Yield and Some Nutritional Elements of *Pleurotus sajor-caju* Cultivated with Golden Apple Snail Supplement

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Abstract

Increasing of yield and essential elements with a supplement on cultivation of mushroom is an excellent food source to reduce nutritional deficiency. The effect of golden apple snail liquid organic fertilizer (GAS-LOF) supplementing on yield and nutritional elements (Ca, Fe, Zn, and Cu) of *Pleurotus sajor-caju* cultivated on the rubber tree sawdust substrate was studied. *Pleurotus sajor-caju* mushroom was cultivated in rubber tree sawdust (RTS) with 0 up to 5% GAS-LOF supplement. The highest yield was obtained from substrate combination 4% GAS-LOF, giving an average increase of 23.40% (210.17 grams per bag) higher than that obtained from the control. The nutritional elements content of *Pleurotus sajor-caju* fruiting bodies, harvested from different substrate combinations. The highest amount of calcium (62.18±0.88 mg/100g dry weight) and iron (27.41±2.16 mg/100g dry weight) were found in RTS substrate supplemented with 4% GAS-LOF. Whereas, zinc (8.50±0.58 mg/100g dry weight) and copper (3.27±0.28 mg/100g dry weight) were highest for the substrate supplemented with 5% GAS-LOF. The addition of GAS-LOF into RTS significantly enhanced the yield and nutritional elements compared to control.

Keywords: *Pleurotus sajor-caju*, Mushroom, Golden apple snail, Yield, Nutritional elements, Rubber tree sawdust.

Introduction

Mushroom is a saprophytic fungus with a high nutritional value that has been widely used as excellent food and food supplements for millenniums. Many mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, and antitumor (Chang, 1996; Hossain et al., 2003; Yoshioka, Tabeta, Saito, Uehara, & Fukoaka, 1985). Famously, mushroom is a rich source of protein (20-35%) (Bellettini et al., 2016), vitamins (Hoa, Wang, & Wang, 2015; Gupta, Sharma, Saha, & Walia, 2013; Alananbeh, Bouqellah, & Kaff, 2014; Wani, Bodha, & Wani, 2010) and some nutritional elements such as calcium, iron, cobalt, copper, manganese, molybdenum and zinc, but low fat content (Gupta, Sharma, Saha, & Walia, 2013) that are required by human. Calcium (Ca) plays a fundamental role in controlling functions. Dietary calcium intakes are often considered to be a primary factor in bone mineralization and metabolism. Other studies also have shown a potential for increased calcium intake and calcium supplementation in the treatment of osteoporosis (Rajput, Wairkar, & Gaud, 2018). Iron (Fe) is an essential metal involved in biochemical processes such as oxygen transport, energy metabolism and DNA synthesis (Wang & Pantopoulos, 2011). Zinc (Zn) is an essential metal and a component of a wide variety of different enzymes in which it is involved in catalytic, structural and regulatory roles (Maret, 2013). Copper (Cu) as stated, is an essential metal, served as a constituent of some metalloenzymes that required in hemoglobin synthesis and catalysis of metabolic growth (Prashanth, Kattapagari, Chitturi, Baddam, & Prasad, 2015; Chellan & Sadler, 2015).

Mushrooms not only play an important role in the ecosystem to biodegrade a substrate for the wastes of agricultural production but also are appreciated as good sources of food and medicine. Cultivation of mushroom has recently increased tremendously throughout the world because of their abilities to grow in a wide range of agro-based residues. The mushroom has been cultivated using different substrate such as wheat straw (Gupta, Sharma, Saha, & Walia, 2013; Pant, Reddy, & Adholeya, 2006; Ruiz-Rodriguez, Soler-Rivasb, Polonia, & Wichers, 2010), bagasse (Hoa, Wang, & Wang , 2015; Pant, Reddy, & Adholeya, 2006), maize stalk, pea residue, banana leaves (Pokhrel, Kalyan, Budathoki, & Yadav, 2013), sawdust (Hoa, Wang, & Wang, 2015; Mallikarjuna et al., 2013; Bhattacharjyal, Paul, Maih, & Ahmed, 2015), perilla stalks (Li, Zhang, Li, Li, & Sun, 2017), cotton stalks, coir fiber and sorghum stover (Ragunathan & Swaminathan, 2003), date palm leaves (Alananbeh, Bouqellah, & Kaff, 2014) and other wastes.

