

Suitability of Water Hyacinth, Rice Straw and Sunflower Residues for the Production of the Edible Mushrooms *Coprinopsis cinerea* and *Volvariella volvacea* in Thailand

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ABSTRACT

Coprinopsis cinerea and *Volvariella volvacea*, both fungi are worldwide used for model organisms and also their nutritional and medicinal benefits throughout the subtropical and tropical region of the world. An existing variety of lignocellulose agricultural wastes after harvesting and supply plant weeds in Thailand can be used for mushroom production. We determined suitability of solid substrates such as water hyacinths, rice straw and sunflower residues for growing these edible mushrooms. Substrate preparations were provided the fermented raw materials for 2 months. Cultivation substrates were mixed with the culture strains for cultivation in a pot shape: 11 cm in diameter, 10 cm height and 2 cm of thickness. The physical properties of all substrates were evaluated: the mean of pH, moisture content and volume of open pores. The range of suitability for both mushroom productions were determined. pH was from 7.6 ± 0.1 to 8.9 ± 0.3 , moisture content was from 80.7 ± 0.5 to $82.9 \pm 0.9\%$, volume of open pores was between 124.6 ± 4.0 and $223.4 \pm 5.6 \text{ cm}^3$, growing temperatures were between 32.4 ± 0.1 to $40.3 \pm 0.0^\circ\text{C}$, relative humidity was between 70.2 ± 0.2 to $89.1 \pm 1.1\%$ and light intensity was between 0.0 ± 0.0 to $26.7 \pm 0.0 \text{ lux}$. A Randomized Complete Block Design experiment with 3 replicates per treatment was used to determine mushroom yield of *C. cinerea* and *V. volvacea* on the different types. The results showed that *C. cinerea* grew well on the containers containing a mixture of rice straw residues, and the yields were significantly higher on rice straw residues than on sunflower residues ($P \leq 0.01$). The best substrate for *V. volvacea* was water hyacinths where the yield was significantly higher ($P \leq 0.05$) than in the other substrates.

Key words: *Coprinopsis cinerea*, *Volvariella volvacea*, Sunflower, Water hyacinths, Rice straw

INTRODUCTION

Mushroom consumption has been increasing world wide when their abundant nutritional contents and the values of medicinal discovery (Zakhary et al., 1984; Belewu and Belewu, 2005). Cultivation of edible mushrooms present an economically increasing business that has expanded all over the world (Marshall and Nair, 2009). Both tropical mushrooms, *C. cinerea* or Hed-cone-noi; in Thai name and *V. volvacea* or Hed-fang; in Thai name and also commonly known as straw mushroom were studied nutritional analysis in Tansania (Mshandete and Cuff, 2007).

C. cinerea and *V. volvacea* have been used as organic model to study such as fruiting body formation, fruiting body development, molecular mechanism, molecular genetics and the potential application of biotechnology in Basidiomycetes. *C. cinereus* was introduced early as an object for studies of development mainly because of its relatively short life cycle, which can be completed in the laboratory within 2 weeks (Kües, 2000; Kües and Liu, 2000; Kües et al., 2007; Srivilai et al. 2005a,b). The hyphae of the initial stage development eventually differentiate to form a primordium that is essentially an embryonic fruiting body. At this stage, structures composing the stipe and cap (pileus) of the immature fruiting body are clearly discernible (Bouliannea et al., 2000). The straw

mushroom fruiting body is white or gray with short stipe and large cap and it is cultivated mainly in South-East Asian countries (Ahlawat et al., 2008).

Recently, several waste materials derived from agriculture and agroindustrial products (cereal straw, vine shoots and grapevine stems, dried olive mill waste, kenaf, cotton and flax wastes) have been utilized as substrates for oyster mushroom cultivation. *C. cinerea* and *V. volvacea* have been studied to grow on various lignocellulosic wastes such as water hyacinths or agro-waste such as rice straw and Sunflower to know more about the selective substrates of mushroom growth in this study. Which had been little known as well in a tropical country such as Thailand which have been little studied (Chisaena, 2009).

MATERIALS AND METHODS

1. Sources of Materials: The mushroom spawns of *C. cinerea* and *V. volvacea* were taken from a mushroom spawn market in Thailand.

2. Growth substrates: Water hyacinths, rice straw and sunflower residues used for substrate preparation were collected from harvesting

3. Preparation of substrates: In order to prepare fermented water hyacinths, rice straw and sunflower residues, all substances were chopped into small pieces and put in plastic bag for 2 months for aerobic fermentation. Before making the culture spawns, both fresh and fermenting substrates were dried by exposing them to sunshine for 3 days.

