A PRACTICAL GUIDE TO MUSHROOM PHARMING

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SEAMEO BIOTROP
CENTRAL LUZON STATE UNIVERSITY 1907

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FOREWORD

This book was written in response to the need of the mushroom industry to have a simple, comprehensive and practical guide on spawn and mushroom production. It consists of the basic and applied aspects of mushroom science and technology. In the first part of the book, the readers are introduce to the world of mushrooms particularly their structure, reproductive biology, biological classification and nutrition. It also provides information regarding the nutritional composition and medicinal attributes of mushrooms. The different species of mushrooms that can be cultivated on various lignocellulosic substrates are also highlighted. The latter part presented the detailed procedure in the preparation of culture media, grain spawn production, and substrate preparation, problems in mushroom production and recommended solutions and economics of mushroom production.

The book goes beyond the traditional knowledge that mushrooms are only regarded as culinary ingredients. The information presented confirms that mushrooms are excellent sources of nutraceuticals. The authors were able to structure and design the book in such a way that even those who are not mushroomologists can understand and easily follow the procedure in mushroom pharming.

This book emphasizes the importance of science behind the art of mushroom pharming.

Dr Irdika Mansur M.For.Sc.
Director of SEAMEO BIOTROP

FOREWORD

As President of the Central Luzon State University, I take pride in the publication of the book entitled *A Practical Guide to Mushroom Pharming*. This book is a clear manifestation that when intelligent people joined together, a scientific masterpiece is formed. It is a product of a very productive scientific collaboration between scientists from the Philippines and Japan with logistics support from SEAMEO BIOTROP and the Central Luzon State University, Philippines.

This book serves as a guide to mushroom pharmers as they adopt the different technologies contained in this book. As you read this book, you can feel that science is working within the art of mushroom pharming. Guided by pictures, procedures are explained clearly in a practical way that even a non-mushroomologist can easily understand and follow. The technical, financial and culinary aspect of mushroom pharming are emphasized in this book.

Let us be mushroom pharmers and let us make pharming an enjoyable and lucrative undertaking!

Tereso A. Abella
President
Central Luzon State University
Philippines

PREFACE

We always say that that there should be science into the art of mushroom pharming activities in order to ensure technical sustainability. This book serves as a practical guide to mushroom pharmers (mushroom growers, development workers and researchers). Every activity in mushroom pharming is presented in pictorial style so that the readers can easily follow the steps. The procedures have been defined and simplified in order to be more popular even to non-mushroomologists. The technologies contained in this book that are products of the scientific collaborative works of the authors can be adopted by mushroom pharmers in the tropics. We intend to bring the reader to the fundamental understanding of the biology and nature of mushroom. Aside from being introduced to the different types of mushrooms, the reader will have knowledge about the nutraceutical attributes of mushrooms. The fundamental requirements in establishing a typical mushroom pharm are emphasized as well as the general flow of mushroom pharming. Upon reading this book, we envisioned that the development of the skills of mushroom pharmers on tissue culture, preparation of culture media, isolation into pure culture and sub-culture, preparation of grain spawn, planting and fruiting spawn as well as production of fruiting bodies will be enhanced. In order to further guide the mushroom pharmers, identification of technical problems is presented. We also present a simple cost and return analysis in establishing a mushroom business. The reader will also be introduced to the different recipes with mushroom as a main dish or side dish. In the last part of the book, mushroom spent is used as the main ingredient in the preparation of compost for organic production of crops.

The Authors

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1 INTRODUCTION

1.1 Mushroom as an Organism


Fruiting bodies of *Pleurotus cornucopiae*

Based on the Cavalier-Smith eight kingdom system of classification, organisms can be divided into three domains; i.e. Domain Archaea, Domain Bacteria and Domain Eukarya. As eukaryotic organisms, mushrooms are classified under Kingdom Fungi of the Domain Eukarya together with Kingdoms Plantae, Animalia, Stramenophila or Chromista, Protoctista and Archezoa. Mushrooms are multicellular, filamentous, eukaryotic organisms where the cell walls are generally composed of chitin. Although considered as relatives of plants, mushrooms are not photosynthetic but rather absorb nutrients from the substratum. Absorption of nutrients is possible due to their ability to secrete extracellular enzymes into the substrates.

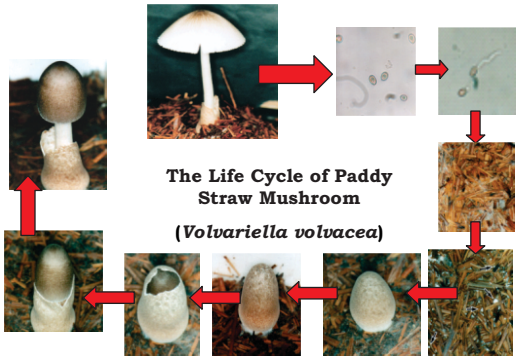
Mushrooms being ubiquitous in nature are known to produce visible fruiting structures which may be epigeous (above ground) or hypogeous (below ground). They may be classified under either Phylum Ascomycota or Basidiomycota. Ascomycetous fungi, also known as sac fungi, are generally known to produce sexual spores called ascospores which are contained in a sac-like structure called ascus (plural: asci). Basidiomycetous fungi also known as club – fungi on the other hand have the ability to produce sexual spores called basidiospores which are attached on a club-shaped structure called basidium (plural: basidia).

Mushrooms which are under Phylum Ascomycota belong to Class Discomycetes (mushrooms with disc or cup-shaped fruit body). These mushrooms are commonly called cup fungi due to their ability to produce asci at the surface of fleshy cup-shaped ascocarps. Ascocarp is the general term for the fruiting body of Phylum Ascomycota. Specifically, it is called apothecium (plural: apothecia). In Class Discomycetes, there are two orders which have edible mushrooms. These two orders are Pezizales and Tuberales. Mushrooms under Order Pezizales are characterized to have covered asci with epigeous ascocarp whereas those under the Order Tuberales have hypogeous

ascocarp. Popular genera under Order Pezizales include *Morchella* and *Peziza*; and *Tuber* under Order Tuberales. On the other hand, most edible mushrooms are found under Classes Hymenomycetes and Gasteromycetes of Phylum Basidiomycota. Mushrooms under Class Hymenomycetes produced basidia in definite layers called hymenium while those under Class Gasteromycetes, the basidia which are typical to *Dictyophora* are produced in an internal hymenium on puffball-type fruiting bodies. Mushrooms of Class Hymenomycetes are further subdivided into two sub-classes namely Phramobasidiomycetes and Holobasiodiomycetes. The Phragmobasidiomycetes are known to possess basidia with cross walls or septa whereas the Holobasidiomycetes have basidia without cross walls. There are two orders under sub-class Phragmobasidiomycetes where edible mushrooms are found. These are Order Tremellales with the genus *Tremella* and the Order Auriculariales with the genus *Auricularia*. The subclass Holobasidiomycetes contains the biggest group of edible mushrooms with two orders namely Order Aphyllophorales in which the hymenium is lining the surface of small pores or tubes and Order Agaricales where the members have hymenia on radiating gills called lamellae. Common genera in the Order Aphyllophorales include *Ganoderma* and *Coriolus.* Other genera of Order Agaricales which have both edible and poisonous species are *Panaeolus*, *Amanita*, *Lepiota and Agrocybe*.

1.2 Basic Structure of Mushroom

Mushrooms differ from other eukaryotic organisms on the basis of the nature of cellular level of organization, mode of nutrition, ultrastructure, biochemical composition of cell wall and molecular profile. The cell walls are generally made up of chitin which is also the chemical constituent of the integument of most insects. Unlike plants, their stored food is glycogen while plants have starch as their stored food. In nature, mushrooms being ubiquitous may act as parasites, saprophytes and symbionts. These groups of fungi are widely known for their ability to produce visible fruiting bodies.



Mushrooms are filamentous multicellular fungi where their vegetative structures are tubular. These tubular and filamentous structures are called hyphae. A hypha (singular for hyphae) originates from the germination of the basidiospore or ascospore. The hypha continuously grows and forms a vast network of filaments called mycelium (plural: mycelia). This network which becomes visible to the naked eye as cottony whitish or dirty whitish growth then permeates the cellulosic substrates. A mycelium may be primary if it originated directly from the germinating spore. A primary mycelium is considered monokaryotic or haploid if it contains only half of the chromosome complement normally designated as n. However, when two compatible primary mycelia fused together through hyphal anastomosis or fusion, a dikaryotic or diploid (2n) secondary mycelium is created thus giving rise to fruiting bodies. In most mushrooms under Phylum Basidiomycota, secondary mycelium can be characterized by the presence of clamp connection in between two compartments within the filament. The presence of clamp connection is an indication that dikaryotic hyphae have been formed. When there is shift in the physical parameters for growth (i.e. from mycelial proliferation to fruiting body formation), pin – head like masses are formed. These masses are called fruiting initials or primordia which are technically referred to as sporocarps (spore containing structures). These primordia eventually develop into structure commonly known as mushroom fruit. Simply speaking, the mycelium is the vegetative stage and the mushroom fruit the reproductive stage.

