectively; at age of 4 days 4.94, 3.77, 2.00, 3.98, 3.82, 3.00, 2.99, 2.00, 1.99, 2.01, 1.97 cm respectively; and at age of 6 days 6.68, 5.99, 3.01, 6.96, 4.87, 4.36, 5.36, 3.01, 2.66, 2.84, 2.74 cm respectively. (Fig. 1) Growth characteristics of mycelium i.e. color, throughout colonized substrates surface and density of mycelium are described as following: 1) Color of mycelium of G. lucidum cultivated in formula 1-7 is found that, mushroom mycelium are white. Cultivation in formulas 8, the mushroom mycelium are white with liquid yellow substance. Cultivation in formula 10 and 11, the mushroom mycelium are white with liquid yellow substance. 2) Growth development thoroughly colonizing on substrates surface at age of 8 days; formula 8-11 found growth development thoroughly colonizing on substrates at age of 7, 8, 14 days respectively; formula 4-7 is found growth development thoroughly colonized substrates at age of 7, 8, 14 days respectively; formula 2 and 4-7 found fairly dense mycelium; formula 8-11 found less dense mycelium. For testing, the researcher selected substrates for G. lucidum with formula proportion by weigh, sawdust 100 (formula 1), V. zizaniodes leaves 100 (formula 2) and sawdust 20: V. zizaniodes leaves 80 (formula 7). (Fig. 2)

According to the test, mycelium of mushroom species grew with V. zizaniodes leaves in substitution of parawood sawdust substrate. The result conformed to [2, 4, 7, 8], who studied on result of mycelium of the following mushroom grown with vetiver leaves substrate; Pleurotus rhodophyllus, P. citrinopileatus, P. cystidiosus, P. tuber-regium, Volvariella volvacea and Lentinula edodes. From the experiment, the comparison was made between V. zizaniodes leaves substrate and V. zizaniodes fermented leaves substrate. It found that V. zizaniodes leaves substrate, mycelium of two mushroom species is more developed than V. zizaniodes fermented leaves substrate. The result did not conform to [9], who presented that three grasses including Phragmites karka var. karka, Saccharum spontaneum and Thysanolaena latifolia were studied as the substrates for Pleurotus sp., Hungarian type and Bhutan type cultivation. It revealed that all composted grasses gave higher yield of Hungarian type than non-composted one.

3.2. Biological efficiency for Cultivation of G. lucidum V. zizaniodes leaves in plastic bags

For production of G. lucidum on substrates with 3 formulas in proportion by weight i.e. sawdust 100, V. zizaniodes leaves 100 and sawdust 20: V. zizaniodes leaves 80 in plastic bags is found that they gave the different mushroom fresh weight are 89.85, 00.00 and 77.56 g/substrate respectively with biological efficiency of 42.72, 00.00 and 40.71% successively. (Table 2; Fig. 2)

According to the test, V. zizaniodes leaves could be substitution of sawdust substrate for G. lucidum. The result conformed to [8] who reported that Pleurotus could be cultivated by V. nemoralis leaves dispensing with fermentation and sterilization. According to [2] presented that V. zizaniodes leaves could cultivate Pleurotus euos, P. citrinopileatus, P. suior-caju and P. platyatus. Furthermore, the reports of JUNCAO stated that several grasses species could be the substrates for Pleurotus, e.g. V. zizaniodes, Pennisetum pupureum, P. sinense, Phragmites communis, Miscanthus floridulus, M. sinense, Saccharum arundinaceum, S. sinense, Paspalum watsteinii, P. dilatatum, Sorghum propinquum, S. sukanense, Cymbopogon citrates, Triticum aestivum, Oryza sativa, Arundinella hirta and A. nepalensis. As a result of that vetiver leaves composed of cellulose, hemicelluloses, lignin, protein and other minerals decomposed by microorganism for growth development [3, 4, 7]. In addition, the experiment found that producing of G. lucidum inoculated by V. zizaniodes leaves 100 (formula 2) to prepare for substrate in plastic bags might induce slower growth of button stage of G. lucidum to be fruiting body of mature stage; so the production efficiency became 0.00. Producing G. lucidum on sawdust 100 substrate in plastic bags was found the most efficient trial. For the reason that two species could grow well on wood substrate, especially sawdust. The findings were that parawood sawdust contains cellulose and lignin with 57.99%, 41.24% (dry weight) respectively [5]. While vetiver leaves contain hemicelluloses,
cellulose and lignin with 40, 30-35 and 10% (dry weight) respectively [1]. According to [6] who studied on result of 17 vetiver varieties which revealed that there were cellulose and lignin 32.2–37.0 and 4.6–6.2% (dry weight) respectively. Based on the experimental data, producing *G. lucidum* mostly efficient with colonization in sawdust 100 might be in consequence of more cellulose and lignin in sawdust than vetiver leaves.

