The Cultivation of Indian-Oyster Mushroom (*Pleurotus pulmanarius*) in Coffee-Pulp Substances Bags with Sawdust, Rice Straw, Rice Husk and Coir Fiber

By

Ms. Lalita Manokullanant

A special project submitted to the Faculty of Biotechnology
Assumption University in part fulfillment of the requirements for
The degree of Bachelor of Science in Biotechnology
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ABSTRACT

The preliminary studying of cultivation Indian-oyster mushroom mixed coffee pulp as a major substance with other 4 materials (sawdust, rice straw, rice husk and coir fiber) in different percentage and the different mixture from three substances. The experiment was separate into two parts. The first part was studied the potential of two substance by mixing coffee pulp with 4 substances at 100:0,75:25, 50:50, 25:75 and 0:100 (substance :coffee pulp). The second part, studied the potential of three substances by mixing coffee pulp with different substances in the ratio 1:1:1. The result showed that mushroom from 100% coffee pulp substance could grow but gained lowest yield and longest incubation time among samples. The sawdust substances provided higher yield with moderate incubation time. Rice husk and rice straw substances provided the shorter incubation time but gave lower yield comparing with sawdust substances. However, rice husk and rice straw when combined with coffee pulp promoted the spawn growth and improve yield. At 25% rice husk with coffee pulp gain percent close to sawdust substance. The cultivation of Indian-oyster mushroom on coffee pulp substance was possible but required further studying to improve percent yield and incubation time.

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INTRODUCTION

Indian-oyster mushroom (*Pleurotus pulmonarius*) is origin at Bhutan and was imported to Thailand by Dr. Anon Urtagul. It can grow in many materials. The Indian-oyster mushroom, same family with oyster mushroom has characteristics similarly to oyster mushroom. But Indian-oyster mushroom can grow easily at room temperature and preserve longer. It provides large yield production and shorter incubation time.

According to standard formula of substance bags, mushrooms normally grow in sawdust substance which contains cellulose and lignin as nutrients source of mushroom. However, sawdust resources are limited. There should be seeking alternative substances in order to cultivate mushroom. Coffee pulp is waste from coffee brewing processing of extraction flavor by using hot water. The consumption of coffee is now increased and resulted the increasing coffee pulp every year. There is utilization of coffee pulp such as cosmetic, fertilizer, pesticides, etc. If possible, the additional of coffee pulp in mushroom substances should be studied because of the lower production cost. Otherwise, Thailand produce, consume and export rice all year round. Therefore, rice straw, rice husk and coir fiber should be alternative resources of mushroom production. Rice straw, stem of rice, gains after harvesting rice about 20 million tons per year. Rice husk is waste that gained from rice milling process. Coir fiber or coconut fiber is a fiber between outer shell and husk of coconut flavor. Coir fiber is the waste from industrial such as coconut milk industrial. Somehow coir fiber can be a good fertilizer of plant and can make a mattress.

This project is studied the potential of the cultivation Indian-oyster mushroom with coffee pulp as a main substances combining with other 4 materials (saw dust, rice straw, rice husk and coir fiber).

OBJECTIVE

1. To study the effects of two substances on the cultivation of Indian-oyster mushroom by combining coffee pulp as a main substances with four substances in 5 levels of percentage.

2. To study the effects of three substances on the cultivation of Indian- oyster mushroom by combining coffee pulp as a main substances with four substances in a ratio 1:1:1

LITERATURE REVIEW

1. Indian-Oyster Mushroom (*Pleurotus pulmanarius*)

Mushroom or fungi is an organism in Eukaryote group, fungi cannot synthesis chlorophyll and food by their function. Mushroom is a Heterotrophic saprobes, cells of hyphae secrete digestive enzymes and absorb products of digestion. Cell wall of mushroom made of chitin and the hypha is composing of chain of cell with or without separating septa. Some fungi are important decomposers can digest lignin and cellulose of wood (ex. Oyster mushroom and Indian oyster mushroom)

Indian oyster or phoenix oyster mushroom (*Pleurotus pulmanarius*), Fig. 1, is classified in Pleurotus family. The mushroom grows wild in subtropical and tropical regions India and Bhutan. Indian-oyster mushroom is first import to Thailand by Dr. Anon Urthagul who position of Mushroom expert in Food and Agriculture Organization at Bhutan. He cultivated this species in Thailand. Indian oyster mushroom is consumed about 45% of mushrooms in the fresh form. 55% is processed with 5% in the dehydrated form and 50% in the canned form (Mushroom Exports in 2008-2009 of Thailand).

Figure1. Indian oyster mushroom

(source: http://healthy-life.narod.ru/mush-e15.htm)

1.1 Mushroom Structure

(1) Cap: Indian-oyster mushroom have thick pale-brown color and about 1-5 cm. diameter. Cap germinates individual or group.

(2) Stalk: Stalk and cap is close together and have a thick white layer. If mushroom grow in wood, the stalk will grow and organize in a layer. There is no ring surround stalk.

(3) Gill: Gill has a white color and long size attack to the stalk.

(4) Mycelium: Mycelium is growing very fast in PDA media and sorghum seed.

(5) Habitant: These mushrooms are saprotrophic, it can grow on dead materials and logs or sick or dying trees. Most often found on deciduous hardwoods (trees that lose their leaves). Beech and aspen trees are common. Sometimes found on conifers as well.

1.2 Advantage of Indian-Oyster Mushroom

Indian-oyster mushroom mycelium can develop faster in PDA media and sorghum seed. Mushroom is germinated faster after inoculation 20-30 days and the next mushroom can germinate again after 5-7 days (Department of Agriculture, Government of Tamil Nadu). Indian-oyster mushroom have a same taste as oyster mushroom, good smell and sweet taste. Moreover Indian-oyster mushroom can keep longer under the low temperature. In cultivation is spent a low cost production due to mushroom can grown in many materials.

1.3 Growing Parameters

(1) Mycelium growth: Indian-oyster mushroom has optimum temperature during 24-29°C for the Incubation period. Relative humidity condition should be about 90-100% to develop the growth of mycelium. Fresh air and light is also requiring for this process. Duration time is about 8-14 days.

(2) Development of mycelium in substance bag: The initial temperature should be control at 24-27°C and relative humidity should be about 95-100%. This step will take 5-7 days

(3) Fruit body Development: During fruit body development mycelium will spread well during temperature at 18-27°C and control Humidity around 85-90%, fruit body will take 5-7 days.

1.4 Life Circle of Mushroom

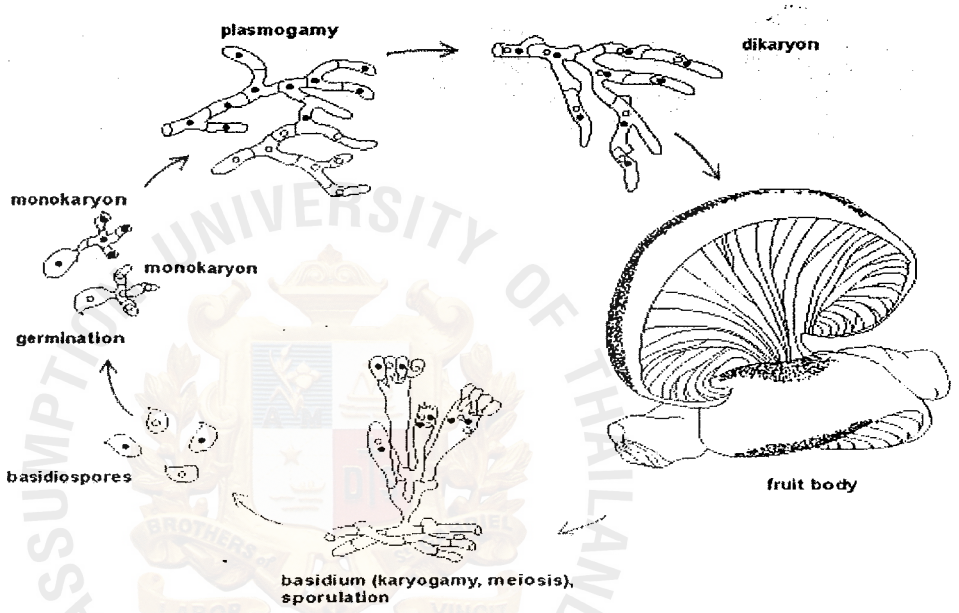

Figure 2. Life circle of Indian-oyster mushroom
(source: Biswas Palikhey, 2011)

Life circle of Indian oyster mushroom (Fig. 2) starts form germination of a basidiospore in a suitable substance. The monokaryotic mycelium rises and contains genetically identical nuclei. When monokaryotic is contact together, it establishes a fertile dikaryon by hyphal fusion or plasmogamy. When the temperature condition is appropriate, the dikaryotic mycelium will different into a fruit body and have a structure called basidia. The four resulting haploid nuclei move to the sterigmata on the basidium to form four new basidiospores. When the fruit bodies are mature, basidiospores are discharged, starting the sexual life cycle again.

1.5 Mushroom House

Mushroom house is another main factor of mushroom germination. The house will enhance mushroom growth and can control the environment such as humidity and also prevent the insect, fly. The house is require a space which suitable to contain all mushroom bags. After that it divided a space to separate room between spawn running and cropping rooms.