Fruiting bodies of *Agaricus bitorquis* showing its annulus

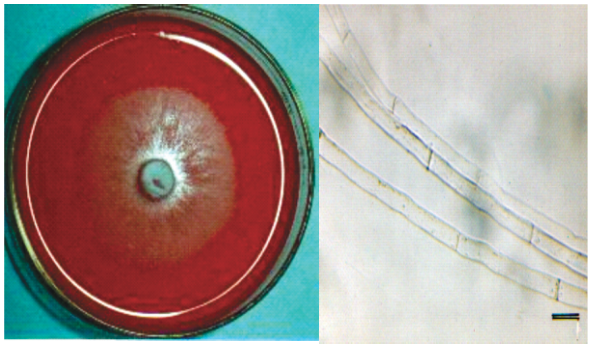
In the case of mushroom under Phylum Basidiomycota, the mushroom fruit which is called basidiocarp is entirely made up of branched differentiated mycelia. The basidiocarp is composed of a cap or pileus, gills or lamellae, stalk or stipe, sometimes a volva or base tissue and ring or annulus. There are genera where the basidiocarp is initially surrounded by a layer of tissue cover called veil. Veil can be universal if it fully covers the entire fruit as in the case of *Volvariella* or partial as in *Agaricus*. Other most common mushrooms do not have veils such as *Pleurotus*, *Lentinula*, *Flammulina* and others. As the basidiocarp develops, the veil ruptures. If the veil is a universal type, it is usually left at the base and thus called volva. However, when the veil is left on the stalk and has formed ring or annulus, it is called partial type. Different species of *Agaricus* produced rings. Also common among Order Agaricales of Phylum Basidiomycota are radiating gills at the ventral side of the cap. These gills, which are also called lamellae, are technically known as hymenophore. When seen under the microscope, the lamellae are made up varied types of cells. Basidia which are club-shaped and bear basidiospores are found on the lamellae. It is in this basidium where nuclear processing happens thus resulting to the formation of 2 to 4 haploid basidiospores which are normally found at the tip of the basidium. Since the basidiospores are found at the tip of the basidium, these fertile structures are easily detached and are blown by the wind current. The basidiospores are resistant structures that hibernate when the physical conditions become unfavorable (i.e. insufficient moisture, high temperature.

During the onset of rainy season in the tropics or when moisture coupled with optimum temperature in the presence of appropriate substrate are prevailing, the spores germinate into germ tube and transformed into mass of hyphae and ultimately form fruits. A 4-cm mushroom cap produces as many as 20 million spores within an hour. Spores of mushroom vary in shape and size depending on the genus. The stipe that holds the cap with millions of spores underneath is usually elevated from the ground thus facilitating the dispersal of the spores by wind. This fast and easy dissemination of the spores ensures the perpetuation of the species from season to season. The mushroom fruit is also naturally designed to protect the gills that hold the spore from the damaging effects of rain, wind current and solar exposure. Please bear in mind that not all mushrooms under Phylum Basidiomycota have the spore bearing gills or hymenophore. For instance, mushrooms under Order Aphyllophorales do not form gills but produce pores or tubes comparable to a honeycomb where the basidia are found in the inner surfaces. Typical examples are shelf or bracket fungi such as *Ganoderma*, *Coriolus* and *Boletus*. Also, *Cantharellus* has a funnel-shaped fruiting body with folds that resemble gills on the underside. Likewise, *Hydnum* has a dentate hymenophore. Gasteromycetes

like *Dictyophora* possesses internal hymenophore which is locked in a closed fruiting body. Its peridium which serves as a protective envelope covers the spores which are only dispersed upon maturation.

1.3 Reproductive Biology of Mushroom

Mushroom can reproduce sexually and asexually. However, sexual reproduction generates variation within the species. Understanding fully the sexuality of a particular mushroom will ensure the sustainability of the species under changing physical and nutritional conditions thus leads to an efficient and effective strain maintenance and development programs. The biology of any mushroom starts with the formation of sexual spores. There are three major events in spore formation namely: plasmogamy, karyogamy and meiosis. Plasmogamy is a process of protoplast fusion; karyogamy on the other hand is the fusion of two different nuclei. Meiosis is a reductional division of nuclei resulting to the formation of haploid nuclei. When these three events are completed, the nuclei migrate to the spore cells in the basidium to become the basidiospores. The produced basidiospores may be self-fertile like in *Volvariella* that can produce mushroom fruit upon germination and mycelial colonization or self-sterile which can not produce a mushroom fruit unless a compatible spore fuses depending on the species. Mushrooms with self-fertile spores are called homothallic and the self-sterile are heterothallic**.** In homothallic species, the spore may germinate to form the mycelia and ultimately produce a fruit body. Hyphal fusion or mating is not necessary among homothallic species before a fruiting body is formed. There are two types of homothallic mushrooms, the primary and the secondary. A primary homothallic mushroom has uninucleated spore (i.e. single nucleus in a spore)**.** *Volvariella volvacea* is a typical primary homothallic mushroom. A secondary homothallic mushroom, on the other hand is characterized by having a binucleated spore resulting from the movement of the two compatible nuclei into a single basidiospore during spore formation. Compatibility control or a



Typical cultural character of mushroom on the left and hyphae of *Volvariella volvacea* on the right

mating system operates in secondary homothallism. Remember that four haploid nuclei are produced after meiosis.

Thus if the four post-meiotic nuclei are marked arbitrarily with the following labels: Rr1,Rr2, Ry1 and Ry2, then the spores of the following nuclear assemblage are possible: Rr1Rr2; Rr1Ry1; Rr1Ry2; Rr2Ry1; Rr2Ry2 and Ry1Ry2. Only those spores bearing both Rr and Ry labels (alleles) or are heterozygous will produce fertile mycelia; spores homozygous for Rr (Rr1Rr2) or Ry (Ry1Ry2) will give rise to sterile mycelia. Thus, in secondary homothallism, a mating type system is always necessary. The two compatible meiotic nuclei penetrate a single basidiospore. Normally, only two binucleated basidiospores are present per basidium. This is typical to *Agaricus bisporus* being a secondary homothallic mushroom. On the other hand, heterothallic mushrooms produce four uninucleated self-sterile basidiospores. Thus in this condition, it is necessary for two compatible monokaryotic mycelium (spore that gave rise to a mycelium) to mate before a fruit body is formed. A different mating system operates in heterothallic mushrooms. There are two types of heterothallism; the bipolar and tetrapolar. In bipolar heterothallism, a compatibility gene normally designated as "A" controls this type**.** In order to be compatible, two monokaryotic mycelia should bear both the A gene. This type of bipolar heterothallism is common to *Pholiota nameko* and *Auricularia auricula*. Two unlinked AB genes control the tetrapolar heterothallism. Thus, compatibility happens on mycelia carrying the same mating type genes. The following mushrooms exhibit tetrapolar heterothallism: *Lentinula edodes, Pleurotus ostreatus, Flammulina velutipes, Pleurotus sajor-caju, Coprinus comatu* and *Auricularia polytricha*.

Fruiting bodies of *Ganoderma lucidum* showing the pores (upper right photo) and *Termitomyces* with its gills (lower right photo)

1.4 Mode of Nutrition

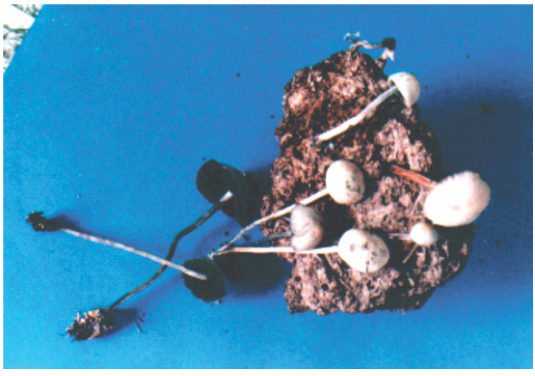
Mushrooms are ubiquitous in nature. As heterotrophic organisms, they can be found growing on cellulosic substrates, roots of living trees or may be found associated with other living organisms. Thus, they are generally grouped as parasitic, saprophytic and symbiotic.

Parasitic mushrooms

Only few edible mushrooms are pathogenic to living host plants. As such, these mushrooms are not recommended for commercial cultivation due to their parasitic nature to host plants. Popular edible mushroom which is a plant pathogen is *Armillaria mellea*, the honey-colored mushroom which causes root rot of trees.

Saprophytic mushrooms

Majority of the commercially cultivated mushrooms are saprophytes i.e. they are dependent on dead organic matter for their nutrition. They grow on varied substrates ranging from decomposed plant and animal materials. Saprophytic mushrooms can be divided into different groups according to the kind of substrates they attack. Saprophytic mushrooms: can be classified as lignicolous, humicolous and coprophilous. Lignicolous mushrooms grow on substrates rich in lignin which are generally wood inhabiting. These mushrooms include different species of *Pleurotus*, *Ganoderma*, *Coriolus*, *Schizophyllum commune, Auricularia* and *Lentinula edodes* that grow on dead wood and fallen logs. Humicolous mushrooms or humus-inhabiting are mushrooms that grow on decomposing leaf-based plant residues. These include species of *Volvariella*, *Dictyophora* and *Collybia* which grow on decomposing piles of rice straw and leaves of trees. Coprophilous mushrooms or animal dung-inhabiting are mushrooms that normally grow on dried animal dung which has been moistened. Mushrooms under this type include species of *Agaricus* and *Coprinus.* In nature, saprophytic mushrooms are commonly found in the forest, lawns and gardens.



