CHAPTER 2

LITERATURE REVIEW

2.1 Paddy Straw Mushroom (*Volvariella volvacea*)

2.1.1 History of *V. volvacea* Mushroom

V. volvacea is a basidiomycete fungal which classified under Pluteaceae family. *V. volvacea* mushroom is well-known as straw and chinese mushroom (Ahlawat & Tewari, 2011). In 1822, it was first cultivated in the Northern Guangdong Province, China and was named after a Nanhua Temple which was known as "Nanhua mushroom". Initially, it was just an ordinary protein source for the Buddhist monk. Soon after, the fame of straw mushroom gross to the whole Chinese nation (Hsu *et al.*, 1997). In 1875, it was sent as a tribute to the royalty due to its medicinal and dietary effect Straw mushroom was successfully introduced in 1932, through the Chinese labour who came to Southeast Asia countries (Diamantopoulou *et al.*, 2016)

2.1.2 Production of *V. volvacea* in Malaysia

In Malaysia, mushrooms are high value crops which were cultivated intensively. The growing mushroom industry in Malaysia is still considered to be very new and small. There are 17 types of mushrooms is being cultivated and *V. volvacea* is ranked 9 in Malaysia (Rosmiza *et al.*, 2016). Table 2.1 shows the types of mushroom cultivated in Malaysia in year 2014.

Table 2.1: Cultivated mushrooms in Malaysia for the year 2014 (Department of Agriculture Malaysia, 2015).

Mushroom type	Total cultivated (%)
Grey oyster (*Pleurotus pulmonarius*)	90.89%
Ling Zhi (*Ganoderma sp.*)	1.64%
King oyster (*Pleurotus eryngii*)	1.17%
Black Jelly (*Auricularia sp.*)	1.17%
Enoki/ Golden needle (*Flammulina velutipes*)	0.70%
White oyster (*Pleurotus florida*)	0.70%
Button (*Agaricus sp.*)	0.70%
Shiitake (*Lenticus endodes*)	0.70%
Paddy straw (*Volvariella volvaceae*)	0.47%
Abalon (*Pleurotus cystidiosus*)	0.23%
Chestnut (*Agrocybe sp.*)	0.23%
Red oyster (*Pleurotus flabellatus*)	0.23%
Yellow oyster (*Pleurotus citronipileatus*)	0.23%
Fungus (*Schizophyllum sp.*)	0.23%
Shaggy mane (*Coprinus sp.*)	0.23%
Monkey head (*Hericium erinaceum*)	0.23%
Morning glory (*Citocybe sp.*)	0.23%

Note: Total percentage of *V. volvacea* is being cultivated in Malaysia.

2.1.3 Morphological Characteristics of *V. volvacea*

V. volvacea is a basidiomycete fungal which classified under Pluteaceae family. It has six distinctive morphological phase for a complete life cycle which are the pinhead, tiny button, button, egg, elongation and mature stage as shown in Figure 2.1.