Rubber tree (*Hevae Brasiliensis* (Willd ex. A. Juss.) Muell.-Arn.) is an industrial tree cultivated in South, East and Northeast of Thailand. Every year, a large of rubber-exhausted trees are cut for new planting and producing furniture. A lot of sawdust waste that may cause serious environmental problems. Now it is recognized that very suitable for mushroom cultivation in Thailand. Rubber tree sawdust has a uniform size and structure so it is not only suitable for utilization in plastic bag cultivation, but the structure also facilitates the enrichment of the substrate. A mushroom can degrade lignocellulose to nutrient and nutritional elements, while a human can't degrade.

Many mushroom species are popularly cultivated on rubber tree sawdust. *Pleurotus* spp. is the most talented group among the cultivated mushrooms, which have the ability to degrade many lignocellulosic substrates and capable to colonize successfully on the rubber tree sawdust substrate (Patrabansh & Madan, 1997). *Pleurotus* spp. is an edible mushroom, the third largest commercially important mushroom species (Tesfaw, Tadesse, & Kiros, 2015). It is a popular and widely cultivated throughout the world, mostly in Asia, Europe and America, that appreciated for its delicious (Zhang, Xiujin, & Fadel, 2002), excellent flavor, taste and higher biological efficiency (Shah, Ashraf, & Ishtiaq, 2004; Mane, Patil, Syed, & Baig, 2007). Various *Pleurotus* species have been shown to possess a number of medicinal properties such as antitumor, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet- aggregating, antihyperglycaemic, immunomodulatory, antimicrobial and antiviral activities (Gregori, Svagelj, & Pohleven, 2007).

The amount of nutritional elements in mushrooms of the same species is directly related to the substrate. Generally, lignocellulosic materials are low in nitrogen/protein and mineral content, thus they required some additives to provide them with different minerals, and thus, enhance mushroom production (Alananbeh, Bouqellah, & Kaff, 2014; Markson, Madunagu, Akpan, & Eshiet, 2012). The various additives are recommended as supplements to the basal substrates for enhancement of mushrooms yield and nutrition (Naraian, Singh, & Ram, 2016; Pardo-Gimenez, Pardo-González, & Cunha Zied, 2017; Curvetto, Figlas, Devalis, & Delmastro, 2002).

The golden apple snail (*Pomacea canaliculata* Lamarck) was introduced almost 30 years ago as a dietary protein supplement and as a pet in the ornamental aquatic animal industry. The spread of golden apple snails in Thailand as a result of intentional and accidental introduction has caused enormous economic and ecological impacts. The farmers' enemies can be used as animal feed, fertilizer and others. Moreover, golden apple snail is also a good source of protein, a major nutrient, minor nutrient and nutrient supplements (Kaosol, Kiepukdee, & Towatana, 2012). Therefore, the reuse of agricultural residue for composting is the sustainable waste management way and to benefit further. The golden apple snail fertilizer is a nutritious food. The exchange of charge is relatively high in nitrogen, phosphorus, and potassium. Liquid organic fertilizer of golden apple snail that enriches protein and mineral is potentially used as a supplement for *Pleurotus sajor-caju* cultivation.

Therefore, the cultivation of *Pleurotus sajor-caju* on rubber tree sawdust that low mineral content by the various ratios of golden apple snail liquid organic fertilizer supplement could be offered high-value products with nutritional and medicinal properties. The aim of this study was to evaluate the use of rubber tree sawdust with golden apple snail liquid organic fertilizer supplement an additive to improve the productivity and nutritional elements of Ca, Fe, Zn and Cu content in *Pleurotus sajor-caju* mushroom.