Fruiting bodies of *Paneolus sp* on carabao dung

Symbiotic mushrooms

There are two types of symbiotic mushroom based on the type of habitat that they prefer: living root – associated and termite- associated. Mycorrhizal mushrooms are found living into the roots of most flowering plants without inflicting injury to the plant. Once infected, the feeder roots are transformed into unique morphological structures called mycorrhizae and form mutually beneficial relationship with the roots of the plants. The roots provide the mushroom with moisture and organic compounds while the mushroom facilitates the absorption of different nutrients and are believed to increase the resistance of the plant to diseases. Edible mushroom which are mycorrhizal include *Tricholoma matsutake, Amanita caesarea*, species of *Cantharellus, Boletus* and *Tuber.*

In the tropical countries, termite – associated mushrooms are very common especially during rainy season where moisture is abundant. Genus *Termitomyces* inhabit the core of the termite mound which is characterized to be sponge – like in appearance. This sponge – like structure which serves as food of termites is made from minute pellets of chewed leaves which has been acted upon by the mycelia of *Termitomyces*. Once the lignified tissues of leaves have been simplified by the mushroom, the termites can assimilate free cellulose for their nutrition.

1.5 Nutritional Contents of Mushrooms

Mushrooms are considered as special foods especially during the earlier times where cultivation technologies were not yet available. Earlier people rely only on wild species for food, medicines and source of strength. For instance, the Greeks ate mushrooms before going to war because they believed that mushrooms can provide physical strength. Some, like the Romans revere mushroom as food of the gods and the pharaohs of Egypt regarded mushroom as a delicacy. If the Chinese considered mushroom as a source of life, the earlier Mexicans on the other side, used mushroom in witch crafting and religious ceremonies. Perhaps, they had used the hallucinogenic mushrooms! The beneficial uses of these mushrooms are being exploited in the modern world as drugs.

Mushrooms are consumed because of their good flavor and nutritional value. Though preference of mushrooms is based on palatability by consumers may be subjective, this may be judged based on the color, texture, flavor, and taste. The nutritional status of mushrooms is definitely precise because this is based on scientific investigation**.** Proximate analysis of the nutritional composition coupled with the determination of the functional activities of mushrooms is important parameters in establishing the nutritional status of

mushrooms. The nutritional composition and functional activities of a certain mushroom may vary depending on species, strain, stage of development, geographical location, nature of substrate used during cultivation and post harvest processing. Because of the previously mentioned factors, the nutritional composition of certain mushroom may fall at certain range.

Mushrooms are considered both as nutriceuticals and functional foods. A food is said to be functional if it can provide the body with the required amount of vitamins, fats, proteins, carbohydrates and minerals which are necessary for healthy living. Thus, when a functional food can prevent or treat diseases or disorders, it is referred to as nutriceutical. Several studies have been conducted in the US, Japan and China on the medicinal effects of mushrooms to humans. These studies have clearly established the nutriceutical position of mushroom.

Similar to other countries, in the Philippines mushrooms are favorite culinary ingredients because they contain varying amount of different nutrients such as proteins, amino acids, carbohydrates, fats, fibers, vitamins, minerals and nucleic acid. The amount of these nutrients present in the fruiting bodies may vary from species to species and the substrate used in growing. Moreover, mushrooms have distinct aroma and unique taste.

Carbohydrate

One of the main components of mushroom fruiting bodies is carbohydrates. Carbohydrates are the primary source of energy of the body. Breakdown of this compound will produce energy in the form of adenosine triphosphate (ATP) that will be used in different cellular activities. Generally, mushrooms contain high amount of carbohydrates. Most of the carbohydrates in mushrooms are non-digestible carbohydrates including oligosaccharides such as trehalose and cell wall polysaccharides such as chitin, β-glucans and mannans (Cheung 2013). On a dry weight basis, *Pleurotus* species contains carbohydrates, ranging from 46.6 to 81.8%, *Agaricus bisporus*, 60% and *Schizophyllum commune* contains 59.56%. Carbohydrates from mushrooms may be in the form of pentoses, methylpentoses, hexoses, disaccharides, amino sugars, sugar alcohols and sugar acids. Lately, the anti tumor properties of the water soluble polysaccharides produced by mushrooms have been documented. For instance, schizophyllan produced by *S. commune*, lentinan by *Lentinula edodes* and collyban by *Collybia confluens* were reported to suppress tumorous cells.

Fibers

Mushroom fruiting bodies also contain a considerable amount of fiber. The crude fiber contents of *Pleurotus* species ranged from 11.99% to 17.48% (Kalaw and Albinto, 2015) while *Volvariella volvacea* contain 5.9 to 6.1% crude fiber (Eguchi *et al*., 2015). Moreover, *Calocybe indica* and *Agaricus bisporus* contain 13.2% and 18.23%, respectively (Pushpha and Purutshothama, 2010). Higher fiber content was reported in *Agaricus bisporous* (18.23%), *Pleurotus florida* (23.18%), *Rusula delica* (15.42%) and *Lyophyllum decastes* (Amabye and Bezabh, 2015).

Protein

Proteins are macromolecules consisting of a long chain of amino acids. Usually, the protein requirements are obtained from meat and legumes. Proteins are important in different biological processes of the body. In general, mushrooms are very good sources of non-animal based protein. The fruiting bodies of mushrooms contain high amount of proteins. Depending on species, protein content may range from 19 to 35% (dry weight basis). For instance, Kalaw and Albinto (2015) reported differences in protein content of the different *Pleurotu*s species namely: *Pleurotus cystidiousus* (16.95%), *Pleurotus florida* (22.89%), *Pleurotus sajor-caju* (21.80%), *Pleurotus ostreatus* (22.06%) and and *Pleurotus pulmunarius* (27.93). Likewise, variation in the amount of protein in different stages of development of *Volvariella volvacea* was also reported by Eguchi *et al*. (2015). They noted highest protein content in fully expanded fruiting bodies (38.9%) while the lowest was observed in egg shaped stage (32.9%).

Fruiting bodies of mushrooms contain standard and non-standard amino acids. The standard amino acids consists of essential and non-essential amino acids. Essential amino acids are amino acids that are not synthesized by the body and therefore should be present in the diet. On the other hand, non-essential amino acids are those that are produced by the body. Based on studies conducted both essential and non-essential amino acids are present in mushroom fruiting bodies. For instance, *Schizophyllum commune*, *Lentinus tigrinus*, *Lentinus sajor-caju*, and *Pleurotus florida* contain nine essential amino acids such as methionine, phenylalanine, threonine, histidine, leucine, lysine, isoleucine, valine and tryptophan and 12 non-essential amino acids namely: glutamine, glutamic acid, serine, aspartic acid, serine, asparagine, glycine, alanine, cysteine, tyrosine, arginine and proline in different concentration (Reyes *et al*., 2013). Moreover, all the essential and non-essential amino acids were found on the fruiting bodies of *Volvariella volvacea* (Eguchi *et al*., 2015). Glutamic acid is used as food enhancer in the food industry is also present on

most species of mushrooms. Non-standard amino acid such as ornithine and γ aminobutyric acid (GABA) which are proven to exhibit anti-hypertensive activities in animal experiments are also occurring in most mushrooms.

Fat

Mushroom fruiting bodies contain small amount of fat compared to carbohydrates and proteins. The fats present are dominated by unsaturated fatty acids (Wani and Wani, 2010). Mushrooms do not contain cholesterol but instead produce enzymes that prevent cholesterol biosynthesis in the body. For instance, *S. commune* produce schizostatin, a potent inhibitor of squalene synthase which is an enzyme involved in the biochemical pathway of cholesterol and triglycerides biosynthesis. Like any other plant-based commodities, mushrooms generally contain all classes of lipid compounds including free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters, and phospholipids.

The crude fat present in *Pleurotus* species ranged from 0.9% to 1.40% (Kalaw and Albinto, 2015); *Agaricus bisporus*, 2.12%; *Pleurotus florida* 1.54%; *Rusula delica*, 5.38%; and *Lyophyllum decastes*, 2.14% (Amabye and Bezabh, 2015).

Vitamins

Vitamins are organic nutrients needed by the body in small amount for normal growth and metabolic activities. Cultivated mushrooms were found to be good sources of vitamin B_2, niacin, and folates, with contents varying in the ranges 1.8 to 5.1, 31 to 65, and 0.30 to 0.64 mg/100 g dry weight, respectively (Mattila *et al*., 2001). Mushrooms generally contain vitamins where their forms and quantity vary depending on the species and strains. For instance, the thiamine content (mg per 100 gram dry weight) of *V. volvacea* is 0.35, 7.8 in *L. edodes*, and 1.14 in *A. bisporus* and 1.16 to 4.8 in *Pleurotus* spp. The niacin content (mg per 100 gram dry weight) of most mushrooms also varies; 54.9 in *L. edodes* 64.88 in *V. volvacea*, 55.7 in *A. bisporus* and 108.7 in *Pleurotus* spp. Among the cultivated mushrooms, *A. bisporus* has the highest riboflavin content having 5 mg per 100 gram dry weight followed by *L. edodes* (4.9 mg) and *V. volvacea* (1.63-2.98 mg). Several vitamins have also been reported in *Volvariella volvacea* such as thiamin, riboflavin and niacin (Eguchi *et al*., 2015).