4. Spawning: The fresh and fermenting substrates were soaked into water overnight and then water was drained off. Fresh and fermenting substances were blended in a ratio of 1:1. These substrate blends were mixed with spawns of *C. cinerea* and *V. volvacea* and put into plastic pots or metallic molds in order to obtain a pot-like shape with a diameter of 11 cm, height of 10 cm and wall thickness of 2 cm (Figure 1A). These cultural pots were incubated in plastic containers (Figure 1B). A Randomized Complete Block Design (RCBD) experiment with 3 replicates per treatment was used to determine mushroom yield of *C. cinerea* and *V. volvacea* on the different experiment with 3 replicates per treatment was used to determine mushroom yield of *C. cinerea* and *V. volvacea* on the different substrates.

5. Culture condition: All pot cultures were incubated in plastic containers with four large plastic drawers as mushroom incubator. Size of each plastic container was 52 x 37 x 18 cm (length x width x height) During the cultivation time of a year of the growth of the fruiting body was evaluated and temperature, relative humidity and light intensity were assessed. In order to provide ventilation to the culture spawns, which was needed for fruiting body development, the drawers were opened once a day for 5 minutes (Figure 1B).

6. Fruiting and harvesting: Approximately 7-15 days later incubation of *C. cinerea* and *V. volvacea*, first mycelial stages appeared from the culture spawn.



Figure 1. Spawning was prepared by blending fresh and ferment-substrates of water hyacinths and rice in a ratio of 1:1 and inoculated with the culture strains follow from each methods. (A) Mushroom pots were made from a blend of substrates and fungal spawns by using plastic pot or metallic mold to provide the shape; the pots had a diameter of 11 cm, height of 10 cm and wall thickness of 2 cm. (B) All pot cultures were incubated for fruiting body development in plastic containers as mushroom incubator. The size of each plastic container was 52 x 37 x 18 cm.

Data collection

The following parameters of growth/yield were measured:

1. Moisture content and pH value of all mushroom pots before incubation were determined.
2. Growth condition such as temperature, relative humidity and light intensity were recorded every day during 2-3 weeks.
3. The number of fruiting bodies was determined; this was done by directly counting the number of fruiting bodies produced on each substrate.
4. Weight of fruiting bodies: An electronic balance was used to determine the average weight of the fruiting bodies. The obtained results were recorded and subjected to analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Physical properties and environmental condition of the mushroom pots:

The results showed the feasibility of mushroom cultivation of Hed-cone-noi (*C. cinerea*) and Hed-fang (*V. volvacea*) by making natural pots from rice straw residues, water hyacinths and mixed with 0.5% aqueous solution of tamarind wood vinegar in cultivation time during 8-25 April 2010 (Figure 2). When the proper environmental conditions for mushroom cultivation were obtained, fruiting bodies formed after 1 week. The cultivation conditions were as follows: the mean of pH value was from 7.6 ± 0.1 to 8.9 ± 0.3 , moisture content from $80.7 \pm 0.5\%$ to $82.9 \pm 0.9\%$, volume of open pores was between $124.6 \pm 4.0 \text{ cm}^3$ and $223.4 \pm 5.6 \text{ cm}^3$, growing temperatures from $32.4 \pm 0.1^\circ\text{C}$ to $40.3 \pm 0.0^\circ\text{C}$, relative humidity from $70.2 \pm 0.2\%$ to $89.1 \pm 1.1\%$ and light intensity from 0.0 ± 0.0 lux to 26.7 ± 0.0 lux.

Study on growth of Hed-cone-noi (*C. cinerea*), Hed-fang (*V. volvacea*) grown on natural pots made from water hyacinths, rice straw and sunflower residues

The results showed that *C. cinerea* grew well on the containers containing a mixture of rice straw residues, and the yields were significantly higher on rice straw residues than on sunflower residues ($P \leq 0.01$) (Table 1). The best substrate for *V. volvacea* was water hyacinths where the yield was significantly higher ($P \leq 0.05$) than in the other substrates (Table 1-4).

After incubation of all mushroom pots it was found that the temperature had more influence on the growth of fruiting bodies than other cultivation factors (Table. 1-4). Chang (1974) reported that the best temperature for the growth and fructification of straw mushrooms was 26 to 30°C and that a bed temperature of 34 to 37°C was needed. At proper growing temperature in the periods of 8-25 April 2010 and during 21-28 February 2011 and 1-4 March 2011 first fruiting bodies of *C. cinerea* were detected after 7-10 days and of *V. volvacea* after 9-13 days of incubation (Figure 2-4).