Methods and Materials

Preparation of gold apple snail liquid organic fertilizer (GAS-LOF) supplement

The gold apple snail liquid organic fertilizer (GAS-LOF) supplement was prepared by mixing each of 3 kg of pounding fresh gold apple snail (Khun Taleay reservoir, Surat Thani) and molasses (M Molasses, Mitr Pol Sugar Cor., Ltd.) in 1.0 L of drinking water (3:3:1), kept in 20 L of polyethylene (PE) bottle. The mixture was fermented at ambient temperature, indoor for 3 months and filtered with thin cloth of wool. The filtrate of GAS- LOF was placed in 2.0 L PE bottle and stored at 4°C in the refrigerator. This was freshly diluted with drinking water to 1, 2, 3, 4 and 5% before used as supplements for *Pleurotus sajor-caju* cultivation.

Substrate preparation, inoculation and incubation

The substrate has consisted of 300 kg rubber tree sawdust (RTS), 24 kg rice bran, 0.3 kg magnesium sulfate and 1.8 kg of each pumice sulfate, gypsum and lime. All of the materials were purchased from the agricultural shop, Surat Thani. Then, the substrate was spread to six portions of 0 (control), 1, 2, 3, 4 and 5% concentrations of golden apple snail liquid organic fertilizer (GAS-LOF) supplement. The water content of the final mixture was adjusted to about 65-70%. Then, each of 0.9 kg substrate was filled into a 10 × 23 cm polypropylene bag and pasteurized by local methods with homemade apparatuses. Sixteen culture bags of each portion were used for each substrate formula (N=16). These substrates were sterilized at high temperature using an autoclave at 100°C for 3 h. After the substrates were cooled to room temperature, they were inoculated with 2 g spawn per bag. These inoculated substrates were incubated in a dark incubation room at 24-28°C and related humidity about 80-90% by wetting the walls of the mushroom house regularly. Harvesting of mushrooms was at maturity.

Yield of mushroom

The fresh fruiting bodies of three flushes of *Pleurotus sajor-caju* mushroom for 45 days were harvested using a scalpel, weighed to determine the mass using analytical balance (AG204, Mettler Toledo, Switzerland).

Determination of nutritional elements

Each flushed of all fruiting bodies of mushroom samples was dried in an oven (Memert, Germany) at 70°C for 48 h and pulverized using a mortar and pestle. 0.5 g sample was placed into a beaker and added 6 mL of 65% HNO_3 and 1 mL of 30% H_2O_2, then digested using a hot plate. After the completed digestion, the solution was transferred to a 50 mL volumetric flask and diluted with deionized water to a final volume.

The nutritional elements of Ca, Fe, Zn and Cu in raw samples were analyzed by the calibration method. All measurements were carried out by atomic absorption spectrophotometer (AA-6200, Shimadzu, Japan). Triplicate analyses were performed for each element. All data were represented in mg/100 g dry weight basis as a mean ± standard deviation.

Statistical analysis

The difference between the mean of individual groups was assessed by a one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT).

Results

Total yield

Pleurotus sajor-caju were cultivated on a rubber tree sawdust substrate with GAS-LOF supplement in different ratios for about 45 days (Figure 1). In this study, only 3 flushes of *Pleurotus sajor-caju* were harvested. The total yield was significantly different among the *Pleurotus sajor-caju* cultures with other supplement concentration (Table 1). The substrate with 4% GAS-LOF gave the highest total yield of fresh fruiting bodies of three flushes of *Pleurotus sajor-caju*, higher than obtained the control for 23% of the fresh weight.

Figure 1 Shape of fruiting bodies of *Pleurotus sajor-caju* with 0% (control), 1, 2, 3, 4, and 5% GAS-LOF.

Nutritional elements

The *Pleurotus sajor-caju* fruiting bodies, harvested from rubber tree sawdust mixed with different ratios of golden apple snail liquid organic fertilizer were determined the content of Ca, Fe, Zn and Cu in mg/100g dw mushroom samples were listed in Table 2.