Minerals

Minerals are simple inorganic nutrients which are also needed by the body in small quantity. Mushrooms are also good sources of minerals. The

potassium, phosphorous, sodium, calcium and magnesium contents constitute about 56 to 70% of the total ash. Potassium is the most abundant which accounts for nearly 45% of the total ash content. Minerals such as potassium, phosphorous, calcium sodium iron, zinc, copper, manganese, and selenium are present in *Lentinula edodes, Lentinus cladopus, Pleurotus florida* and *Pleurotus djamor* (Mallikarjuna *et al*., 2013). *Pleurotus florida*, *Pleurotus pulmonarius*, *Pleurotus salmoneostramineus* and *Pleurotus cystidiosus* contain magnesium, phosphorous, sulfur, chlorine, iron and potassium (Umagat *et al*., 2016). Magnesium, potassium, sulfur and phosphorous were also present in the fruiting bodies of *Pleurotus djamor* (Reyes *et al*., 2016) Different stages of fruiting body development of *Volvariella volvacea* contain calcium, phosphrous, iron, sodium, potassium (Eguchi *et al*., 2015) Alam *et al*. (2008) reported that calcium, iron, zinc, magnesium, manganese, selenium and arsenic are present in *P. florida*, *P. sajor-caju, P. osteratus* and *Calocybe indica*

Nucleic Acid

Since nucleic acid is an important biomolecules in a living system, fungi also contain this important component. Generally fungi including mushroom contain 3.2 – 4.7%. Among the commercially cultivated mushrooms, *P. sajor-caju* possesses the highest nucleic acid content (0.51% on a weight basis). Thus, it is still safe to eat 400 grams of fresh *P. sajor-caju* daily. Nucleic acid is associated with the high plasma concentration of uric acid. The limit for the daily intake of mushroom could even be beyond this amount for other mushrooms with lower nucleic acid content. Thus the tolerable content of nucleic acid in mushroom is not a limitation for its use as a daily vegetable. In fact, mushrooms such as *Agaricus blazei* exhibits anti arthritic activity. Excess nucleic acid in the body system may lead to the precipitation of uric acid in tissues and joints thus resulting to stone formation in the kidney and bladder.

Functional and Nutraceutical Activities of Mushrooms

Apart from being a nutritious culinary ingredients, mushrooms are recently considered as a nutriceutical food with functional activities Mushrooms exhibit different functional activities such as antioxidant, antibacterial, platelet aggregation inhibition, inhibition of angiotensin converting enzymes, anti-hypertensive activity and hypoglycemic activity (Eguchi *et al*., 2015; Kalaw and Albinto, 2015; Dulay *et al*., 2014; Reyes *et al*., 2016; Dulay *et al*., 2014).

Compounds isolated from *Pleurotus djamor* have reported anti-tumor and antioxidant activity (Ragasa *et al*., 2016). The ethanolic extract of fruiting

bodies of *Lentinus tigrinus*, *Coprinus comatus* and *Pleurotus cystidiosus* exhibited antibacterial activities against *Staphylococcos aureus* (Dulay *et al*., 2014; Kalaw and Albinto, 2015). Rai *et al*. (2005) reported that *Lentinula edodes* can lower both blood pressure and free cholesterol in plasma as well as accelerate accumulation of lipids in liver by removing them from circulation. Polysaccharide extracts from higher basidiomycetes mushrooms showed potent antitumor activity against sarcoma, mammary adenocarcinoma, leukemia and a host of other tumors (Daba and Ezeronye, 2003). Wild mushrooms from India exhibited potent antioxidant, anti-inflammatory, and antimicrobial activities under assay conditions (Khaund and Joshi, 2015). *Schizophyllum commune*, *Lentinus tigrinus*, *Lentinus sajor-caju*, *Ganoderma lucidum*, *Pleurotus florida* and *Volvariella volvacea* exhibited anti-platelet coagulation and anti-inflammatory activities (Reyes *et al*., 2013). Moreover, *Lentinus tigrinus* also showed anti-diabetic properties (Dulay *et al*., 2014).

2 MUSHROOM PHARMING FOR A LIVING

2.1 Species of Cultivated Mushrooms

Mushroom production is already a very established industry in developed countries and is becoming a growing industry in developing and least developed countries. Though it started as an agri-based industry where mushroom was considered as food on the table, its use has already been realized in other non-agri based industries including cosmetics, drugs and even environmental clean – up.

Asian countries in the Far East such as Japan, China, Taiwan and Korea have developed the fascination of consuming mushrooms since time immemorial with *Auricularia* as the oldest mushroom so far cultivated. Historically, the Europeans and the North Americans have enjoyed collecting mushrooms from the wild. Their fondness of mushroom has led to the development of production technologies for *Agaricus bisporus* whose production has led to a very established industry in the world today. The Chinese and the Japanese have developed the technology for the production of *Lentinula edodes* and *Flammulina velutipes*. The success of developing production technologies through extensive research and the development of efficient marketing system have paved way to increase consumption and curiosity of trying other species of mushrooms. From *A. bisporus* to *L. edodes*, the world is now enjoying other species of cultivated mushrooms!

Agaricus sp.

There are three species of *Agaricus* that are commercially cultivated in the world. These are *A. bisporus, A. bitorquis* and *A. blazei*. From these species of *Agaricus*, *A. bisporus* or popularly known as button mushroom is the most widely cultivated mushroom in the world spanning almost every continent. *A. blazei* which is known as *himematsutake* in Japan is cultivated for its medicinal attributes. It exhibits antihypertensive, antirheumatic, anti – inflammatory, anti-cancer and anti-diabetic activities. Thus, with its functional

Fruiting bodies of *Agaricus bisporus*

attributes, it is one of the highly priced medicinal mushrooms in Japan. *A. bitorquis* is a warm strain that can be cultivated in the tropical conditions on composted rice straw enriched with rice bran and dried animal dung such as carabao and cow. Composted mushroom spent of *V. volvacea* is also used for the cultivation of *Agaricus* in the tropical regions of the world.

Lentinula edodes

Lentinula edodes which is popularly known as shiitake is the second most widely cultivated mushroom in the world where commercial production is concentrated in the Far East particularly in Japan, China and Korea. This medicinal mushroom can be cultivated year-round under environmentally controlled condition on enriched sawdust-block. Traditionally, this mushroom grown on logs of Eucalyptus, Alnus and other temperate species of trees.

Fruiting bodies of Lentinula edodes on sakura logs

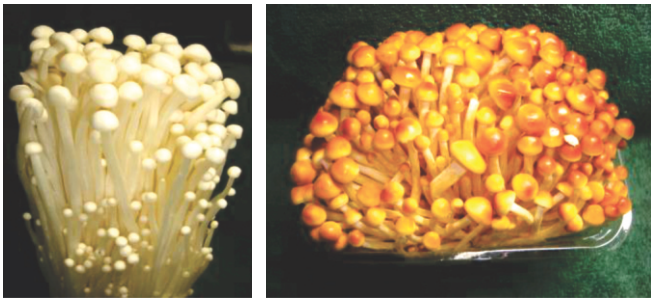
Volvariella volvacea

The most popular mushroom in tropical and subtropical regions of the world is *Volvariella volvacea*. It is commonly grown on decomposing piles of rice straw, banana leaves, cotton wastes and other lignocellulosic plant residues. Though it has lower biological efficiency compared to other mushrooms, *V. volvacea* is one of the fastest growing mushrooms. Its fruiting bodies can be harvested 14 days after spawning provided that the favorable temperature (30 to 35°C) with 65% moisture content of the substrate, under very minimal ventilation with profused light are fulfilled.

Fruiting bodies of *Volvariella volvacea*

Flammulina velutipes

This mushroom which is called as enokitake or winter mushroom is the most popular in the Japanese cuisine. It is considered as a temperate species (10-12^{o}C) of mushroom and is widely cultivated in Japan in plastic containers. Aside from being used for culinary purpose, this mushroom also exhibits an ability to inhibit the angiotensin converting enzyme. Its ability to inhibit this enzyme proves that it has antihypertensive properties.



White (left photo) and brown (right photo) strains of *Flammulina velutipes*

***Auricularia* spp.**

Historically, *Auricularia* is the first to be commercially grown. It is known for its jelly texture and medicinal attributes. Three species of *Auricularia* are commercially cultivated, namely: *A. polytricha*, *A. auricula* and *A. fuscosuccinea*. This mushroom is widely cultivated in Southeast Asia on sawdust-based formulated substrates contained in heat resistant plastic bags.

***Pleurotus* spp.**

This mushroom has the most number of commercially cultivated species and is the most widely cultivated type of edible mushrooms. These species include *P. sajor-caju, P. florida, P. cornucopiae, P. eryngii, P. nebrodensis, P. ostreatus, P. pulmonarius* and *P. cystidiosus*.