In spawn running room, the temperature should maintain at 23-25 °C. Inside the room should provide ventilation such as air and water. Light is not require in this stage so the room should close and not allow light though inside.

In a Cultivation room, temperature will maintain at 23-25°C while the humidity should above 75-80 %. The humidity will maintain by spraying water 3 times a day. Light and aeration is requiring moderately allowing mushroom germinates.

1.6 Nutrient's Mushroom Requirement

Table1. The nutrition require in germination of mushroom

Nutrient	Material		
Organic	C-source	Cellulose	humus materials such as wood, straw, leaf, etc.
		Hemicellulose	Same as cellulose
	N-source	Protein	Same as cellulose
		Amino acid	Same as cellulose
Inorganic		K, P, Si, Fe, Mg, etc	Same as cellulose

(Source: Cha et al., 1997)

The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin. C-source and N-source ratio are important factor for optimal substrate composition for Indian-oyster mushroom. It requires high amount of carbon but less amount of nitrogen source. Most of main substrate materials such as cereal straw, cotton waste, and sawdust need supplementation of nitrogen source such as wheat and rice bran to reach optimal C-source and N- ratio for mushroom. Amino nitrogen is used during spawn run, but it is not suitable for fruiting, therefore, growers commonly do not need additional apply of amino nitrogen during mixing (Cha., et. al., 1997).

Indian-oyster mushroom can utilize various agro-wastes with its enzyme so Indian-oyster mushroom uses lignin and cellulose together. Therefore, any type of organic matters containing lignin and cellulose can be used for oyster mushroom substrates, and this includes almost all agricultural wastes. (Seung Woo Kang, 2004, Mushroom grower handbook 1)

1.7 Mushroom Biotechnologies

(1) Spawn production

The complete procedure of spawn production involves preparation of the medium, filling the test tubes or Petri dishes and sterilizing them, and the process of inoculating larger containers with this culture.

The starter culture (or mother culture) can be made from a fresh and healthy fruiting body or obtained from a spawn producer or laboratory. More agar cultures are then made from this starter culture. These serve to inoculate larger containers (like bottles) with mother spawn, which can be used to inoculate the final spawn substrate.

Grain, sawdust and compost contain large numbers of contaminants. A single grain kernel may contain thousands of bacteria, fungi and Actinomycetes. A heat treatment of 1 hour at 121°C is usually sufficient to kill all organisms.

The first steps in spawn production are performed on artificial media. These should contain sufficient nutrients for the mushrooms to grow, like saccharides and a solidifying agent (agar or gelatin). The mycelium grows on the surface of the medium and will later be used to inoculate larger amounts of substrates like sawdust or grain. Test tubes or Petri dishes (or flat whiskey bottles) can be used as culture containers. Young and vigorous mycelium can be obtained from a young fruiting body using a scalpel, alcohol, sterilized agar slants, Petri dishes or bottles with agar, flame, and a clean table to work on, or preferably a laminar airflow cabinet or inoculation box.

Mother spawn is spawn that cultivate in the sorghum seed which ability to develop mycelium. Sorghum seed was soaked for 1 night before process then boil and cool down. After that fill sorghum seeds to the container (glass bottle) which resist to sterilization. Inoculation spawns from PDA media to sorghum seed and incubation 5-7 days.

(2) Mushroom production technology

First prepare raw substance material such as sawdust, rice straw and rice husk. Second prepare main ingredient for mushroom cultivation. Mixing all dry ingredient together then add water 60-65% in order to increase moisture content. Packing the mixture to the substance bag ready for the sterilization. After that Spawning is performed by lifting the plugs from the bags containing the substrate (thus opening the bags) and putting in a small amount of spawn to bag.

(3) Incubation

Substance bag will stay at low temperature 23-25 °C which could provide a good environment to allow spawn grow. After complete spawn grow in substance bag, the plug is remove in order to provide space for mushroom germination. Watering is requiring as usual, at least 3 times a day.

1.8 Mushroom Nutrition

Indian oyster mushroom has many nutrients which could be benefit to the consumer. The protein content of mushrooms can be considered their main nutritional attribute, average values ranging 10.5–30.4%, on a dry weight basis. The concentration of essential amino acids varies from 33.4 to 46.0 g per 100 g of corrected crude protein, showing significant amounts of lysine, leucine, and methionine. The fat content report is 1.1–2.2% on a dry weight basis, having a high proportion of unsaturated fatty acids (79.3%). The carbohydrate content varies from 46.6 to 81.8% on a dry weight basis.

Main vitamins present in 100 g dry weight of oyster mushrooms are thiamine (1.16–4.80 mg), niacin (46.0–108.7 mg), and ascorbic acid (7.4 mg). Fiber (7.4–27.6% on a dry weight basis) and minerals (potassium, phosphorus, iron, copper and zinc) are also present in good proportion. Several compounds from oyster mushrooms, potentially beneficial for human health, have been isolated and studied. Polysaccharides show strong antitumor activity, a lectin called pleurotolysin with hemolytic properties, and extracts with hypotensive action on renal functions.

2. Coffee

Coffee is a brewing that people are usually consume in dairy life. Around 143,500 tones around the world is generate from coffee processing in year 2008. Coffee pulp is obtained from brewing process by using hot water to extract flavor from coffee cherries.

2.1 Structure of Coffee Bean

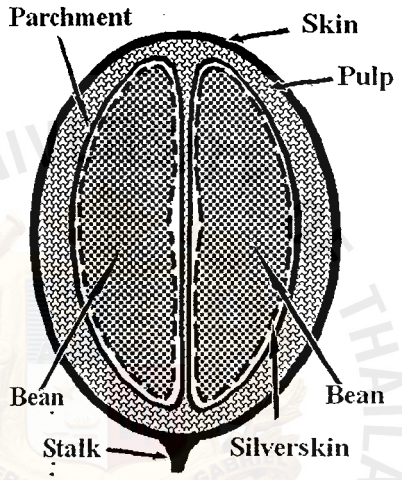

Figure 3. the structure of coffee bean

(source: www.kupajoe.com/espressocafe/plants.htm)

Coffee berry will contain two seeds inside face together from figure 3.The outer skin of the coffee berry is generally tough and can withstand handling. The inner pulp of the coffee berry is generally mushy. In a few types of coffee plants, the pulp is more valuable than the bean itself. The coffee berry parchment shell is fairly tough. This is taken off in the last coffee bean processing stage. However, the coffee silver skin is so thin and attached so well it tends to stay with the coffee bean right up to roasted. When roasted, the silver skin can, and usually does, crack off the coffee bean. The silver skin cracks off because it not expand like the inner coffee bean does when roasted. First it is a thin messy chaff which is undesirable and must be removed from the batch of roasted coffee beans for cosmetic reasons. Second, it can easily catch fire.

2.2 Species of Coffee

Species of coffee can effect to a different chemical composition of coffee pulp. Coffee has a two main species in world consumption which are Arabica and Robusta. The both two species is come from different cultivation country. (Fig. 4)

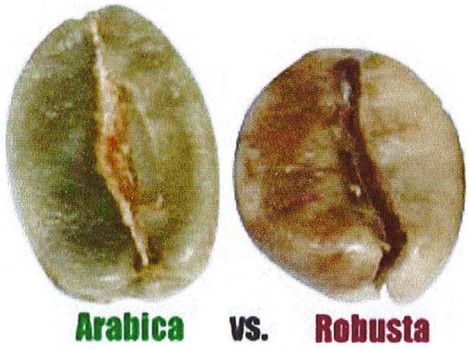

Figure 4. Coffee bean of robust and Arabica

(source: bluebagcoffee.com/2011/07/31/chiapas-european-grade-coffee/)

(1) Arabica

Arabica is coffee to be cultivated, being grown in Southwest Arabia for over 1,000 years and represent a three-quarters of world coffee production. The Arabica coffees produced in Brazil take the collective name of Brazilian Coffees; those from Colombia, Venezuela, Peru, Guatemala, Salvador, Haiti and Santo Domingo are called *Milds*. The Arabica makes a full-bodied coffee, sharp in taste, with rather low caffeine content. There are, however, different tastes, due to the different crop varieties. There are so many varieties on the market can assert that some low-quality Arabica species are actually inferior to the best qualities of coffee Robusta. Arabica beans look slightly elongated, with greenish-blue shades.

(2) Robusta

Robusta species is growing in the Easter hemisphere and thrive in equatorial climate. Robusta berry have twice in caffeine content in Arabica and less flavor. Robusta is 20 % of coffee production in the world. Robusta is greater crop than other major species and low cost product. Robusta is mostly grown in Vietnam where French colonists introduced it in the late 19th century, though it is also grown in Africa and Brazil, where it is often called "conillon". In recent years Vietnam, which produces mostly robusta, has surpassed Brazil, India, and Indonesia to become the world's single largest exporter of Robusta coffee. Brazil is still the biggest producer of coffee in the world, producing one-third of the world's coffee, though 80% of that is Coffee Arabica

Robusta coffee is hardier plant, tolerating lower and less favorable climate. Robusta is use to produce instant coffee in the market. The smell of bean is similar with oat and pea nut.