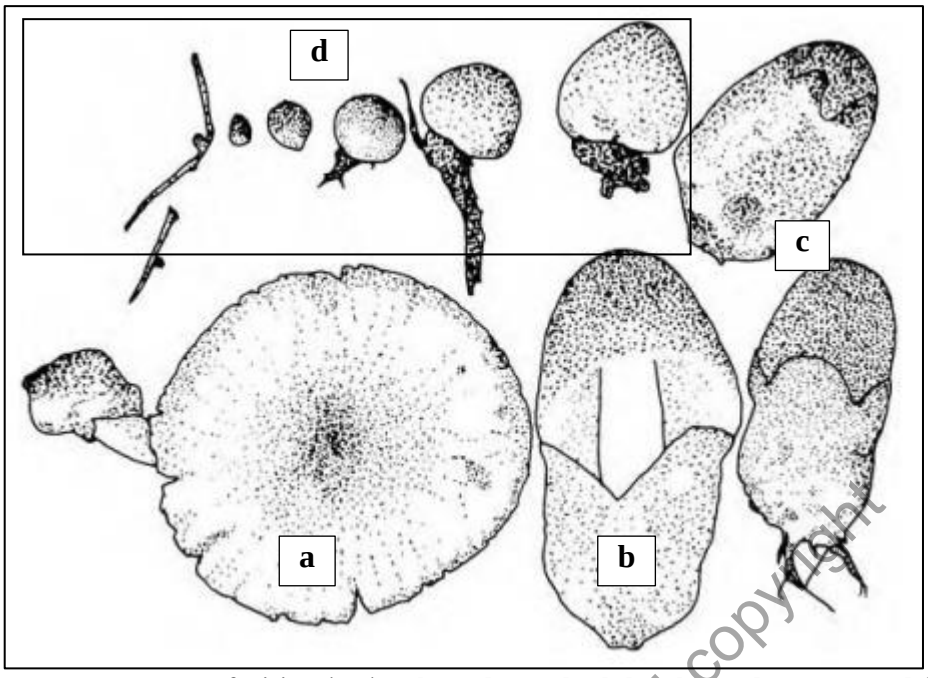

Figure 2.1: (a) "mature" fruiting body, (b) volva, which has been disconnected from the pileus by rapid elongation of the stipe and is also the "elongation" stage, (c) "egg" stages indicating that the universal veils have been damaged, (d) "button," "tiny button," and "pinhead" stages (Miles & Chang, 2004).

2.1.3.1 Pinhead Stage

The pinhead stage is formed from interwoven hyphae. The veil is white and spotless and the size is relatively small, similar to a pinhead. From the vertical view, the pileus and stipe are hidden from vision, while horizontal view, both pileus and stipe can be observed. The whole structure is a knot of hyphal cells (Ahlawat & Tewari, 2011).

2.1.3.2 Tiny Button Stage

The tiny button stage is also formed from interwoven hyphae. It is round in shape and the veil is brown. At the bottom section of the thick pileus, lamellae can be seen if a vertical cut is made through the button (Miles & Chang, 2004).

2.1.3.3 Button Stage

At button stage, the ovoid stipe can be served as culinary food, therefore, it can be sold at a good price. The stipe is not easily found but in longitudinal section of mushroom, it is visible. In this stage, the whole body is covered by a coat, which is called as the universal veil and the inner surface of the universal veil consist of a closed pileus (Ahlawat & Tewari, 2011).

2.1.3.4 Egg Stage

At egg stage, the pileus is pushed out from the veil while the veil remains as volva. The stipe is not easily found but in longitudinal section of mushroom, it is visible. There are no basidiospores in the lamellae of this stage. Under microscope view, although there may be basidia in the stage of sterigmata formation. But, only the cystidia and the paraphyses can be seen (Ahlawat & Tewari, 2011).

2.1.3.5 Elongation Stage

In this stage, the shape of the fruiting body is almost the same as the mature stage. The pileus remains close and the size is smaller than mature stage while the stipe attains the maximum length. Elongation mainly occurs in the upper section of the stipe (Ahlawat & Tewari, 2011).

2.1.3.6 Mature Stage

At this stage, the whole body can be divided into three regions: (i) the fully grown pileus or cap, (ii) mature stipe or stalk and (iii) the ovoid volva or cup. The pileus and stipe are situated in the middle with 0.06 to 0.12 m in diameter.

The fully grown pileus is round in shape. It has a smooth surface with an entire margin. The surface of the pileus is dark grey in colour at the center, meanwhile, near the margin it appears in light grey. On the lower surface of it, it has lamellae which exist around 280 to 380 lamellae. Lamellae exist in different sizes from one quarter size of pileus to full grown size. It can be observed that each lamellae is made up of three layers of interwoven hyphae under microscope. The outer layer is hymenium and it forms the club-shaped basidia and the cystedia. The basidia hold basidiospores. Usually, one basidium bears four basidiospores. The basidiospores vary in shape and colour; egg shaped, spherical or ellipsoidal and light yellow, pink or dark brown (Ahlawat & Tewari, 2011).