Ganoderma lucidum

Due to an extensive research regarding its medicinal attributes, this bracket fungus became popular in the US and Asia. High value products from this mushrooms are already available in the market which include Ganoderma-based tea, capsules, lotions, soap, toothpaste and others.

Pholiota nameko

P. nameko which is known as viscid mushroom or nameko in Japan is a wood rotting mushroom with a very distinct viscid or slimy cap. Like *F. velutipes,* it requires low temperature for fruiting.

Tremella fuciformis

This white jelly fungus is sometimes referred to as "silver ear" mushroom. Though popularly known in China as a medicinal mushroom, it is considered as special dessert in Thailand and other Asian countries. Large scale production of this mushroom is located in China.

Tricholoma matsutake

Like *Tuber melanosporum*, *T. matsutake* is a typical example of mycorrhizal mushroom that has symbiotic relationships with the roots of pine trees. The difficulty of cultivating this type of mushroom under artificial condition coupled with its very peculiar aroma made this mushroom as one of the most expensive mushrooms in Japan. It is usually available in the Japanese market during autumn.

2.2 Other Species

Due to extensive research on the biophysiology of wild edible mushrooms which lead to the development of production technology, a number of edible mushrooms are successfully grown on an array of lignocellulosic residues. Among these species with commercial potential include, *Coprinus comatus*; *Dictophora indusiata*; *Hypsizigus marmoreus*; *Hericium ramosum*; *Stropharia rugoso-annulata*, *Agrocybe aegerita, Collybia reinakeana, Schizophyllum commune.*

Pleurotus florida

Pleurotus cystidiosus

Pleurotus cornucopiae

Pleurotus sajor-caju

Pleurotus nebrodensis

Pleurotus pulmonarius

Commercially cultivated species of *Pleurotus*

Dictyophora indusiata

Schizophyllum commune

Lentinus sajor-caju

Auricularia polytricha

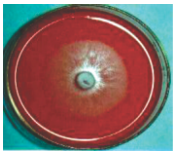
Other tropical species of mushrooms which can be cultivated for their nutraceutical properties

2.3 The General Perspective of Mushroom Pharming

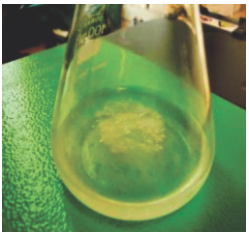
Successful mushroom growing starts from a very vigorous and healthy fruiting body. Through the proper execution of the tissue culture technique, mycelia from the tissue of the desired mushroom can be rescued in the laboratory. The rescued mycelial culture is further nurtured in the laboratory on an appropriate culture medium in its pure culture form which subsequently sub - cultured for mass production. The pure culture may be used to prepare grain spawn or may be used as inoculum in the submerged culture for metabolite production from mushroom. This may also be utilized as starting material in the lawn culture for mycelial mat production. In order to industrialize the process, grain spawn is prepared from enriched granular materials like sorghum seeds, palay seeds and even feed conditioner (a mixture of various seeds of sorghum, wheat, corn, sunflower etc.) which is commonly available in commercial feed suppliers. Depending on the type of mushroom to be grown, spawn which may either be planting spawn or fruiting spawn can be prepared from formulated cellulosic substrates such as rice straw, sawdust, tobacco midrib, sugarcane bagasse, coffee hull and other plant residues which are abundant in the locality. These formulated bagged substrates are inoculated with the preferred grain spawn in order to enhance the colonization of mycelia thus shortening the incubation period for the mycelia to fully ramify the bagged substrates and ultimately produced fruiting bodies. In mushroom business, it is necessary to shorten the incubation period and enhance the biological efficiency of mushroom. Biological efficiency refers to the ability of the mycelia of mushroom to convert cellulosic spawn substrate into fruiting bodies.

The general flow chart in spawn and mushroom production is presented in the next page.

Tissue culture



Preparation of pure culture



Production of metabolites

Preparation of grain spawn

Production of fruiting spawn

Production of planting spawn

Production of fruiting bodies

Production of fruiting bodies

General flow chart on spawn and mushroom production

2.4 Basic Requirements in Mushroom Laboratory

For spawn and mushroom production business to take off, it is necessary that the basic equipment and materials should be installed in the pharm. Since spawn and mushroom production are technical activities, a mushroom businessman should satisfy the following basic requirements:

1. Preparation room
2. Clean room
3. Working shed
   - Soaking tank
   - Pasteurizer
4. Incubation rooms
5. Mushroom growing house
6. Post harvest processing area

The Preparation Room

The preparation room is basically the clean kitchen of the mushroom pharm. It is in this area of the pharm where preparation of culture media takes place. Cleanliness and proper disposal of garbage should always be done before and after working. The size of this room varies depending on the extent of operation of the pharm but usually a 10 meter by 10 meter size is recommended. It should be equipped with central concrete laboratory table with sink, and side concrete tables. Hanging cabinets should also be installed for the storage of glassware, supplies and materials in the preparation of culture media.

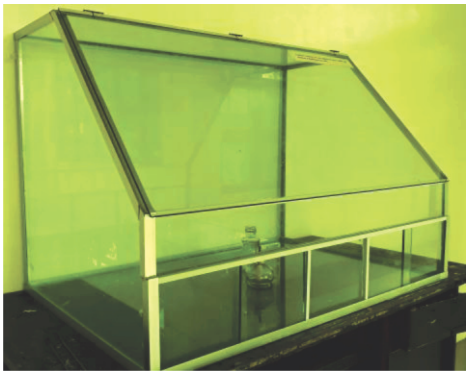
Inside this room, the following equipment and fixtures should be in placed:

1. One table type - autoclave
2. One refrigerator which serves as storage chamber for stock cultures
3. One or two casseroles
4. One gas or electric stove
5. pH and temperature meter,
6. One or two-Liter capacity graduated cylinder
7. One kg weighing scale
8. 500 g weighing scale
9. Empty flat bottles
10. Other kitchen utensils (knife, ladle, chopping board etc.)

The Clean Room

The clean room is an area in the mushroom pharm where isolation and inoculation works take place. It is a highly restricted room where only authorized person is allowed to enter. Usually this room is directly attached to the preparation room. Cleanliness and proper disposal of wastes in the laboratory are the major requirements in a clean room. Technology for the cultivation of mushroom entails an exceptionally clean environment. Many microorganisms are only suspended in the air which you cannot see with your naked eyes but you can feel their presence the moment they create havoc to your cultures. Most of these microorganisms which are called contaminants require the same physical and nutritional requirements with your mushroom thus posing problem to your culture. Thus, it is very important not to give chance to these contaminants to settle in your culture. Proper precautionary measures should always be followed. In the laboratory, it is always a rule to clean your mess before and after working. Contaminants usually come from the room, the improperly prepared culture medium, the equipment used, the mushroom itself and the worker himself. The size of the clean room depends on the volume of operation in the pharm. A 5 meter x 6 meter size of room can handle 500 spawn bags a day. It is equipped with an exhaust fan, air conditioning unit with air filter (optional), fluorescent lamp, long table with formica, laminar flow hood or isolation chamber and racks.

The Isolation Chamber



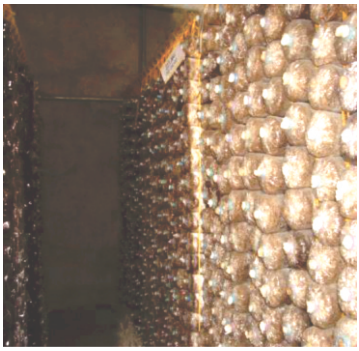
For a starter, this chamber may be very helpful. It is in this chamber where tissue culture, isolation and revival of pure cultures are being done. It should be equipped with a fluorescent lamp. The surface of the working area should be smooth and possibly coated with a formica in order to facilitate cleaning. Preferably, the

chamber should be made from glass framed with aluminum or wood. Inside the chamber, alcohol lamp, inoculating/transfer needle, matches, and disinfectant should be placed on the surface. In disinfecting the surface of the chamber as well as the hands of the worker, rubbing alcohol is recommended as a disinfectant.

The Laminar Flow Hood

Although more expensive than the inoculation chamber, this equipment is most advisable to be installed in the clean room especially when large operation is being undertaken. Its hood consists of an air duct with filters, fan, UV light and the laminar flow hood. Its air filters have 99.99% efficiency of filtering the contaminants. Thus with this provision, the air that circulates inside the hood is practically sterile. Even though sterile air prevails inside the hood, it is highly recommended to observe cleanliness and orderliness. Too much movement of hands is not also desired whenever you are working inside the hood. Proper precaution should also be observed when you switch on the UV light. UV light is dangerous! It should only be switch on from 5 to 10 minutes before working. Make sure that it is switch off when your hands start working inside this equipment. Switch on the fluorescent light instead.

Incubation Rooms



Four 5 meters x 6 meters size- incubation rooms are ideal as start – up structures which can accommodate 4,000 fruiting spawn bags per room. A number of incubation rooms which are made from light materials such as woven bamboo poles, woven dried leaves of cogon and nipa, plywood or even hollow blocks are desirable. The incubation room should be enclosed with minimal ventilation and light penetration. Cleanliness should

always be observed. It should also be designed in such a way that pests such as rats will not have accessed to the inoculated bags.