2.3 Caffeine

Caffeine (1, 3, 5-trimethylxanthine) is belong to a family of naturally occurring component know as xanthines. Caffeine has ability to increase alertness, put off sleep and to improve attention in study. The amount of caffeine in a cup of tea or coffee differs greatly, depending on the method of preparation. According to the FDA, the limitation of caffeine is not over 200 ppm. in order to maintain healthy. Caffeine can effect to the body if consume in high dose, first caffeine is stimulate function of heart, respiratory system and central nervous system. Moreover, the level of fatty acid in blood will increase and stimulate of blood circulation and blood pressure. Stomach is produce more acid and the digestion less effective by relaxing the muscles of intestinal system. Drinking of 8 cups coffee will cause increase urination. Caffeine can stimulate the cortex of brain heightening the intensity of mental activity. This can result in a temporary feeling of alertness and, in the short term, banishes drowsiness and feelings of fatigue. Caffeine can decreased bone density or osteoporosis in women.

3. Coffee Pulp

Coffee pulp or husk is a fibrous material obtained during the processing of coffee cherries by wetting or drying process, respectively. Coffee pulp/husk contains some amount of caffeine and tannins, which is toxic and pollute in the environment. Caffeine also result the disposal problem. However, caffeine is rich in organic nature, which makes it an ideal substrate for microbial processes for the production of value-added products. Several solutions and alternative uses of the coffee pulp have been attempted. These include as fertilizers, livestock feed, compost, etc. Attempts have been made to detoxify it for improved application as feed, and to produce several products such as enzymes, organic acids, flavour and aroma compounds, and cultivataion of mushroom. Solid state fermentation has been mostly employed for bioconversion processes.

Coffee pulp is estimate to be 29% of the weight of the whole berry. After obtain the process coffee pulp is contained very high moisture and the high moisture will allow the potential of utilization. Moreover coffee pulp is composing of many organic compounds which can use as animal feedstuffs. Potassium content present high level in the pulp, so coffee pulp is suitable to feed animal such as cattle. Furthermore coffee pulp contain of carbohydrate which the main constituent is cellulose 27.65%, reducing sugar 12.40% and non reducing sugar 2.02%. The pulp also contains pectin substance 6.52%. The level of constituent indicates that coffee pulp is superior to various other feed.

3.1 Constituent of Coffee Pulp

Table 2. chemical composition of coffee pulp

Constituent	Percent
Protein	8.5-12.1
Lipid	1.5-2.0
Cellulose	15.1-20.3
Ash	5.5-6.8
Extract not-nitrogen	45.5-54.3
Tannins	1.8-2.4
Caffeine	0.5-0.7

(Source: Leifa Fan and Carlos R. soccol, 2005)

In the coffee pulp contain many nutrients which can use to cultivation Indian-oyster mushroom such as cellulose which can provide energy source to mushroom grow and protein is a source of nitrogen which benefit to mushroom during spawn run. Moreover, coffee pulp need to fermentation before process, Martinez-Carrera, 2000 reported that coffee pulp should be ferment up to 10 days in order to have a good structure and consistency to be used for Pleurotus cultivation.

3.2 Utilization of coffee ground

According to chemical composition of coffee pulp it will enhance a variety of utilization in different way.

(1) Decomposition

Coffee pulp feed the beneficial bacteria in compost pile. Bacteria consume the added nitrogen in the grounds to aid and speed decomposition. This generates a hotter compost pile can achieve without coffee grounds. The extra heat also kills weed and vegetable seeds and pathogens. However, to maintain compost balanced in carbon Pseudomonas and pin molds (Mucorales), prevent pathogenic fungi from establishing and nitrogen, coffee pulp should not make up more than 25 percent of compost. Pulp can also mix coffee grounds into a worm bin as worm food.

(2) Disease suppression

Coffee pulp was part of a compost mix, in one case comprising as little as 0.5 percent of the material. It suggests that the bacterial and fungal species normally found on decomposing coffee grounds, such as non-pathogenic. A similar bio-control effect was noted on bacterial pathogens including *E. coli* and *Staphylococcus* spp., which were reduced on ripening cheeses covered with coffee grounds. Currently, disease suppression from coffee grounds has only been demonstrated under controlled conditions on a handful of vegetable crops, including bean, cucumber, spinach, and tomato. Their efficacy in gardens and landscapes is unknown.

(3) Effects on plant growth

Given their antimicrobial activity, it's not surprising that attempts to cultivate mushrooms in coffee grounds have been variable and species-specific. Likewise, their effects on plant growth are unpredictable. Coffee ground composts and mulches have enhanced sugar beet seed germination and improved growth and yield of cabbage and soybeans. It's been an effective replacement for peat moss in producing anthuriums. Increases in soil nitrogen as well as general mulching benefits, such as moderating soil temperature and increasing soil water, are proposed mechanisms for these increases. In sprite, there are some plant are not available to use coffee ground as cultivative such as Chinese mustard (*Brassica juncea*), Komatsuna (*Brassica campestris*) and Italian ryegrass (*Lolium multiflorum*) were all inhibited by coffee grounds. One investigator speculated that toxic substances released from decomposing coffee grounds were responsible for their inhibitory effect. This effect also reduces weeds, and perhaps in a landscape dominated by large shrubs and trees, only germinating seeds and seedlings would be injured.

Analysis of used coffee grounds and raw coffee seeds indicated that the protein and fat contents were nearly the same. Unsaturated fatty acids constituted nearly 50%, while polyunsaturated fatty acids accounted for one third of the total fatty acid content of the seed fat. During roasting of coffee seeds and extraction of soluble coffee, some of the amino acids were destroyed. Tryptophan was the limiting amino acid of raw coffee seed proteins. Increasing the used coffee pulp in the diets from 0 to 15% had no effect on the food intake of animals. However, as the level of used coffee pulp increased, gain in body weight, protein efficiency ratio, net protein utilization, protein and dry matter digestibility decreased significantly, the former being significantly negatively correlated to all the later attributes. Coffee pulp is a great addition to the garden and compost pile. Help to recycle this great organic resource and reduce the amount of organics going to the landfill.

4. Sawdust

Sawdust, particle of wood, is gain from wood cutting or sawing. Sawdust is mostly found in the furniture industrial. After furniture make sawdust is produce in high amount (Fig.5). Sawdust can called as a particle of wood so the constituent of sawdust is similar to wood.

Figure 5. Sawdust gain from sawing process

(source: http://7thgradedigitalportfolios.wikispaces.com/Andrew)

Sawdust is mainly composed of cellulose and lignin which is suitable for mushroom cultivation (Table3). Due to Mushroom can digest cellulose and lignin as their energy in growth development (Viziteu Gabriel, 2004). Sawdust is the main substance that use in cultivation of mushroom due to its composition is similar with a hard wood. Hard wood is the material that mushroom usually discovered such as in hardwood forest. (Stamets, 1983)

4.1 The chemical composition of sawdust

Table 3. The constituent of sawdust

Constituent	Percent
Ash	0.21%
Cellulose	58.2%
Hemi-cellulose	28.4%
Moisture	4.8%
Lignin	12%

(Source;D. L. Showalter, Chemical Composition of Sawdust from Lunar Rock 12013 and Comparison of a Java Tektite with the Rock)

Table 3 show that the sawdust composes of cellulose as their main constituent, hemicelluloses also present in the sawdust. Lignin is other compounds in sawdust which can use as a main nutrition for mushroom grow. Moreover saw dust contain moisture at 4.85 with ash 0.21%.

4.2 Utilization of sawdust

Sawdust can be a main substance in cultivation of mushroom because sawdust could provide a good source to mushroom. Moreover, sawdust is a waste so it will have a low price and can produce a higher yield of production. Sawdust can be usage as biomass energy replaces the wood (Ranta, 2007). The project show that wet sawdust can use in burning process. Sawdust can be a Nitrogen fertilizer added to soils to provide such plant nutrients as nitrogen and to improve the physical nature of the soil. Owing to sawdust constituent of high nitrogen content which could be a good fertilizer to improve the soil. (Barbarick, 2011)

5. Rice straw

Figure 6. Rice straw gain after rice harvesting

(source: http://www.phitsanulokhotnews.com/6459)

Thailand is a country that both consumption and export rice to over the world. Rice can grow continue grow 2-3 times per year in Thailand so rice straw will present in high amount. Rice straw is agriculture product gain from rice harvesting (Fig.6). Rice straw is a crop residues are high incellulose, hemicellulose and lignin, but low in pectin and silica. Lignin forms a ligno-cellulolic complex with some carbohydrates and proteins. This complex, specially the crystalline structure of cellulose in cell walls, is highly resistant to breakdown by enzymes, rumen microorganisms in the small intestine (Langar *et al.*, 1980; Henics, 1987). Lignin not only inhibits ruminal digestion of polysaccharides, but protects other highly digestible compounds (Hadar *et al.*, 1992; Karunanandaa *et al.*, 1995; Montaez *et al.*, 2004). The basidiomycetes fungi have the capability to degrade lignin in cell walls (Yamakawa *et al.*,1992b).

During the colonization of the substrate by the fungi easily digestible carbohydrates are converted into simpler sugars, a process known as fungus primary metabolism. The sugar is totally consumed by the fungus and then begins the secondary metabolism, which consists of the breakdown of structural carbohydrates and lignin from substrates by the extracellular enzymes like laccase, manganese peroxidase and peroxidase (Moyson and Verachtert, 1991; Karunanandaa *et al.*, 1995; Cohen *et al.*, 2002). In the rice straw contain many nutritions, silicon is present in high amount. Silicon is not effect to mushroom percent yield, size distribution and cap diameter (Thongsook T, Kongbangkerd T. 2011) so mushroom have a potential in cultivate Indian oyster mushroom.