Based on analysis of nutrition value from fruiting of *G. lucidum* on colonized substrates with proportion by weight of formula 1 in plastic bags, the protein contents found is more nutritive than formula 7 with 10.29 and 8.45 mg/100 g dry weight respectively. For amino acids contents form fruiting of *G. lucidum* on colonized substrates of formula 1 a variety of quantitative essential amino acids are found i.e. Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine with 235.01, 137.29, 313.64, 343.49, 260.00, 175.27, 185.86, 316.56, 58.64 and 212.19 mg/100 g dry weight respectively. Non-essential amino acids are also found namely Alanine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Proline, Serine and Tyrosine with 318.98, 542.57, 148.65, 566.62, 291.15, 242.64, 383.25 and 123.48 mg/100 g dry weight respectively. Based on analysis of nutrition value from fruiting of *G. lucidum* on colonized substrate of formula 7, a variety of quantitative essential amino acids is found with 214.41, 115.31, 311.03, 324.83, 236.20, 164.67, 165.37, 266.51, 47.60 and 266.51 mg/100 g dry weight respectively. For essential amino acid from fruiting of *G. lucidum* on colonized substrate of formula 7, a variety of quantitative essential amino acids is found with 214.41, 115.31, 311.03, 324.83, 236.20, 164.67, 165.37, 266.51, 47.60 and 200.35 mg/100 g dry weight respectively. Quantitative essential amino acids are also found with 282.45, 354.14, 64.88, 408.57, 230.06, 190.83, 311.09 and 103.26 mg/100 g dry weight successively. (Table 3).

Following the nutritional analysis of mushroom inoculated in sawdust 100, more nutrients are found than those on sawdust 20: *V. zizaniodes* leaves 80. This complied to [9] who reported on result of *L. squarrosulus* cultivated from sawdust had higher protein percentage than *P. karka* var *karka*. However, the result did not conform to [3, 4] who reported that variation of grasses species e.g. *V. zizaniodes*, *Pennisetum sinense*, *M. sinense*, *S. sinense*, *Themeda gigantean*, *Paspalum wettsteinii*, *S. sudanense*, *Triticum aestivum*, *Arundinella hirta*, *Spartina anglica* and *Setaria anceps* are high molecular mass grasses with high nutritional contents. Furthermore, these grasses are herb species. Hence, the cultivated mushroom from which such grasses produce contains higher protein and some minerals contents than those from sawdust and wood log e.g. *Agaricus bisporus*, *Coprinus comatus*, *L. edodes* and *G. lucidum*.

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**Table 2. Mushroom fresh weight and biological efficiency of *G. lucidum* cultivated on substrates in plastic bags**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Mushroom fresh weight (g/Substrate)</th>
<th>% Biological efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89.85±18.01 a</td>
<td>42.72</td>
</tr>
<tr>
<td>2</td>
<td>00.00±0.00 c</td>
<td>00.00</td>
</tr>
<tr>
<td>7</td>
<td>77.56±8.52 b</td>
<td>40.71</td>
</tr>
<tr>
<td>CV %</td>
<td>20.58</td>
<td></td>
</tr>
</tbody>
</table>

**Remark:** 1/ Means average of 100 duplicating value each formula. a, b and c define the alphabets after the same number in column. None of difference with statistically significantly P-value < 0.05 were found.

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**Fig. 1.** Growth characteristics of *G. lucidum* mycelium on substrates in petri dish

**Fig. 2.** (a) *G. lucidum* cultivated on substrates with sawdust 100; (b) *V. zizaniodes* leaves 100; (c) sawdust 20 : *V. zizaniodes* leave 80 proportion by weight in plastic bags
4. Conclusion

From the experiment, the researcher selected 3 formulas with proportion by weight: sawdust 100, V. zizaniodes leaves 100 and sawdust 20: V. zizaniodes leaves 80. And then in the production efficiency of mushroom with V. zizaniodes Leaves in plastic bags of G. lucidum in substrates is production efficiency equivalent to 42.72, 00.00 and 40.71% respectively. Nutrition analysis of fruiting body of mushrooms cultivated on substrate, particularly sawdust 100 in plastic bag, found more protein than cultivated in sawdust 20: V. zizaniodes leaves 80. In amount of essential amino acid and non-essential amino acid of G. lucidum cultivated sawdust 100 more amino acid than in sawdust 20: V. zizaniodes leaves 80. Recommendation; Respond to the Royal Initiative for enhancement and development of vetiver utilization according to the Royal Initiative. Benefit for study on Bachelor of Science Program in Biology or other related subjects of Chandrakasem Rajabhat University such as subjects of Biology, Microbiology, Mushroom Biology, Industry and Food Microbiology.

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References