Table 1 Effect of GAS-LOF supplement on total yield of *Pleurotus sajor-caju* mushroom (N=16)

Substrate	Flush (g/bag)			Total yield
	1st	2nd	3rd	(g/bag)
RTS (control)	76.95±24.18	54.04±17.34	39.33±18.01	170.32±59.53
RTS + 1% GAS-LOF	70.71±19.65	49.64±22.09	37.87±20.57	158.22±62.31
RTS + 2% GAS-LOF	77.69±15.49	55.08±29.90	34.22±15.74	166.99±61.13
RTS + 3% GAS-LOF	83.49±24.63	51.96±16.61	32.62±21.21	168.07±62.45
RTS + 4% GAS-LOF	96.43±29.80	52.09±18.45	61.65±19.75	210.17±68.00
RTS + 5% GAS-LOF	56.18±24.53	51.63±28.05	41.84±24.99	149.65±77.57

Table 2 Nutritional elements of Ca, Fe, Zn and Cu in mg/100 g dry weight of *Pleurotus sajor-caju* mushroom

Substrate	Ca	Fe	Zn	Cu
RTS (control)	26.31±5.95^{a}	16.09±0.46^{a}	5.97±0.54^{a}	2.36±0.47^{a}
RTS + 1% GAS-LOF	32.82±2.61^{a}	18.43±2.62^{a}	6.45±0.45^{a}	2.51±0.30^{a}
RTS + 2% GAS-LOF	45.19±5.62^{b}	22.06±2.01^{b}	6.69±0.64^{a}	2.41±0.27^{a}
RTS + 3% GAS-LOF	46.97±2.29^{b}	23.08±2.22^{b}	6.34±0.27^{a}	2.61±0.21^{a}
RTS + 4% GAS-LOF	62.18±0.88^{c}	27.41±2.16^{c}	6.95±0.90^{a}	3.01±0.48^{ab}
RTS + 5% GAS-LOF	61.00±2.23^{c}	23.56±1.45^{b}	8.50±0.58^{b}	3.27±0.28^{b}

Note: mean values under the same column that bear different superscript letters significant different (p=0.05) by DMRT

This study, all nutritional elements in the fruiting bodies of *Pleurotus sajor-caju* cultivated from all mixed RTS with 1, 2, 3, 4 and 5% GAS-LOF were higher contained than the control. The result indicated that the amount of Ca, Fe, Zn and Cu contents were depended upon the supplement concentration of golden apple snail liquid organic fertilizer (Figure 2).

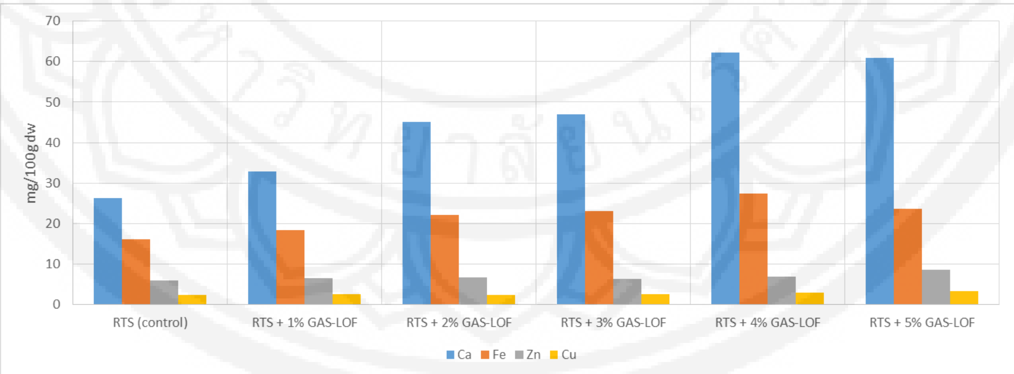

Figure 2 Nutritional contents of Ca, Fe, Zn and Cu in mg/100 g dw fruiting bodies of the *Pleurotus sajor-caju* cultivated with 1, 2, 3, 4 and 5% of GAS-LOF supplement

Discussion

The yield performance of the mushroom depended upon the supplement concentration of golden apple snail liquid organic fertilizer (GAS-LOF). That might be due to the enhanced supply of nutrients available from the organic fertilizer to the fungus. However, at 5% GAS-LOF the total yield was decreased. That corresponds with Gothwal reported that it probably due to the fact that excess forms of nutrients and/or degradation products might have become toxic to *Pleurotus* fungus (Gothwal, Gupta, Kumar, Sharma, & Alappat, 2012). This study agreed with the study of different supplements were used to enhance mushroom production, including rice bran, wheat bran and maize powder (Alam, Amin, Khair, & Lee, 2010), raw/detoxified mahua cake (Gupta, Sharma, Saha, & Walia, 2013), rice bran and chicken manure (Pokhrel, Kalyan, Budathoki, & Yadav, 2013), wheat bran and corn meal (Alananbeh, Bouqellah, & Kaff, 2014).