The Mushroom Growing Houses

There are a number of designs of a mushroom house. The size and design are primarily based on the physical requirements of mushroom to be grown and the financial capability of growers. In tropical countries, mushroom houses are made from indigenous materials which abound in the locality.

Typical mushroom houses in the tropics which are made from light materials. The house on the left is made from cogon and on the right is made from bricks.

Shelf – type set up of a growing house for the bag cultivation of wood rotting mushrooms such as *Pleurotus, Ganoderma, Auricularia, Schizophyllum* and others.

The car garage can also be used as a growing area for plastic bag cultivation of mushrooms as shown in the above photos. Make it sure that the structure is provided with a wind breaker such as woven dried leaves of cogon, bamboo or any light materials that may protect the bags from dehydration and excessive exposure to sunlight which may lead to sun scalding of the fruiting bodies.

A low cost mushroom house can be constructed from dried cogon or any other indigenous materials in the locality as shown in the above photos. Mushroom growers may have the option to hang their bags instead of using a shelf or a rack.

The Working Shed

The working shed is an open area with roofing. Soaking, composting and bagging activities are being undertaken in this area. It is desirable not to enclose this area in order to provide sufficient ventilation to the workers. It is in this shed where the soaking tank, and pasteurizer are installed.

A concrete soaking tank

A composting area

A cabinet type pasteurizer which is being fueled by rice hull (left photo) and recycled drums as pasteurizer (right photo).

Fueled by firewood

Fueled by wood shavings

Fueled by kerosene

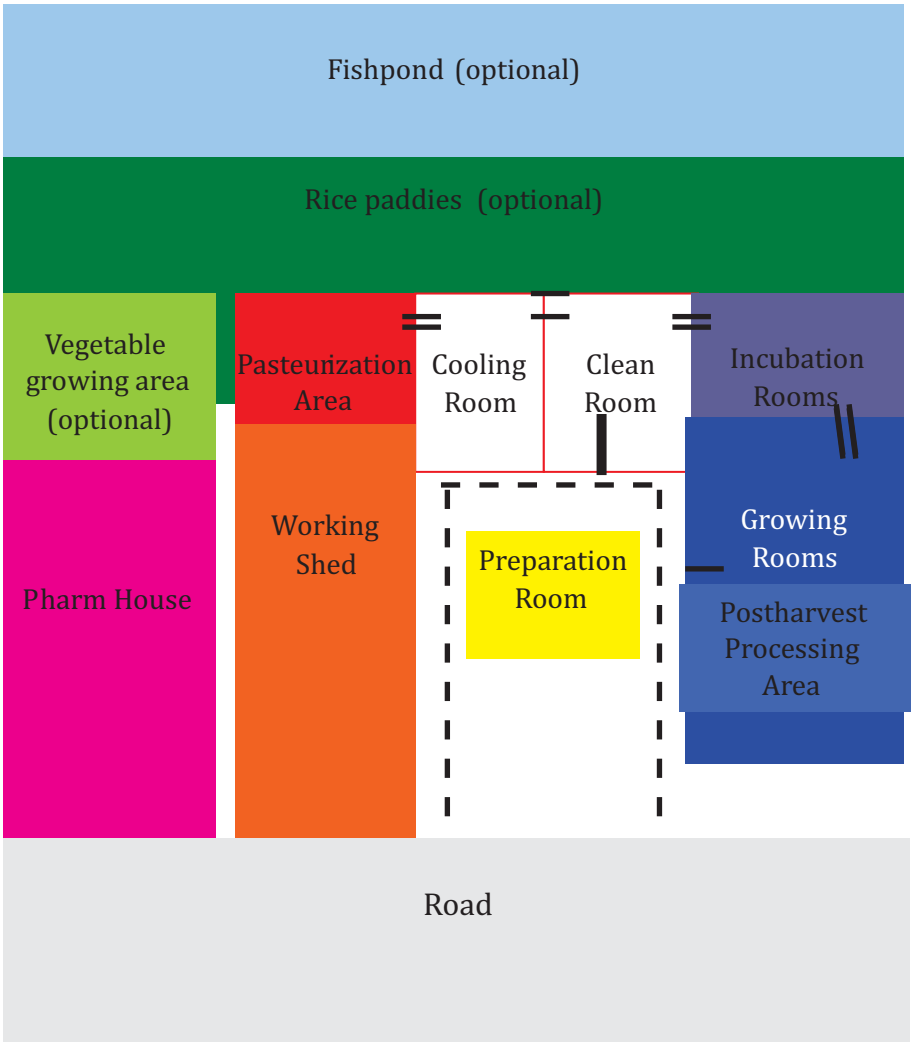
Fueled by liquefied petroleum gas (LPG)

Post Harvest Processing Area

Harvested mushrooms should be handled carefully and should be processed immediately in order to prevent post harvest losses due to deterioration and weight loss. A post harvest processing area should be equipped with a lavatory, processing table and basic equipment such as weighing scale, plastic sealers etc.

Lay – out of a Small to Medium Scale Mushroom Pharm

A small to medium scale mushroom pharm should have a production capacity of 1,500 spawn bags per week or 6,000 spawn bags per month in order to be economically viable. The correct lay-out of the pharm is important in order to attain the target production without compromising the technical requirements of every operation. The size of each room may be increased depending on the target volume of production and financial capability of the owner of the pharm. Fishpond, rice paddies and vegetable areas are optional. These may serve as buffer areas of the pharm as such, it is advisable to plant banana, madre de cacao or *Sesbania* as border plants. These plants may also act as botanical barriers against strong wind currents.



Legend:

Concrete and covered pathway for the technician

Concrete pathway for visitors and other workers

Door

3 MUSHROOM LABORATORY TECHNIQUES

3.1 Preparation of Culture Medium

Mushroom has to be returned back into its mycelial (thread-like) form as a prelude to the successful cultivation of its fruiting bodies. In doing so, it is necessary to consider the formulation of an appropriate culture medium. A culture medium is an enriched material which is often times derived from plant based sources that can promote and sustain the mycelial growth of the desired mushroom. This is usually being prepared in the laboratory under aseptic condition. It is necessary that a culture medium is sterile in order that a pure culture (i.e. without the other forms of microbial growth) of mushroom be obtained. In that case, the use of an autoclave or sterilizer is a requirement to render the medium free from undesirable microorganisms. The prepared medium is needed in rescuing the mycelia of mushroom through tissue culture and in the revival of culture for stocking/maintenance purposes.

Bottled culture media

The following materials and equipment are required in the preparation of culture medium:

1. Specific materials from plant-based sources (these may vary depending on the type of mushroom)
2. Autoclave
3. Cotton plugs
4. Pieces of old newspaper
5. Rubber band
6. Clean flat bottles
7. Casserole
8. Graduated cylinder or any measuring cup, 1 liter capacity
9. Weighing balance
10. Knife
11. Stove

An array of culture media from plant based sources have been formulated and successfully used in the propagation of the mycelia of mushroom.

Coconut water agar/gelatin (recommended for most types of mushrooms)

Components: One liter of coconut water derived from a newly cracked matured coconut; 20 grams shredded gelatin or agar which ever is available. However, agar is preferred than gelatin as a solidifying agent because gelatin easily melts when it reaches 40°C.

Procedure:

1. Boil the coconut water in a casserole.
2. Drop the shredded gelatin or agar into the boiling coconut water and let it melt.
3. Once melted, dispense 50 ml in clean flat bottle.
4. Plug the opening of the flat bottle with tightened roll of cotton.
5. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
6. Fasten it with rubber band. Sterilize in an autoclave at 15 psi, 121°C for 20 minutes.
7. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
8. During slanting, make sure that the melted medium will not touch the cotton plug.
9. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Potato sucrose agar/gelatin (recommended for most types of mushrooms)

Components: 250 grams potatoes, 15 grams table sugar and 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:

1. Peel the potatoes and cut into cubes.
2. Boil the potatoes until tender in a casserole containing a liter of water.
3. Once done, separate the broth from the potato cubes.
4. Discard the cubes.
5. During boiling, a portion of the water has evaporated, measure the collected broth and bring it back to one liter and boil again.
6. Place the table sugar and the shredded gelatin into the boiling broth to melt.