Rice straw is a waste that could cultivation of straw mushroom (*Volvariella volvacea)* is a type of mushroom found widely distributed throughout Asia. The thumb sized mushrooms are heavily cultivated for food and export in Asia, and can be found in canned and dried forms in other parts of the world. The mushroom takes its name from paddy straw, the straw left over after growing rice. Usually the Straw Mushroom is cultivated for consumption on a mixture of cotton fiber and paddy straw. When mature for eating, a Straw Mushroom is approximately thumb sized, and distinguished by its pale pink gills and white spore print. The mushrooms have long white stems with bulbous bases, and drooping yellow to brown caps with a partial veil.

5.1 Composition of rice straw

Table 4. chemical composition of rice straw

Constituent	Percent
Ash	25%
Cellulose	37%
Hemicellulose	24%
Lignin	14%

(Source: (Chandel et al., 2009) Siti humairahbintisalmi, University Teknologi MARA)

Rice straw is composing of 4 main materials which are ash at 25%, Cellulose 37%, hemicelluloses 24% and lignin 14 %. The composition of rice straw is mostly are carbohydrate which can enrich mushroom to grow.

5.2 Benefit and utilization

Rice straw has many nutrients that can improve soil and have nutrient after harvesting. Soil will cover by rice straw in order to protect soil from insect and weed. Straw can also be a good fertilizer when mix with soil and provide a good source of nutrition value, rice straw could maintain a moisture content to soil Moreover rice straw could be a new alternative source of energy (Bio external), rice straw will extract external by distillation process. Normally farmer will use rice straw to feed animal instead of burning which could provide a great pollution. Danial J. Drake, university of California, reported that rice straw have very high silica content which help to decrease digestibility of the feed. Now in furniture industrial, rice straw is using to make a furniture and door. The innovative and advanced technology to encapsulate the straw, covering it in recyclable Kraft Paper while using biodegradable wheat/rice flour as adhesive. The resulting product is biodegradable, typhoon resistant, insulated, weatherproofed, sturdy, vermin resistant and easy to erect. This technology requires less or no wood and therefore reduces deforestation while using renewable raw materials. (Yvan Perrin, Minh Quyen JSC, 2005)

6. Rice husk

Rice husk is the natural sheath or productive cover, which forms the cover of rice grains during their growth. Rice husk represents about 20 % by the weight of the rice harvested, about 80 by weight of the raw husk is made of organic components (Anonymous, 1979).

Figure 7. Characteristic of rice husk

(source: http://www.indiamart.com/aditya-agro-industries/rice-products.html)

Rice husk is an agricultural residue abundantly available in rice producing countries (Fig. 5). The annual rice husk produce in Thailand amounts is generally approximately 60 million tons. Rice husk is generally not recommended as cattle feed since its cellulose and other sugar contents are low. Furfural and rice bran oil are extracted from rice husk. Industries use rice husk as fuel in boilers and for power generation. During rice refining processes (Fig. 5), the husks are removed from grains. It is little commercial value and because of its high silicon dioxide content, it is not useful to feed either human or cattle. Silica is the major constituent of rice husk ash and the following tables gives typical composition of rice husk and rice husk ash. With such a large ash content and silica content in the ash it becomes economical to extract silica from the ash, which has wide market and also takes care of ash disposal.

Incorporation of rice husk into soil mixture was found to affect many crops (Sharma et al.1988). Soil organic matter content is gradually declining due to high cropping intensity which causes quick decomposition of organic matter. Use of rice husk as an organic fertilizer, might be play a vital role not only in improving soil physical condition but also in improving the plant nutrients (Abo-Soliman et al., 1990). Rice husk is highly resistant to moisture penetration and fungal decomposition. Husk therefore makes a good insulation material. Moreover, Rice husk has a high average calorific value of an 3410 kcal/kg and therefore is a good, renewable energy source.

Table 5. the constituent of rice husk

Constituent	Percent
Cellulose	35%
Hemi-cellulose	35%
Lignin	20%
Ash	10%

(Source: Luh,1980, Jutarat Prachayawarakorn and Niracha Yaembunying)

Rice husk is difficult to ignite and it does not burn easily with open flame unless air is blown through the husk. It is highly resistant to moisture penetration and fungal decomposition. Husk therefore makes a good insulation material. Rice husk has a high silica (SiO_2) contents which means that it decomposes slowly when brought back to the field. Rice husk has low bulk density of only 70-110 kg/m^3, 145 kg/m^3 when vibrated or 180 kg/m^3 in form of pellets. It thus requires large volumes for storage and transport, which makes transport over long distances un-economical. When the ash content burned is 17-26%, a lot higher than fuels (wood 0.2-2%, coal 12.2%). This means when used for energy generation large amounts of ash need to be handled. Rice husk has a high average calorific value of a 3410 kcal/kg and therefore is a good, renewable energy source. Because of the high silica contents rice husk is very abrasive and wears conveying elements very quickly. Rice husk is not an easy fuel.

7. Coir fiber or coconut fiber

Coir fiber is a natural fibre extracted from the husk of coconut and used in products such as floor mats, doormats, brushes, mattresses etc. Technically *coir* is the fibrous material found between the hard, internal shell and the outer coat of a coconut. Other uses of brown coir (made from ripe coconut) are in upholstery padding, sacking and horticulture. White coir is harvested from unripe coconuts, and is used for making finer brushes, string, rope and fishing nets.

7.1 Structure of coir fiber

Coir fibres are found between the hard, internal shell and the outer coat of a coconut. The individual fibre cells are narrow and hollow, with thick walls made of cellulose. They are pale when immature but later become hardened and yellowed as a layer of lignin is deposited on their walls. There are two varieties of coir. Brown coir is harvested from fully ripened coconuts. It is thick, strong and has high abrasion resistance. It is typically used in mats, brushes and sacking. Mature brown coir fibres contain more lignin and less cellulose than fibres such as flax and cotton and so are stronger but less flexible. White coir fibres are harvested from the coconuts before they are ripe. These fibres are white or light brown in color and are smoother and finer, but also weaker. They are generally spun to make yarn that is used in mats or rope.

The coir fibre is relatively water-proof and is one of the few natural fibres resistant to damage by salt water. Fresh water is used to process brown coir, while sea water and fresh water are both used in the production of white coir.

Figure 8. Coir fiber after de-husk process

(source: http://www.ehow.com/how_6702879_clean-coir-carpets.html)

(1) Brown fibre

The fibrous husks are soaked in pits or in nets in a slow moving body of water to swell and soften the fibres. The long bristle fibres are separated from the shorter mattress fibres underneath the skin of the nut, a process known as *wet-milling*. The mattress fibres are sifted to remove dirt and other rubbish, dried in the sun and packed into bales. Some mattress fiber is allowed to retain more moisture so that it retains its elasticity for twisted fibre production. The coir fibre is elastic enough to twist without breaking and it holds a curl as though permanently waved. Twisting is done by simply making a rope of the

hank of fibre and twisting it using a machine or by hand. The longer bristle fibre is washed in clean water and then dried before being tied into bundles or hunks. It may then be cleaned and 'hackled' by steel combs to straighten the fibres and remove any shorter fibre pieces. Coir bristle fibre can also be bleached and dyed to obtain hanks of different colours.

(2) White fibre

The immature husks are suspended in a river or water-filled pit for up to ten months. During this time micro-organisms break down the plant tissues surrounding the fibres to loosen them a process known as retting. Segments of the husk are then beaten by hand to separate out the long fibres which are subsequently dried and cleaned. Cleaned fibre is ready for spinning into yarn using a simple one-handed system or a spinning wheel.

Researchers at CSIR's National Institute for Interdisciplinary Science and Technology NIIST) in Thiruvananthapuram have developed a biological process for the extraction of coir fibre from coconut husk without polluting the environment. The technology uses enzymes to separate the fibres by converting plant compounds into soluble compounds and hence curbs the pollution of water-bodies caused by retting of coconut husks.

7.2 Fibre Structure

Individual fibres are 0.3-1.0 mm long and 0.01-0.0.2 mm in diameter; the ratio of length to diameter being 35. The lumen is medium to large, polygonal-rounded, or elliptic. The vascular bundle is collateral and is surrounded by thick sclerenchymatous sheath. Lignin and hemicelluloses, which form the cementing materials of fibre cells, increase with the age of the fibre and the pectin decreases. As the lignin content increases, the fibre becomes stiffer and tougher.

Length of the fibre determines its spinnability and commercial utility. Spinnability may be defined as the ease with which textile fibres may be twisted into continuous, uniform yarns, having commercially acceptable properties.

Fineness of a fibre is usually expressed by its diameter in microns or by the weight of the fibre per unit length- dinier. The compactness and strength of a yarn or cord depends on the cohesion between individual fibres. Strength or tensile strength of a fibre is determined by its ability to resist strain or rupture induced by tension, and is a determining factor in the selection of a fibre. Elongation at rupture is a criterion of practical value and is an index of the work that could be performed by the fibre within the limits of its breaking load. Stresses in the fibre due to twisting and bending or important factors which affect the diameter of the yarn, its ability to snarl, its pliability and elastic recovery from small strains and internal pressures.