Growth to fruiting body of *C. cinerea* and *V. volvacea* on water hyacinths, rice straw residues adding 0.5% aqueous solution of Tamarind wood vinegar as growing substrates effected the vegetative phase of mycelial development. Since mycelia covered the whole substrates in the first stage of colonization, the second stage of nuclear fusion lead to produce fruiting bodies (Kües, 2000; Chaisaena, 2009).

C. cinerea more widely grown on rice straw residues was better on water hyacinths and also in case of the mixture of substrates with adding wood vinegar capable to promote fruiting bodies (Amusa and Muhammad, 2003; Mu et al., 2006; Ku and Mun, 2006; Justo et al., 2011).

Fruiting bodies were detectable on their likely substrates (Figure 3-5 and Table 1-4). The efficiency of culture growing was shown significant ($P \leq 0.05$), by the method of adding tamarind wood vinegar. The results were increased the growth rate of the mushroom hyphae and of the fruiting bodies of both fungi significantly as compared to the cultures without vinegar (Table 4).

Table 1. Number and weight of fruiting bodies for each method as well as the percentage of the cultural pots in which mushroom growing occurred. The results were subjected to analysis of variance (ANOVA) (experimental time was 25 April-10 May 2010).

Method	Mean no. of fruiting bodies	Mean fresh weight of fruiting bodies (g)	Percent of cultural pots with fruiting bodies (%)
Rice straw + <i>C. cinerea</i> (1:1) - in a pot shape	12.7 ^a	6.7 ^a	100 ^a
Rice straw + <i>C. cinerea</i> (1:1) - in a round thickshape	7.5 ^a	3.3 ^a	66.7 ^a
Sunflower + <i>C. cinerea</i> (1:1) - in a pot shape	1.2 ^c	0.5 ^d	33.9 ^b
Sunflower + <i>C. cinerea</i> (1:1) - in a round thick shape	3.0 ^{bc}	0.7 ^{cd}	44.4 ^c

a, b,... different letter compare the mean values in a column within the significant level $\alpha = 99\%$

Table 2. Number and weight of fruiting bodies for each method as well as the percentage of the cultural pots in which mushroom growing occurred. The results were subjected to analysis of variance (ANOVA) (experimental time was 8-25 April 2010).

Method	Mean no. of fruiting bodies	Mean fresh weight of fruiting bodies(g)	Percent of cultural pots with fruiting bodies (%)
Water hyacinths + <i>C. cinerea</i> (1:1)	1.7 ^b	1.1 ^c	14.8 ^b
Water hyacinths + <i>V. volvacea</i> (1:1)	7.7 ^a	64.9 ^a	55.6 ^a
*Water hyacinths + <i>C. cinerea</i> + <i>V. volvacea</i> (1:0.5:0.5)	3.7 ^a	15.5 ^b	25.9 ^b

a, b,... different letter compare the mean values in a column within the significant level $\alpha = 95\%$

* The values show only fruiting bodies of *V. volvacea*, No fruiting bodies of *C. cinerea* detectable.

Table 3. Number and weight of fruiting bodies for each method as well as the percentage of the cultural pots in which mushroom growing occurred. The results were subjected to analysis of variance (ANOVA) (experimental time was 6-25 December 2010).

Methods	Mean no. of fruiting bodies	Mean fresh weight of fruiting bodies (g)	Percent of cultural pots had fruiting bodies (%)
Water hyacinths + Rice straw + <i>C. cinerea</i> (0.5:0.5:1)	33.3 ^{ab}	19.1 ^{ab}	100 ^a
Water hyacinths + Rice straw + <i>V. volvacea</i> (0.5:0.5:1)	ND	ND	ND
* Water hyacinths + Rice straw + <i>C. cinerea</i> + <i>V. volvacea</i> (0.5:0.5:0.5:0.5)	23.0 ^b	8.3	100 ^a

a, b, ... different letter compare the mean values in a column within the significant level $\alpha = 95\%$

ND=No fruiting bodies detectable

*Results show only fruiting bodies of *C. cinerea*. No fruiting bodies of *V. volvacea* detectable.

Table 4. Number and weight of fruiting bodies for each method as well as the percentage of the cultural pots in which mushroom growing occurred. The results were subjected to analysis of variance (ANOVA) (experimental time was 21 Febuary-4 March 2011).