The white, fleshy and without any annulus stipe connects the volva and the pileus. The length of the stipe depends upon the size of the pileus and it is usually about 0.03 to 0.08 m in length and 0.05 to 0.15 m in diameter. At the base of the stipe remains the fleshy, white and cup shaped with irregular margins volva, which is a thin sheet of interwoven hyphae around the bulbous base of the stipe. The base of volva carries rhizomorphs, which is used to absorb the nutrient from the substrate (Miles & Chang, 2004).

2.1.4 Cultivation Process of *Volvariella volvacea*

2.1.4.1 Spawn Production

Mushroom spawn is simply any substance that has been transferred and inoculated to the compost substrate for colonization or grow with the mushroom fungus or with mycelium. By this way, two monokaryotic mycelia can be fused and developed into a dikaryotic mycelium and moved on to fruiting body (Leiva *et al.*, 2015).

Usually, when harvest of mushroom is not good, unsatisfactory spawn is always to be blamed. This happened when the spawn has not been cultivated from a genetically suitable fruiting culture, a degenerated stock or too mature, the yield of mushrooms will be less. In order to produce a good spawn, cultivators should use a stable strain or stock

possessing the genetic characteristics required for the cultivation of mushroom spawn (Miles & Chang, 2004).

In short, in the production of spawn, there is a few criteria's cultivators must considered. The genetic capabilities of the fruiting culture for vegetative growth both in the spawn substrate and in the bed material following inoculation and for a satisfactory yield and quality of mushrooms produced. Consideration must also be given to the nature of the spawn material, because this influences rapidity and thoroughness of mycelial growth in the spawn container as well as spawn running in the bed following inoculation (Miles & Chang, 2004).

2.1.4.2 Substrate Preparation

The substrates used in spawn production may or may be different from the materials used in the cultivation of the mushroom. Substrates can be used singly or in mixture. Some of the substrates used in the spawn production include various grains (rye, wheat, and sorghum), paddy straw, cotton waste, rice husks, cotton seed husks and etc. Spawns. Grain spawns or straw spawns are referred to spawn as well, where it is equally as important as substrate too. Spawn substrate is used to transport the vegetative mycelium of the mushroom that is used to inoculate the growing beds (Leiva *et al.*, 2015).

A substrate rich in available nutrients does not necessarily contains a complete medium for cultivating mushrooms. This is because bacteria and molds will grow rapidly which will affect the growth of mycelium and the development of mushroom, therefore the material must be sterilized first. Whenever a spawn is inoculated into an unsterilized substrate, the naturally occurring microorganisms quickly gain dominance and halt the development of mushroom mycelium. Thus, substrate preparation function as promote the growth of mushroom mycelium to the practical exclusion of other organisms. In order to achieve this, certain chemical properties and physical properties must be meet. These features of the substrate are equally important and are interdependent. When all the nutritional requirements of the mushroom have been achieved, the correct chemical state of the substrate can then be achieved (Andersen *et al.*, 2010).

Most mushroom species cannot utilized nitrates, therefore the substrates must be in a form that is readily use for mushrooms. Thus, it is best to eliminate a substrate with most of the nitrogen source due to nitrogen source is another essential building block element required for good mushroom cultivation.. (Samandeep *et al.*, 2015).

2.1.4.3 Incubation Process

During the incubation process, the air temperature is cooled to 35 °C, while the bed temperature is 36 °C – 38 °C. The spawn is crush into peanuts size inserted into several cobs at a depth of about 0.02 m – 0.025 m and cover it with a loosened cap to promote aeration uptake. The temperature is maintained at 32 °C – 34 °C during the spawn running period. Full growth may take 3 - 4 days, depending on the substrate quality and the temperature.

During the 3 – 4 days period of spawn run, no water or light is added. Only a limited amount of fresh air is required during this period. A few days later fluorescence light and additional ventilation are provided. On fifth day after spawning, mushroom primordia usually appear on the surface of the beds. Four to five days later, the first flush of mushroom is ready for harvest (Miles & Chang, 2004).