7.3 Chemical Compositions

Table 6. chemical composition of coir fiber

Constituent	Percent
Pectin	14.25 %
Hemi-cellulose	8.5%
Cellulose	23.81%
Lignin	29.23%
Total water soluble	26 %

(source: Dr. Balakrishna Gowda, Univerity of Agricultural Sciences Hebbal, GKVK Campus)

7.4 Utilization

Brown coir is used in floor mats and doormats, brushes, mattresses, floor tiles and sacking. A small amount is also made into twine. Pads of curled brown coir fibre, made by *needle-felting* (a machine technique that mats the fibres together) are shaped and cut to fill mattresses and for use in erosion control on river banks and hillsides. A major proportion of brown coir pads are sprayed with rubber latex, which bonds the fibres together (rubberized coir) to be used as upholstery padding for the automobile industry in Europe. The material is also used for insulation and packaging.

The major use of white coir is in rope manufacture. Mats of woven coir fibre are made from the finer grades of bristle and white fibre using hand or mechanical looms. White coir also used to make fishing nets due to its strong resilience to salt water.

In horticulture, coir is a strongly recommended substitute for sphagnum moss because it is free of bacterial and fungal spores, and produces good results without the environmental damage caused by peat mining. Coir is also useful to deter snails from delicate plantings. Coir is also used as a growing media in intensive glasshouse horticulture.

Coconut coir from Mexico has been found to contain large numbers of colonies of the beneficial fungus *Aspergillus terreus* which acts as a biological control against plant pathogenic fungi.

Coir is an allergen, as well as the latex and other materials used frequently in the treatment of coir. This should be noted specially for people with allergies using mattresses and other furniture made with coir.

Materials and Equipments

Material:

1. Coffee pulp
2. Rice straw
3. Rice husk
4. Coir fiber

Equipment:

1. HPLC
2. Autoclave
3. Digital weigh
4. Hot plate

Experimental Design

1. **To study the effects of two substances on the cultivation of Indian- oyster mushroom by combining coffee pulp as a main substances with four substances in 5 levels of percentage.**

Table7. The Variation in percentage of the mixture between each substance and coffee pulp

Mixture	Percentage of Substances				
	100%	75%	50%	25%	0%
Coffee pulp(CP) : sawdust(SD)	✓	✓	✓	✓	✓
Coffee pulp(CP) : rice straw(RS)	✓	✓	✓	✓	✓
Coffee pulp(CP) : rice husk(RH)	✓	✓	✓	✓	✓
Coffee pulp(CP) : coir fiber(CF)	✓	✓	✓	✓	✓

The experiment is studied the variation of coffee pulp with four materials in different percentage. Coffee pulp mixed with sawdust, rice straw, and rice husk and coir fiber in different percentage as shown in the table7. The percentage of each mixture were 100% substance with 0% coffee pulp, 75% substance with 25% coffee pulp,50% substance with 50% coffee pulp, 25% substance with 75% coffee pulp and 0% substance with 100% coffee pulp. Each percentage mixture has 2 replication and 5 duplicate. Substance bag will remove to incubation room which control environment, temperature, humidity and light intensity. After the mycelium develop grow completely, plug and paper cover will removed. Mushroom need to watering at least 3 times per day for control their humidity and moisture proper for the first germination. Mushroom was collected at the first germination and second germination. Mushroom was determined by yield, incubation time (start from inoculate until collect the first mushroom batch), number of mushroom stem and caffeine residue.

2. To study the effects of three substances on the cultivation of Indian- oyster mushroom by combining coffee pulp as a main substances with four substances in a ratio 1:1:1

The experimental studied the variation of 3 substances mixtures in ratio 1:1:1. Coffee pulp combine with other substances as CP:SD:RH, CP:SD:RS, CP:SD:CF, CP:RS:RH, CP:RH:CF, CP:RS:CF. Each sample has 2 replication and 5 duplication. All samples incubated at room temperature until spawn grew completely and the moves to mushroom house. Substance bag will remove to incubation room which control environment, temperature, humidity and light intensity. After the mycelium develop grow completely, plug and paper cover will removed. Mushroom need to watering at least 3 times per day for control their humidity and moisture proper for the first germination. Mushroom was determined by yield, incubation time (start from inoculate until collect the first mushroom batch), number of mushroom stem and caffeine residue.

RESULTS AND DISCUSSION

1. Effects of sawdust combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom

Table 8: Effects of sawdust combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom (%SD:%CF-100:0, 75:25, 50:50, 25:75 and 0:100)

Sample	%yield	Incubation time(days)	Number of stems	Caffeine residue (ppm.)
SD-100	$8.8^{a}\pm2.3$	$40.8^{a}\pm6.4$	89	0
SD-75	$8.6^{a}\pm 1.0$	$36.4^{a}\pm6.4$	71	0
SD-50	$9.8^{a}\pm3.0$	$41.5^{a}\pm12.7$	60	49.9
SD-25	$9.9^{a}\pm3.4$	$42.2^{a}\pm4.4$	61	6.6
SD-0(CP-100)	$3.1^{b}\pm1.0$	$54.2^{b}\pm9.7$	29	52.6

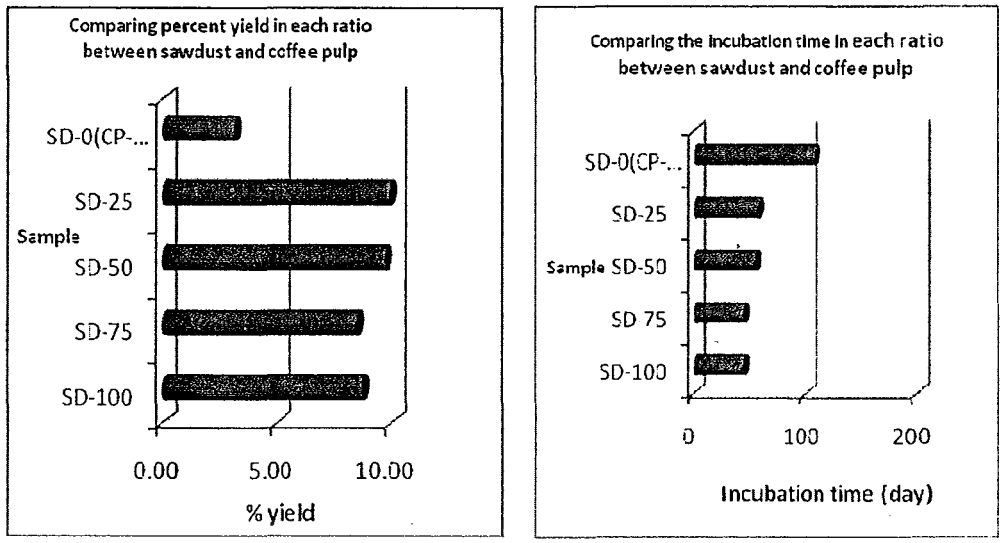

Figure9. Yield and incubation time of the mixture between sawdust and coffee pulp(%SD:%CF-100:0, 75:25, 50:50, 25:75 and 0:100)

This experiment was studied the combination between sawdust (SD) and coffee pulp (CP) substances by varying percent of each substance (%SD:%CF-100:0, 75:25, 50:50, 25:75 and 0:100). Determination parameters were yield, incubation time (the 1st germinated of mushroom), numbers of germinated stem and caffeine residue. The result was shown that there were no significant different in all samples in both of yield and incubation time ($\alpha = 0.05$). Both yield and incubation time of coffee pulp (SD-0 or 100%CP) were lower than any mixtures of SD and CP samples. Germination rate of SD-0 samples was only 50% while mixture of CP with SD samples was 100% of substance bags. Number of mushroom stems of SD-100 sample (or 100% SD) was highest while SD-0 sample (100%CP) was lowest among samples. The increasing of SD content was effected on the increasing number of mushroom stems. The caffeine residues of SD-0 sample (100%CP) were about 52 ppm. while SD-100 was caffeine-free. But the other samples could not conclude because SD-50 samples also contained high caffeine residue. This might be the smaller samples in order to determine caffeine residue. The next special project should be increased the samples to determine it. However, other samples indicate lower CP content leaded to the lower caffeine residue.

Lignocellulolytic enzymes in Indian-Oyster mushroom can convert cellulose and lignin, as a substrate to glucose that can be used as an energy source for the fungi (Christopher and Custodio, 2004). Lignin and cellulose content of SD (about 50%) were higher than CP (about 27%). Hence, the additional of SD might promote the mushroom growth in CP substrate. However, this part of experiment should be further studied the effect of SD in different percent on the mushroom growth. According to the research of Asian J. plant science 2009 showed that time of mycelium germination is depend on the use of substance. Material with high content of lignin and cellulose take a longer time to germinate. As consequence coffee pulp has a lower of cellulose and lignin that should be effect on the mushroom, shorter time than sawdust. However, CP might contain some compound and inhibit the mycelium growth. Martinez-Carrera, 2000 reported that CP should be fermented prior before mixing with SD. Caffeine will be destroy metabolism of mushroom and slow the germination rate. However, the variation of SD content in this experiment do not shown the effect in both yield and incubation time but effect on the number of mushroom stems. This might be the watering in the cultivation of mushroom by pouring direct on substances bags. The top bags will gain more moisture than the inner bags. Hence, the watering of the mushroom should be improved in the next special project.