7. Once done, dispense 50 ml in clean flat bottle.
8. Plug the opening of the flat bottle with tightened roll of cotton.
9. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
10. Fasten it with rubber band.
11. Sterilize in an autoclave at 15 psi, 121°C for 20 minutes.
12. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
13. During slanting, make sure that the melted medium will not touch the cotton plug.
14. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Rice bran decoction agar/gelatin (recommended for most types of mushrooms)

Components: 50 grams rice bran, D1; 15 grams table sugar, 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:

1. Boil 50 grams of rice bran in a liter of water.
2. Once done, filter the decoction using a clean cheesecloth to remove the large particles.
3. Restore the volume of the decoction to 1 liter by adding water. Bring back to boil and add 15 g shredded gelatin.
4. Once done, dispense 50 ml in clean flat bottle.
5. Plug the opening of the flat bottle with tightened roll of cotton.
6. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
7. Fasten it with rubber band.
8. Sterilize in an autoclave at 15 psi, 121°C for 20 minutes.
9. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
10. During slanting, make sure that the melted medium will not touch the cotton plug.
11. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Bean pod gelatin/agar (recommended for *Collybia reinakeana*)

Components: 250 g snap beans/Baguio beans, 15 grams table sugar, 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:

1. Boil 250 g snap beans in a casserole containing a liter of water.
2. Once done, macerate the snap beans.
3. Using a clean cheese cloth, strain the decoction in order to remove the large particles of snap beans.
4. Restore the volume of the decoction to 1 liter by adding additional water.
5. Boil the decoction and add 15 g table sugar and 20 g shredded gelatin until completely melted.
6. Once done, dispense 50 ml in clean flat bottle.
7. Plug the opening of the flat bottle with tightened roll of cotton.
8. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
9. Fasten it with rubber band.
10. Sterilize in an autoclave at 15 psi, 121°C for 20 minutes.
11. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position. During slanting, make sure that the melted medium will not touch the cotton plug.
12. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Corn grit decoction agar/gelatin (recommended for *Agaricus* sp.)

Components: 50 grams corn grit; 15 grams table sugar, 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:

1. Boil 50 grams of corn grit in a liter of water.
2. Once done, filter the decoction using a clean cheesecloth to remove the large particles.
3. Restore the volume of the decoction to 1 liter by adding water.
4. Bring back to boil and add 15 g shredded gelatin.
5. Once done, dispense 50 ml in clean flat bottle.
6. Plug the opening of the flat bottle with tightened roll of cotton.
7. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
8. Fasten it with rubber band.
9. Sterilize in an autoclave at 15 psi, 121°C for 20 minutes.
10. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
11. During slanting, make sure that the melted medium will not touch the cotton plug.

12. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Sorghum seed decoction gelatin/agar (recommended for *Collybia reinakeana*)

Components: 50 g sorghum seeds, 15 grams table sugar, 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:
1. Boil 50 g sorghum seeds in a casserole containing a liter of water.
2. Once done, macerate the seeds.
3. Using a clean cheese cloth, strain the decoction in order to remove the large particles of the seeds.
4. Restore the volume of the decoction to 1 liter by adding additional water.
5. Boil the decoction and add 15 g table sugar and 20 g shredded gelatin until completely melted.
6. Once done, dispense 50 ml in clean flat bottle.
7. Plug the opening of the flat bottle with tightened roll of cotton.
8. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
9. Fasten it with rubber band.
10. Sterilize in an autoclave at 15 psi, 121^{o}C for 20 minutes.
11. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
12. During slanting, make sure that the melted medium will not touch the cotton plug.
13. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Dried carabao/buffalo dung decoction agar/gelatin (recommended for *Agaricus* and *Coprinus*)

Components: 50 grams dried carabao dung; 15 grams table sugar, 20 grams shredded gelatin or agar; 1 liter of water.

Procedure:
1. Boil 50 grams of dried carabao dung in a liter of water.
2. Once done, filter the decoction using clean cheesecloth to remove the large particles.
3. Restore the volume of the decoction to 1 liter by adding water.
4. Bring back to boil and add 15 g shredded gelatin.
5. Once done, dispense 50 ml in clean flat bottle.

6. Plug the opening of the flat bottle with tightened roll of cotton.
7. Cover the roll of cotton with a sheet of used paper in order to prevent the cotton from absorbing moisture during sterilization.
8. Fasten it with rubber band.
9. Sterilize in an autoclave at 15 psi, 121^{o}C for 20 minutes.
10. Once sterilization is complete, remove the newly sterilized bottled media from the autoclave and position the bottle in a slanting position.
11. During slanting, make sure that the melted medium will not touch the cotton plug.
12. Once hardened, the flattened medium may be placed temporarily inside the refrigerator for future use.

Weighing

Hardening

Boiling

Sterilization

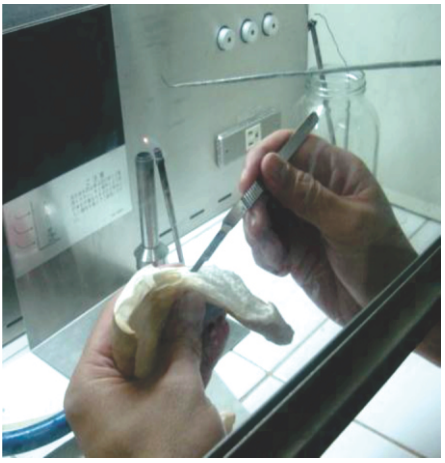
Melting of gelatin and sugar in the broth

Wrapping with used paper

Dispensing of the medium in flat bottles

Plugging with cotton

3.2 Tissue Culture Technique



Tissue culture is an important step in spawn and mushroom production. It is a procedure that ensures the vigor of the mycelia of the desired mushroom. Though delicate and requires skills, spawn producer is advise to perfect this technique in order to have a sustainable supply of vigorous and productive culture.

Every part of mushroom has the capacity to regenerate into mycelia if the right nutritional and physical conditions for growth are provided. But since the number one problem in this technique is the occurrence of microbial contaminants that may impede in the production of a pure culture, extra care should be observed. It is recommended that the unexposed part of a healthy mushroom be used. This procedure should be done in a clean room with illumination and controlled ventilation equipped with an isolation chamber or clean bench.

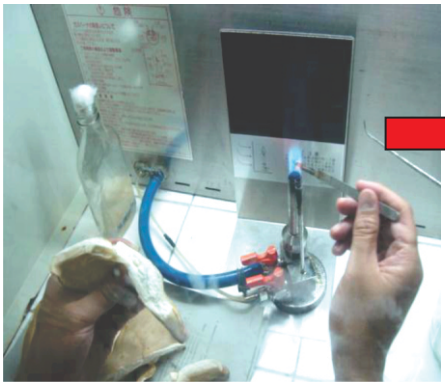
The following materials and facility are required in this procedure:

1. Clean room with illumination and controlled ventilation
2. Clean bench or isolation chamber
3. Previously prepared sterile culture medium
4. Isolation/inoculation needle
5. Sharp scalpel
6. Alcohol lamp
7. Rubbing alcohol as disinfectant
8. Tissue paper
9. Matches
10. Clean and healthy fruiting bodies of mushroom

Procedure:

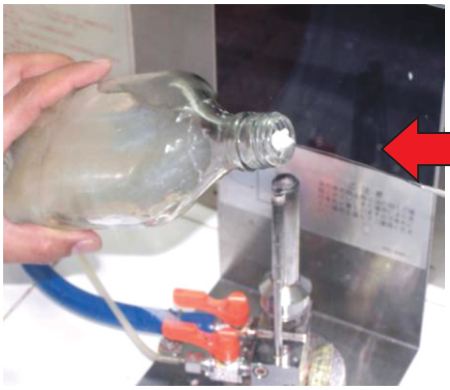
1. Select clean and healthy fruiting bodies of mushroom. Over matured fruiting bodies should not be considered for tissue culture. If freshly collected, let the fruiting bodies to stand by in an open container lined with clean tissue paper for 1 hr inside a well ventilated room to reduce the moisture content of the fruiting bodies.
2. Prior to isolation work, clean the clean bench or isolation chamber with a tissue paper moistened with disinfectant.

3. Set – up the following materials inside the clean bench or isolation chamber: sterile inoculation needle, previously prepared slant culture medium, alcohol lamp, inoculating needle, scalpel and the fruiting bodies of mushroom.
4. Inside the chamber and using a previously sterilized scalpel, expose the unexposed part of the fruiting bodies. Make it sure that your fingers will not have contact with the unexposed parts of mushrooms. It is believed that the unexposed part of the mushroom is free from any contaminating microorganisms.
5. Using a sharp sterile scalpel, cut a square millimeter size of the unexposed tissue in one stroke
6. Using a sterile inoculation needle, lift the sliced tissue of mushroom.
7. With your other hand, hold the bottom of the previously sterilized culture medium and unplug the cotton that covers the bottled medium. Make it sure that the cotton plug will not touch the surface of the chamber and should be held by your other fingers while holding the flat bottle and the sliced tissue of mushroom at the same time.
8. Carefully lay down the sliced tissue on the surface of the culture medium preferably at the center.
9. Plug the inoculated medium with the cotton plug.
10. Incubate the inoculated medium at the appropriate temperature for the desired mushroom. Different mushrooms have different optimum temperature for mycelial growth.
11. Allow the medium to be totally permeated by the mycelia of the desired mushroom. Usually if the culture medium is appropriate and if incubated at the right temperature, the bottled medium will be fully colonized in 7 to 10 days.