Methods	Mean no of fruiting bodies	Mean fresh weight of fruiting bodies (g)	Percent of cultural pots had fruiting bodies (%)
Water hyacinths + <i>V. volvacea</i> + Vinegar (1:1:0.5%)	5.7 ^b	8.1 ^a	15.7 ^b
Rice straw + <i>V. volvacea</i> + Vinegar (1:1:0.5%)	15.0 ^a	5.2 ^{bc}	24.1 ^a
Water hyacinths + Rice straw + <i>V. volvacea</i> + Vinegar (0.5:0.5:1:0.5%)	1.3 ^c	5.8 ^{ab}	3.7 ^d
Water hyacinths + <i>V. volvacea</i> (1:1)	0.1 ^{bc}	2.1 ^c	1.8 ^d
Rice straw + <i>V. volvacea</i> (1:1)	2.3 ^{bc}	5.5 ^b	6.5 ^c
Water hyacinths + Rice straw + <i>V. volvacea</i> (0.5:0.5:1)	ND	ND	ND

The significant level $\alpha = 95\%$, ND = No fruiting bodies detectable

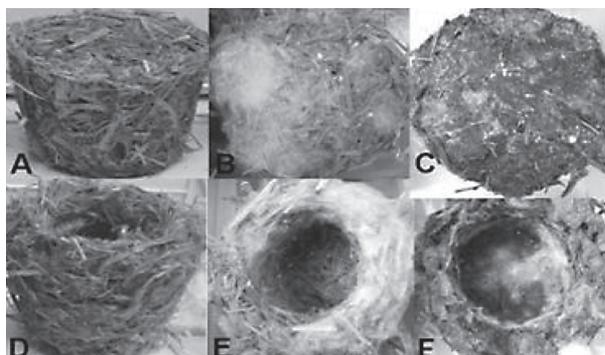


Figure 2. (A - F) *Coprinopsis cinerea* with normal mycelial stage development grown on rice straw and sunflower residues made from a pot shape and a round thick shape after 10 days incubation (total experimental time 25 April-10 May 2011).

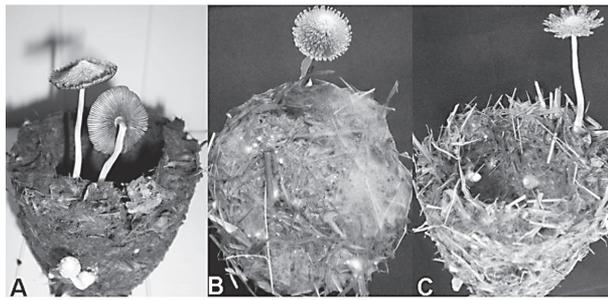


Figure 3. (A) *Coprinopsis cinerea* with normal fruiting bodies were grown on sunflower residues made from a pot shape and (B) rice straw residues made from a round thick shape and (C) made from a pot shape after 9 days incubation (total experimental time 25 April-10 May 2011).



Figure 4. *Volvariella volvacea* with normal fruiting bodies were grown on rice straw residues adding 0.5 % aqueous solution of tamarind wood vinegar after 9 days incubation (total experimental time 21 February-4 March 2011).

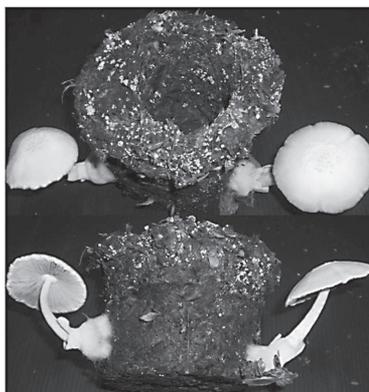


Figure 5. (A-G) *Volvariella volvacea* with normal fruiting bodies grown on water hyacinths adding 0.5% aqueous solution of tamarind wood vinegar after 10 days incubation (total experimental time 21 February-4 March 2011).

CONCLUSION

Coprinopsis cinerea and *Volvariella volvacea* are very well grown on agricultural waste materials as rice straw residues and water waste problem such as water hyacinth in Thailand. Their cultivation are easy edible growing on the different substrates in the laboratory for studying the fruiting bodies which general call mushrooms. The basic need of the study was observed on their bioconversion of the fungal ability to degrade on various substrates. The factors of fruiting body development; such as temperature, humidity and light intensity of the environment, the physical properties of substrates as moisture contents, volume of open pores and acid-base of the substrate were indicted in this study. The experiment was obviously shown the results at same the experimental environment which *C. cinerea* grew well on a mixture of rice straw residues with higher yield than on sunflower and the best substrate for *V. volvacea* yield production was water hyacinth. Suitability of the other substrates for mushroom cultivation is necessary study for the development in the laboratory scale which due to the higher scale for the mushroom farm.

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