2.1.4.4 Harvesting Process

The first crop of mushroom is usually harvested 10 days after planting the spawn. The first flush normally provides three or four successive days of harvesting and produces 85 to 90 % of the expected yield. During the next 3 to 5 days (the rest period), additional water may be added to the substrate. Stable conditions must be maintained in the growing room during the period. Spraying with superfine mist will maintain the desired humidity in the growing rooms and protect the substrate form excessive drying. The temperature can be maintained at the appropriate level by opening or closing the ventilators. The second flush may be also provide 2 or 3 days of harvesting, but the yield is lower (10 to

15% of the total yield). *V. volvacea* is best harvested at the button (egg-shaped) stage when the volva or the universal veil has not broken (K. P. Singh, 2009).

2.1.5 Parameters Affecting the Cultivation Process for *Volvariella volvacea*

2.1.5.1 Carbon to Nitrogen Ratio

The study shows that *Volvariella volvacea* can utilize cellulose materials more effectively than other cultivated mushrooms and the optimum C:N ratio is 40 to 60 (Miles & Chang, 2004). However, another study carried out shows that *V. volvacea* showed a marked preference for asparagine nitrogen with optimum nitrogen supply, cellulolysis was higher at a C:N ratio of 24 to 36 (Fasidi & Akwakwa, 1996). While another study found that a C: N ratio of 60:1 was optimum for the basal medium. However, when 0.5 % yeast extract was added, the maximum growth was at a ratio of 80:1, which was also the maximum ratio tested. It is deduced that the optimum C: N ratio is determined as much by the other components of the substrates as by the primary sources of C:N ratio for *V. volvacea* is about 40 to 60 (Diamantopoulou *et al.*, 2016). Table 2.2 shows the major forms of carbohydrate in three popular substrate for *V. volvacea* mushroom cultivation

Table 2.2: Major forms of carbohydrates in three popular substrates for *V. volvacea* mushroom cultivation (Miles & Chang, 2004).

	Cotton Waste	**Paddy Straw**	**Banana Leaf**
Total N	1.22	0.61	1.71
Total C	49.94	54.26	50.52
C:N	40.90	84.00	29.50
Hemicellulose	8.73	17.11	19.95
Cellulose	56.76	29.68	10.85
Lignin	10.47	12.17	18.21

2.1.5.2 Moisture Content

Water, one of the prime factors which can impact the mushroom growth. Water is needed to transport the nutrients to fruiting bodies from the mycelium. However, excess watering will cause the mycelium to suffocate thus causing lack of oxygen transfer in and out of the mycelium. Moreover, excess moisture content also capable of inducing the competing molds to grow and causes contaminations. Thus the most suitable moisture content is ranged between 50 % - 75 % in substrate (Bellettini *et al.*, 2015).

2.1.5.3 Temperature

The optimal temperature for fruiting *in V. volvacea* is 28 to 30°C and is generally lower than the optimal temperature for mycelial growth (34 to 36°C). However, the cultivation of mushroom in a controlled environment is unsuitable in term of cost. Yet, the suitable temperature in the range is required to have a stable yield of mushroom.

2.1.5.4 Substrate Composition

The substrate composition can be altered based on the suitability and compatibility of the substrate. The common ratio for cultivation is 80:20 or 20:80 of two different substrate (Apetorgbor *et al.*, 2015).

2.1.5.5 pH

pH differs for different stages of growth. For example, mycelium grows the best at pH 4.0 – 7.0. Meanwhile, formation of basidiocarp is optimum at pH 3.5 – 5.0. It was reported that the optimum pH is between 6.5 – 7.0 for mycelium growth and fruiting body growth (Bellettini *et al.*, 2015).

2.2 Paddy Straw (PS)

2.2.1 Total Production of Paddy Straw Wastes in Malaysia

Malaysia is one of the largest producer of paddy in south east countries, therefore there are abundant amount of paddy straw wastes generated each year. Table 2.3 shows the total paddy straw wastes generated in Malaysia in the year 2011.

Table 2.3: Total generation volume of paddy straw wastes in Malaysia, 2011 (Shafie *et al.*, 2014)

Paddy straw waste	Paddy production (tonnes)	Paddy straw production (tonnes)
Perlis	232,674	174,505
Kedah	878,430	658,822
Penang	144,613	108,459
Perak	323,445	242,583

2.2.2 Utilization of PS as Mushroom Cultivation Medium

Mushroom utilizes carbon, nitrogen and inorganic compounds, as its food source to grow and the main nutrient needed are carbon source such as cellulose, hemicelluloses and lignin. While PS contains cellulose, hemicellulose and lignin which is used as media for mushroom cultivation.