CP without fermentation can be cultivated the Indian-oyster mushroom but grow slowly and get lower yield. The addition of SD can promote the growth and yield. During the growth, mushroom can be absorbed caffeine compound within their mycelium but the caffeine content found about 56 ppm.

2. Effects of rice straw combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom

Table 9: Effects of rice straw combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom (%SD:%CF-100:0, 75:25, 50:50, 25:75 and 0:100)

Sample	%yield	Incubation time(days)	Number of stem	Caffeine residue
RS-100	0	0	0	0
RS-75	5.2^{bc}±1.2	24.5^{a}±12.5	56	0.8
RS-50	5.4^{b}±1.2	30.4^{bc}±11.9	31	0
RS-25	7.7^{a}±5.1	36.0^{cd}± 8.6	64	4.1
RS-0(CP-100)	3.0^{c}±0.9	54.2^{d}±9.7	29	52.6
SD-100	8.7^{a}±2.3	40.8^{d}±6.5	89	0

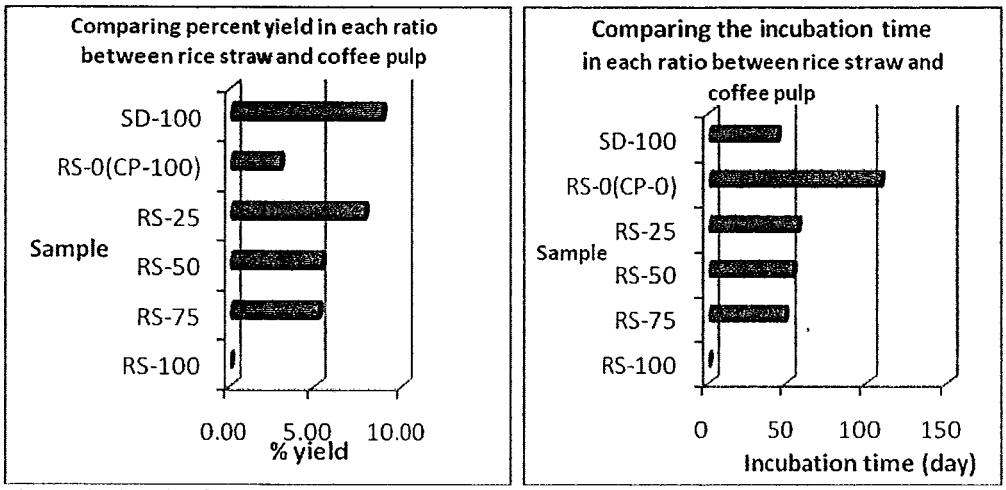

Figure10. Graph of percent and incubation time between mixture of rice straw and coffee pulp

The experimental is studied the combination of rice straw (RS) with coffee pulp (CP) substances by varying percent of each substance (%RS:%CF-100:0, 75:25, 50:50, 25:75 and 0:100). Determination parameters were yield, incubation time, numbers of germinated stem and caffeine residue. The result shown that there are significant different in percent yield and incubation time ($\alpha = 0.05$). Coffee pulp 100% yield were lower yield and longer incubation time of the first germination comparing with other mixture of RS and CP. CP-100 (or RS-0) ranged in the highest position of caffeine residue than other mixture. The germination time and yield production of RS-25 is almost the same as the control (SD-100). Control (SD-100) produced highest a number of stem than the other mixture. Incubation time of RS-75 is shortest times during the first germination and lowest yields as same as CP-100, among samples. RS-75 compared with RS-50, RS-75 had a higher yield, but spent longer time of germination. Moreover, RS-75 had higher amount of stems and caffeine residue than RS-50. However, the result shown that rice straw substance promoted mushroom grow faster than the control (SD-100). At RS-25 shown the same yield with control, therefore Rice straw have a potential in or to cultivate Indian–oyster mushroom similar with control. Ayman S. et.all, 2008 reported that rice straw can be used as organic source for mushroom to provide nitrogen, especially during the formation of fruiting bodies so rice straw can enrich mushroom to grow. Incubation time rice straw at 75%, 50% and 25% were shorter than control.

RS-100 mushroom is not produce in this experiment which might cause from the moisture content inside substance bag was too high and interfere the spawn growth. Rice straw was soaking over night, and absorbs water. There also added water into substrate bags and caused excess moisture inside bag. Moreover, the insect and fruit fly is infected in the substance bag which can inhibit mycelium growth.

Rice straw helps coffee pulp in order to cultivate mushroom in term of shorter incubation time but gain lower yield with control. the production of RS-25 similar to control in yield, incubation time and number of stems.

3. Effects of rice husk combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom

Table10: Effects of rice husk combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom (%RH:%CF-100:0, 75:25, 50:50, 25:75 and 0:100)

Sample	%yield	Incubation time (day)	Number of stems	Caffeine residue
RH-100	0	0	0	0
RH-75	$4.4^{b}\pm 4.9$	22.6^{a}	24	0.9
RH-50	$4.9^{b}\pm 5.5$	39.0^{a}	31	0
RH-25	$5.1^{b}\pm 5.4$	27.0^{a}	25	0
RH-0(CP-100)	$3.0^{c}\pm 0.9$	54.2^{b}	29	52.6
SD-100	$8.7^{a}\pm 2.3$	40.8^{b}	89	0

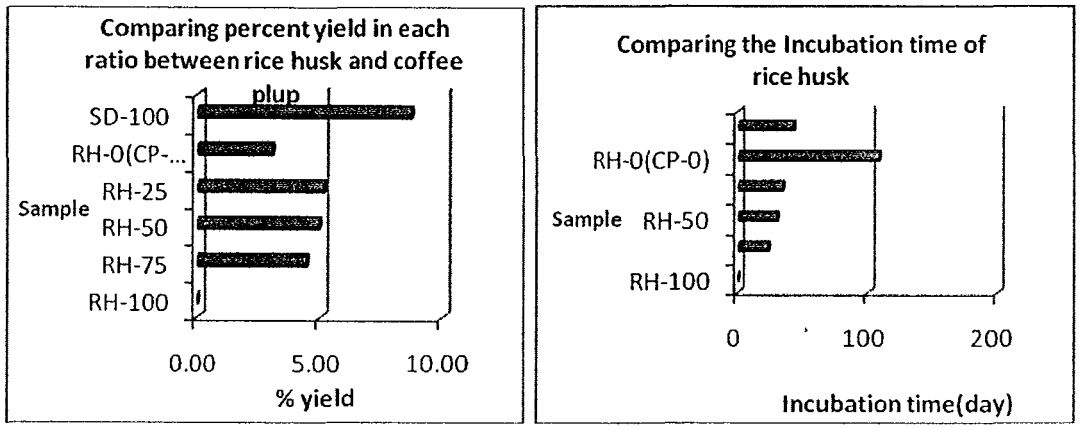

Figure11. Graph of percent and incubation time between mixture of rice husk and coffee pulp

The experimental is studied the combination of Rice Husk (RH) with coffee pulp (CP) substances by varying percent of each substance (%RH:%CF-100:0, 75:25, 50:50, 25:75 and 0:100). Determination parameters were yield, incubation time, numbers of germinated stem and caffeine residue. The result showed the mixture of rice husk was significantly different from the control. Percent of yield of RS mixture were lower than the control (SD-100), whereas incubation time of RS mixture is shorter than control. Nevertheless, yield of RH was higher than CF and also shorter germinate time as the same as RS. Number of stem of control showed higher than the other mixtures. Caffeine residue in coffee pulp is higher than the other mixture. However rice husk can cultivation Indian-oyster mushroom but can produce in low yield and spent a shorter time of germination.

At RH- 100 mycelium is not growth during the incubation. According to the research (Hanai, 2004) showed that the rice husk is both stimulate and inhibit mushroom to grow. In rice husk present momilactone (MILA) which is recognized as one of the phytoalexins which its biological activity is against mycelia to growth. The mushroom cannot grow in rice husk well. RH-100 might contain high level of momilactone which can inhibit grow of the mushroom.

Research of Asian J. plant science 2009 showed that time of mycelium germination is depending on the use of substance. Material with high content of lignin and cellulose take a longer time to germinate. So compare incubation time between control and rice husk, control have a

higher yield but longer time of germination. The amount of nitrogen available in a rice husk after each flush, influence the degree of cellulose degradation which in turn affects the yield (Zadrazil and Brunnert, 1980). The amount of nitrogen cloud effect to the percent yield of mushroom.

4. Effects of rice husk combining with coffee pulp substances on the cultivation of Indian-Oyster mushroom

Coir fiber is another waste material that it constituent can grow mushroom as well reported by Asian J. plant science 2009. All sample of this experiment didn't germinated mushroom. There are many reasons to inhibit germination. First, during incubation moisture content was excess. It came from many sources, water from soaking coir fiber over a night so the fiber can absorb water fully. Second, water form mixing process, according to the standard formula water need to add 60-70% of the media. All of the reasons could cause excess moisture inside the media which inhibit spawn growth. More reason mushroom is cultivated in the open system so it cannot control the humidity of environment. Light intensity and aeration are not suitable for mushroom to grow. There are some insect and warm present in the bag so those animal is consume nutrition of the substance bag which could not allow mycelium to develop themselves.