Calcium is the major nutritional elements found in the fruiting bodies of the edible mushrooms. In this study, the highest calcium content was 62.18±0.88 mg/100g dw with 4% GAS-LOF supplement. This result was higher than Mallikarjuna, Alam and Falandysz reported that were found 8.27 and 34.2 mg/L in *P. Florida and P. djamor*, respectively. (Mallikarjuna et al., 2013), 22.15 mg/100g dw in *P. sajor-caju* (Alam et al., 2008) and 1.8-59.0 mg/100g dw (Falandysz et al., 2001). However, it was less than Gogavekar reported that found 505.0 mg/100g dw of *Pleurotus sajor-caju* on paddy straw used as substrates (Gogavekar et al., 2014). Iron is one of the important nutritional elements for human. The Fe content of fruiting bodies increased significantly with increasing concentrations of the organic fertilizer. In this study, the highest content of Fe was 27.41 mg/100g dw in the 4% GAS-LOF that was 1.7 times of the control. That obtained here was higher than *Pleurotus ostreatus* cultivated on spent beer grain as 7.1 mg/100g dw (Wang, Sakoda, & Suzuki, 2001) and 13.79-14.78 mg/100g dw (Hoa, Wang, & Wang, 2015). In contrast, they were less than oyster mushroom (*Pleurotus ostreatus* FR.) founded 37.87-42.55 mg/100g dw on different sawdust based substrates (Bhattacharjya, Paul, Miah, & Ahmed, 2015). In this work, the highest Zn contents were found in fruiting bodies of mushrooms cultivated on sawdust mixed with organic fertilizer up to 5% (8.50 mg/100g dw). Zn contents found in this study parallel those reported in the literature, have been reported in the range of 7.61-8.77 mg/100g dw (Hoa, Wang, & Wang, 2015), and 0.27-9.46 mg/L (Alananbeh, Bouqellah, & Kaff, 2014), respectively. However, they are less than that reported on 20.56-27.65 mg/100g dw (Bhattacharjyal, Paul, Miah, & Ahmed, 2015), 13.7 mg/100g dw (Wang, Sakoda, & Suzuki, 2001) and 20.9 mg/100g (Alam et al., 2008), respectively. The highest Cu contents in mushroom were recorded of sawdust mixed with 5% organic fertilizer (3.27 mg/100g dw). The quantity of copper in the literature for mushroom were 2.11-2.43 mg/100g dw (Hoa, Wang, & Wang, 2015), 2.5 mg/100g dw (Wang, Sakoda, & Suzuki, 2001) and 0.02 mg/kg (Obodai et al., 2014), respectively. However, these are less than the reported of 16.1 mg/100g dw Cu in *Pleurotus sajor-caju* grown on the paddy straw based substrate (Gogavekar et al., 2014). High levels of calcium were also observed in *Pleurotus sajor-caju* mushroom with the following decreasing order of elements: Ca > Fe > Zn > Cu. However, 5% of the GAS-LOF supplement was given the highest amount of Zn and Cu, but the total yield of fresh fruit bodies of *Pleurotus sajor-caju* was lower than 4% of the GAS-LOF supplement.

The GAS-LOF is an alternative supplement for increasing productivity and some nutrition of *Pleurotus sajor-caju*. In addition, this methodology is the best way to reduce the number of golden apple snail in nature. Furthermore, this method is potentially used to apply for others cultivated mushroom.

Conclusion and Suggestions

The present study has established the feasibility by using golden apple snail liquid organic fertilizer (GAS-LOF) as a supplement for enhancing the yield and nutritional elements of *Pleurotus sajor-caju* on rubber tree sawdust. About 4% of the GAS-LOF supplement was the most suitable for increasing yield and some minerals (Ca, Fe, Zn and Cu) content of fresh fruiting bodies of *Pleurotus sajor-caju*. This study revealed that GAS-LOF can also be used as an alternative supplement with the different substrate for the cultivation of *Pleurotus sajor-caju*.

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