Studies carried out shows that PS has the potential to utilize as an alternate substrate for the cultivation of mushroom. In Malaysia, where PS wastes are abundant is found that mushrooms can be cultivated by using PS and rubber wood sawdust (RSD) as cultivation medium of *Volvariella volvacea* (Apetorgbor *et al.*, 2015b)

2.3 Total Lignocellulosic Content of Paddy Straw (PS)

Based on the findings of Shawky and Garay (Shawky *et al.*, 2011; Garay M *et al.*, 2009), Table 2.4 is the comparison study of total lignocellulosic content of paddy straw.

Table 2.4: Comparison of total lignocellulosic content of paddy straw

Type of Substrate	Extractives (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)	Reference
Paddy Straw	8.88	28.00	32.15	19.64	11.33	Shawky *et al.*, 2011
Paddy Straw	9-14	23-28	43-49	12-16	15-20	Garay *et al.*, 2009

2.4 Medicinal Values of Straw Mushroom

Specific biochemical or bioactive compounds such as polysaccharides, glycoproteins, low molecular weight proteins, tri-terpenoids and immunomodulating compositions which present in mushrooms are responsible for enhancing human health in different ways. Studies have shown that mushrooms can boost health by strengthening immune system, reduce the risk of cancer by inhibiting tumour growth, fight off harmful viruses, bacteria and fungi and help in detoxification in human (Singh *et al.*, 2011)

As for the medicinal values of straw mushroom, a toxin called Volvatoxin A is a cardiotoxic protein isolated from the lectin in the mushroom. This toxin have been proven to decrease hemolytic activity towards red blood cell of Group O. Nevertheless, the lectin self-possessed a few subunits with molecular weight 24,000, 26,000 and 50,000. Lectin with molecular weight 24,000 and 50,000 are proven to be more effective on decreasing the hemolytic activity towards red blood cell of Group O, while lectin with molecular weight 26,000 displayed hemagglutination toward Group O red blood cells(Singh, 2009; Ding *et al.*, 2001). Cardiotoxic protein can be isolated from toad hearts and is proven

quite potent and lethal because at a dosage of 0.1 mg/ml, cardio ventricular systolic arrest can be induced (Bilal *et al.*, 2010; Lin & Chou, 1984).

2.5 Design of Experiment

Design of Experiment (DoE) is an application from Design-Expert® software which is widely utilized in various research fields to screen and optimize the studies. DoE is a systematic application which can be used to screen and optimize the factors affecting the designated responses. The data obtained can be analyzed using the Analysis of Variance (ANOVA) which is a function to numerically score the important factors to produce an optimum output (response) (Yang & El-Haik, 2008).

2.5.1 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) utilizes combination of mathematical and statistical knowledge to build a model based representation on the data gained from factors (input variable) and response (output variable). The aim of RSM is to reduce the cost by reducing number of run of expensive experimental analysis. In finite element method and RSM helps to cancel out their noise effects as noise is considered as an error. RSM is also used to create an approximate model function that can be used to find the relationship between the independent variable. In order words, RSM is used to build a bridge that connects all the independent variables (factor). Turning parameters are the regression coefficient involved in the approximation model and are estimated by reducing the sum of squares errors. After an approximation model is obtained, goodness of fit will determine whether the solution is suitable or not. If it is not, then approximation model is started all over again and further experiment are carried out until a satisfied model is achieved (Gunst, 1996).

2.5.2 Box-Behnken Design (BBD)

Response Surface Methodology (RSM) is one of the pillar in the DoE software which mainly being used for the optimization studies. Meanwhile, Box-Behnken Design is a type of RSM which is used to minimize the range of the parameters in order to produce optimal output. The factors that previously screened were used to optimize in order to minimize the wastage of chemicals, labor work and time. This will eventually will lead to the better cost efficient research work and more précised study and reproducibility.