5. Comparing percent yield each sample

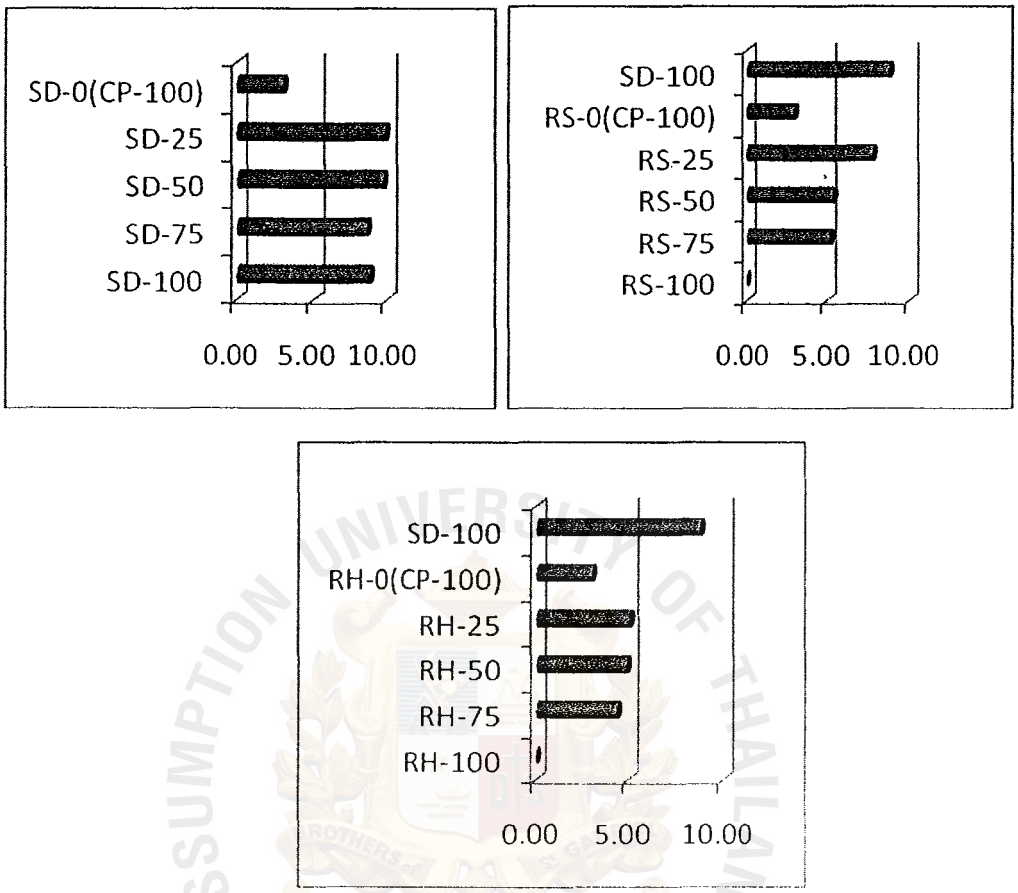

Figure12. Graph of percent yield between mixture of sawdust, rice straw and rice husk

The graph showed the comparison of the percent yield of each substance mix with coffee pulp. The result showed that sawdust was a substance that could provide yield of production higher than the other two substances. According to Whittaker, R.H. 1969, new concepts of kingdoms of organisms, mushroom can be survive and grows in a hard wood which could provide a source of nutrition (cellulose, lignin) for germination. Sawdust was waste from the hard wood which could provide a good source of nutrient to mushroom, therefore yield of mushroom from the mixture provided a good result. If compare the nutrient in rice straw and rice husk from the table, rice straw content nutrient that could provide mushroom to grow higher than rice husk so the yield of rice straw might be more than rice husk.

6. Comparing incubation time in each sample

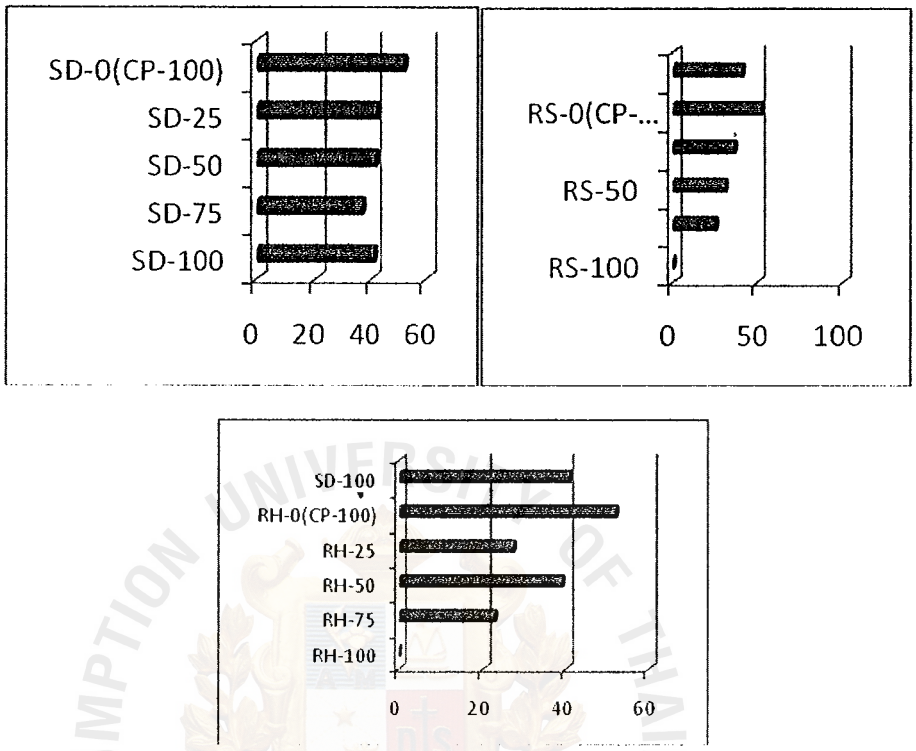

Figure13. Incubation time graph between mixture of sawdust, rice straw and rice husk

Table 11: Compare each percent of 2 substance mixture

sample	% yeild
SD-75	8.5^{a}± 1.0
RS-75	5.2^{b}±1.2
RH-75	4.4^{b}± 4.9
SD-50	9.7^{a}±2.9
RS-50	5.4^{b}±1.2
RH-50	4.9^{b}±5.5
SD-25	9.9^{a}±3.4
RS-25	7.7^{ab}±5.1
RH-25	5.1^{b}±5.4

The graph showed the comparison of incubation time of sawdust, rice straw and rice husk mixture with coffee pulp. As Asian J. Plant Science 2009 research showed that time of mycelium start germination is depend

on the substance. Material with high content of lignin and cellulose take a longer time to germinate. From the graph rice husk and rice straw spent a short time of incubation than sawdust and control due to the constituent value of both substances have lower than sawdust. Coffee pulp 100% was spent a longer time than the control and other substance even its substance was lower than other. Martinez-Carrera 2000 reported that coffee pulp should be fermented before 10 days in order to get good structure and consistency for Pleurotus cultivation. Moreover caffeine after fermentation might suitable for mushroom grow, inspire of improper caffeine can inhibit grow and metabolism of mushroom. Coffee pulp in the experiment had not fermented before utilization so the chemical inside might not suitable for mushroom grow that reason made mushroom grow slowly.

7. Three substance mixture

Table12. Percent yield and incubation time of each three substance mixture

Sample	%yield	Incubation time(day)	Number of stem	Caffeine residue
CP:SD:RS	$5.5^{b}\pm2.2$	$28.9^{a}\pm9.4$	37	18.2
CP:SD:RH	$5.1^{bc}\pm 2.4$	$33.9^{ab}\pm11.2$	57	2.7
CP:SD:CF	$7.1^{ab}\pm3.1$	$44.8^{cd}\pm14.8$	50	0
CP:RH:RS	$4.9^{b}\pm3.1$	$24.6^{a}\pm15.1$	31	0
CP:RH:CF	0	0	0	0
CP:RS:CF	0	0	0	0
CP-100	$3.0^{c}\pm0.9$	$40.8^{cd}\pm6.4$	89	56.5

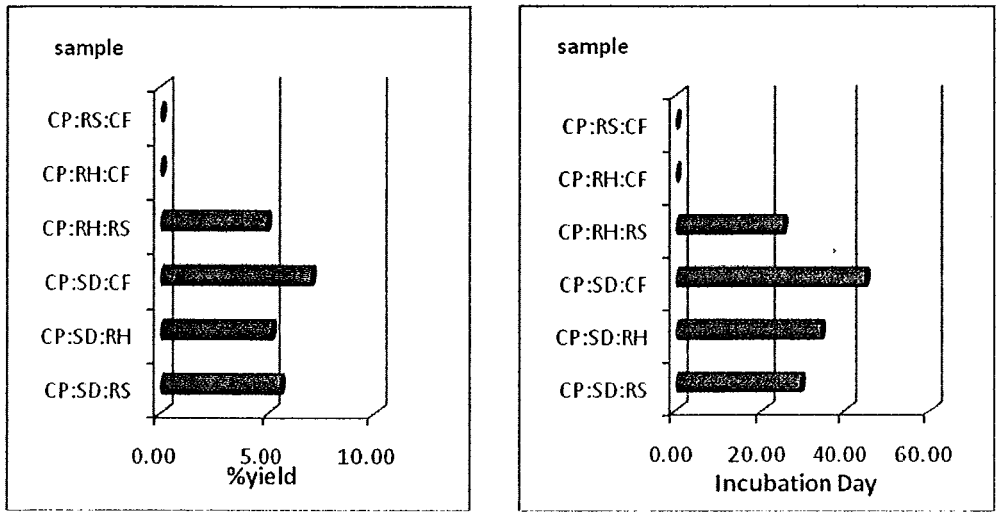

Figure14. Incubation time graph between mixture of sawdust, rice straw and rice husk

This experimental was studied the variation of the 3 substances mixtures in ratio 1:1:1. The result showed that all the sample mixture present lower yield comparing with the control (SD-100), therefore CP:SD:CF have a yield equally with the control. Moreover, yield in sample is higher than CP-100. The incubation time of CP:SD:RS, CP:SD:RH and CP:RH:RS was not significant different while control and CP:SD:CF was almost the same. The control gave a number of stem more than the other mixtures. CP:SD:RS and CP:SD:RH had shown the caffeine residue in the mushroom while the other sample not present any residue.

The result was obviously showed that the combination of rice straw and rice husk in the mixture will provide mushroom growth and germination faster. The mixture of CP:RH:CF and CP:RS:CF had not present any mushroom germination and develop of mycelium. The 3 substance might not support mushroom grow if combine together.

8. Sensory evaluation (triangle test)

Sensory evaluation is test the different character between commercial mushroom and coffee pulp 100% mushroom. Triangle test of the experimental have 50 panelists who test the sample. The sample will blanching in hot water for 30 sec then cool down and cut into a small piece .After that place to a plastic cup and serve to the panelist.the resulted showed that there was no significant different between coffee pulps 100% mushroom with commercial mushroom at α 005.

Conclusion

- There were significant different in all samples in $\alpha = 0.05$
- Compare between sawdust and coffee pulp:
    - Sawdust material promoted the shorter incubation time and gain higher yield when compare with coffee pulp 100%.
- Compare between rice straw and coffee pulp:(with control)
    - Yield of 25 % of rice straw was similar to Control (Sawdust 100%)
    - Rice straw substance had the shorter incubation time but provide lower yield, comparing with control sample .
- Compare between rice husk and coffee pulp:(with control)
    - Rice straw substance had the shorter incubation time but provide lower yield, comparing with control sample .
- Samples from coir fiber is not germinate in this experiment.
- Caffeine residue in all sample do not exceed the standard.
- From Triangle test, mushroom from 100% coffee is no significant different from commercial mushroom

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APPENDIX

1. Cultivation of Indian-oyster mushroom

1. 1 Cultivation spawn in PDA media

Prepare PDA media in a glass bottle then inoculate small piece of mushroom to the media under aseptic technique. Next close the bottle with cotton pulp and paper, Incubation PDA media in room temperature until the mycelium is grow completely.

Figure15. Inoculation fresh mushroom in PDA media

(Source: FAO corporate document repository)

1.2 Cultivation PDA mycelium to the sorghum seed

After mycelium grow completely in PDA media, prepare a sorghum seed in the glass bottle. Next, cut a square (dimension: 1 x 1 cm) piece of PDA to the sorghum seed. Close with a cotton plug and paper, Incubation at room temperature (25°C-30°C) for 7-10 day

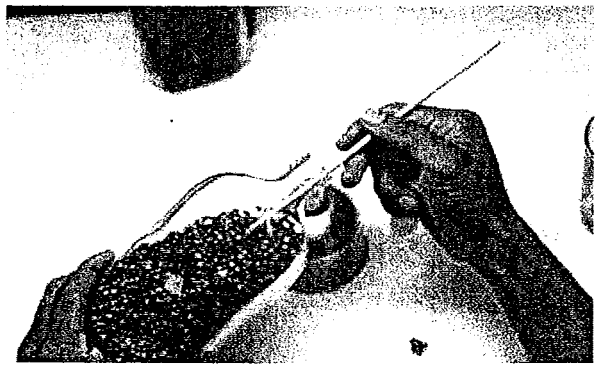

Figure16. Inoculation PDA mycelium in sorghum seed

(Source: FAO corporate document repository)

1.3 Preparation of substance bag

Mix all ingredients in the table with the substance in the bucket until the all substance is mix well. Add water to the dry ingredient mixture, mixed until homogenous. Pack in a substance bag and weigh about 1 kg then sterilize in Auto-clave at 121°C for 1 hr. Cool down the bag prepare for the next step.

Formula

Table 9 Main ingredient for mushroom cultivation

Ingredient	kg
Sawdust	100
Magnesium sulfate	0.2
Lime	1
Rice bran	6
Gypsum	0.2
Water	60-70

1.4 Inoculation mycelium sorghum seed in the substance bag

Mycelium in a sorghum seed will lose by using spatula hit to break the seed. Inoculate sorghum seed to the cooled substance bag approximately 10-20 seed. Close substance bag with cotton plug and paper, The substance bag will incubate at room temperature (25°C-30°C) until mycelium grow completely.

1.5 Cultivation at the shelf

The complete substance bag will move to the shelf and remove the plug in order to allow mushroom germinate. Watering mushroom three times a day in order to maintain humidity and moisture content to environment

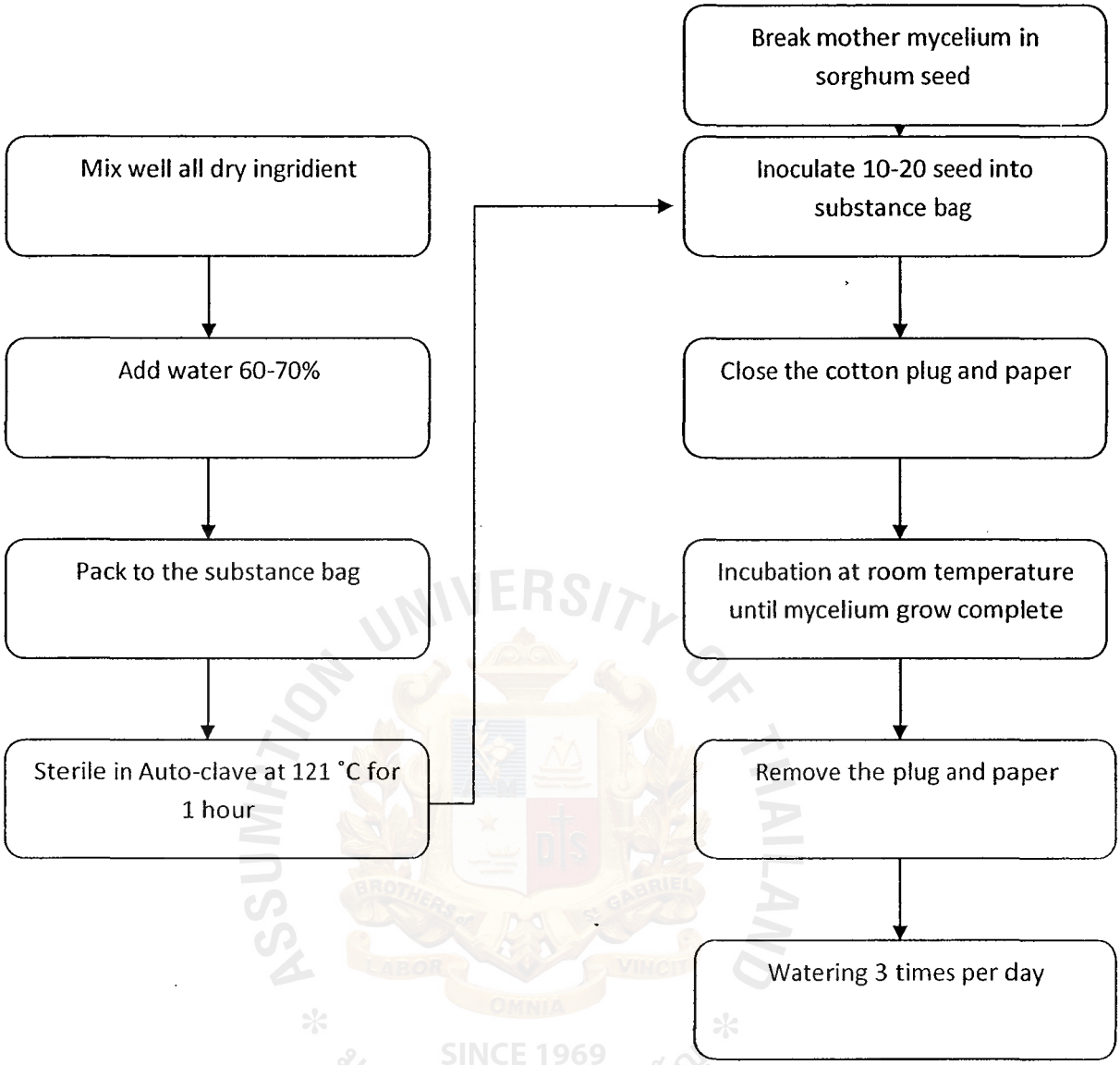

Figure 7. Flow chart of the cultivation of Indian-oyster mushroom

Sensory evaluation (Triangle test)

Evaluation of mushroom test

Name: _____ ID:_____

Instruction:

Three products are presented: two of them are identical, the other is different. Taste the sample in the prescribed order and tick the box of the unique product.

☐ _____ ☐ _____ ☐ _____