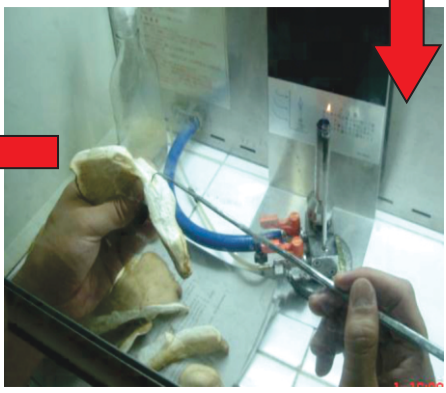


Sterilization of the scapel

Slicing a small section from the internal tissue of mushroom



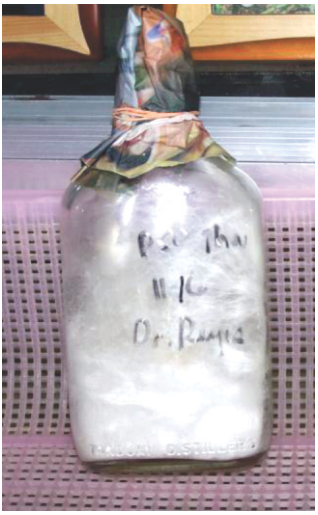
Transferring into the prepared slant medium



Picking the sliced tissue

Pictorial guide on the basic procedure of tissue culture

Pure Culture and Sub-culture Technique



Pure culture is a type of culture that shows a uniform and single cultural character in the medium. This can be distinguished easily by the presence of one type of mycelial growth that spreads on the medium. This type of culture is very important in mushroom production because contaminated culture when used in mass cultivation will lead to technical trouble due to the proliferation and spread of unwanted microorganisms in the production batch.

Sub- culture is a procedure of reviving the old stock culture into a newly prepared culture medium. Please note that it is always necessary to sub-culture your stock culture every time you prepare grain spawn. Once a pure culture is produced, luxuriant and vigorously growing mycelia is revived in another set of bottled slant medium. A fully colonized flat bottled culture could produce 50 sub-cultures. Sub-culturing is only recommended for several transfers, usually up to 7 to 8 generation transfers or until such time that the mushroom exhibit degeneration, sectoring and poor growth. The vigor of the mycelia after several transfers is dependent on strain. Strain degeneration is usually expressed as weak growth in culture medium and in spawn bag which resulted to poor yield. In this case, a new tissue culture should be produced as source of vigorous culture.

It is very necessary to label your cultures, for easy identification, mark your culture as name of mushroom - C_0 if it is from tissue culture, name of mushroom - C_1 when derived from C_0, name of mushroom - C_2 when it came from C_1 and so on and so forth. For example if your mushroom is *Volvariella volvacea,* your culture should be labeled: *V. volvacea* - C_0; which means that it originated from its tissue.

Please take note that this activity is delicate due to the possible contamination from other microorganisms. Thus extra care in executing this activity should be observed taking into consideration the following requirements:

- Disinfected hands with rubber alcohol
- Inoculating needle and scalpel should have been flame-sterilized and cooled.
- Reduced air movement
- Disinfection of surfaces of the working area. Rubbing alcohol may be used.

The following materials and facility are required in this procedure:

1. Clean room with illumination and controlled ventilation
2. Clean bench or isolation chamber
3. Previously prepared sterile culture medium
4. Inoculating needle with flattened l-shaped tip
5. Alcohol lamp
6. Rubbing alcohol as disinfectant
7. Matches
8. Tissue paper
9. Stock culture or tissue culture

Procedure:

1. Clean the clean bench or isolation chamber with a tissue paper moistened with disinfectant.
2. Set – up the following materials inside the clean bench or isolation chamber: sterile inoculation needle, previously prepared slant culture medium, alcohol lamp, inoculating needle and stock culture.
3. Inside the clean bench, carefully unplug the stock cultured bottle with your fingers and flame the opening of the bottle.
4. Using an inoculating needle with flattened L-shaped tip, carefully slice a square millimeter mycelial block from the stock culture.
5. With your other hand, hold the bottom of the previously sterilized culture medium and unplug the cotton that covers the bottled medium. Make it sure that the cotton plug will not touch the surface of the chamber and should be held by your other fingers while holding the flat bottle and the inoculating needle at the same time.
6. With the inoculating needle, slice a one square millimeter section from the stock culture and carefully take it out. Aseptically lay it down on the surface of the newly prepared slant medium preferably at the center.
7. Plug the inoculated medium with the cotton plug.
8. Incubate the inoculated medium at the appropriate temperature for the desired mushroom. Different mushrooms have different optimum temperature for mycelial growth.
9. Allow the medium to be totally permeated by the mycelia of the desired mushroom. Usually if the culture medium is appropriate and if incubated at the right temperature, the bottled medium will be fully colonized in 7 to 10 days.

Maintenance of Stock Culture

After a painstaking tissue culture activity, a pure culture is produced. In this case, it is very important to keep the pure culture. Pure culture should be properly maintained in order to ensure its vigor and yield potential. The essence of storing culture is to delay the metabolism of your mushroom thus also delaying its aging and degeneration. Refrigeration is still the most practical means of maintaining culture in small and medium scale production. Usually most mushrooms can be stored in the refrigerator for 6 to 12 months without losing their yield potential. However, please take note that *V. volvacea* should not be kept in the refrigerator since this mushroom is very sensitive to cold temperature as its culture becomes watery as a result of chilling injury.

In this case, *V. volvacea* should be kept at room temperature and should be revived before the solid medium dries up. When you are reviving your stock cultures, prepare at least two bottled cultures. Do not forget to indicate on the flat bottle the name of the mushroom, date when it was subcultured and the medium used. This information will facilitate easy monitoring and prevent possible mismatching of your cultures.

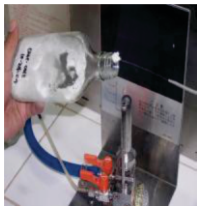
Degeneration of Culture

Prolong sub culture may lead to the degeneration of culture. Degenerated culture will subsequently lead to poor production and ultimately result to the sterility of your culture. Degeneration is manifested on the form of growth of mycelia. These include sectors of slow growth and varied mycelial density within the colony. Degenerated cultures should be disposed off properly by autoclaving.

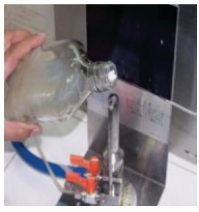
Sterilization of the transfer needle by flaming

Careful removal of the cotton plug

Flaming the opening of the bottled culture



Obtaining a portion from the bottled culture



Inoculation into the bottled culture medium

Labeling of culture

4 MASS PRODUCTION OF MYCELIA OF MUSHROOM

4.1 Preparation of Grain Spawn

The grain spawn is the starter for the mass/bulk production of mushroom. Its facilitates the rapid colonization of the mushroom substrate thus reducing the days of mycelial colonization. This material is generally made from cereal-based substrates such as unmilled rice seeds, corn, wheat, rye, barley, sorghum and others. Among these materials, unmilled rice seeds and sorghum seeds are the most commonly used especially in Southeast Asia where their availability is not a problem. In other countries where mushroom production has become mechanized, sawdust based starters are used. The idea of using grain spawn is to facilitate the inoculation of many bags of substrates in just one sitting compared when pure culture in flat bottle is used as source of inoculum.

In the preparation of grain spawn, the following materials and equipment are needed:

1. Autoclave
2. 6 inches x 12 inches heat resistant polypropylene bags
3. One inch pvc neck
4. Rubber band
5. Cotton plug
6. Sheet of paper
7. Clean room with illumination and controlled ventilation
8. Clean bench or isolation chamber
9. Pure culture of mushroom
10. Inoculating needle with flattened l-shaped tip
11. Alcohol lamp
12. Rubbing alcohol as disinfectant
13. Matches
14. Tissue paper
15. Unmilled rice seeds or sorghum seeds

Procedure:

1. Boil two kilograms of unmilled rice seeds or sorghum seeds in water. Make sure that the seeds will not crack. It is desirable that seeds should maintain their granular appearance therefore seeds should not be sticky.

2. Drain the boiled seeds until no water runs off on the draining board. Seeds should not be dried. Remember that 65% moisture content of the substrate is necessary to sustain the growth of mycelia of mushroom.

3. Place the seeds inside the heat resistant plastic bag (i.e. 300 g seeds per bag).

4. Put the pvc neck on the plastic bag and fastened it with a rubber band.

5. Plug the pvc neck with cotton ball and cover the cotton ball with sheet of paper.

6. Sterilize the bagged seeds in an autoclave at 121°C, 15 psi for 30 minutes.

7. After sterilization, place the previously sterilized bags in an isolation room to cool down. Normally cooling takes overnight inside the room.

8. Inoculate the bags with a mycelial block of the desired mushroom.
9. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the seeds. Incubation normally takes two weeks before the grain spawn can be used.

4.2 Preparation of Fruiting Spawn

Fruiting spawn is the propagating material of wood rotting mushrooms which is usually contained either in heat resistant plastic bags or bottles. These wood rotting mushrooms naturally grows on fallen logs, dead trees or wood shavings and sawdust. In order to be successful in the cultivation of these types of mushrooms, it is very necessary to consider their nutritional as well as physical requirements for growth. Understanding fully their natural habitat will lead to their successful cultivation. A fruiting spawn should mimic the natural substrates of the wood rotting mushrooms where fruiting bodies directly emerge from the spawn, thus its name.

Wood rotting mushrooms which are grown in fruiting spawn include different species of *Pleurotus* and *Auricularia*, *Lentinula edodes*, *Schizophyllum commune*, *Ganoderma lucidum*, *Agrocycbe* and *Coriolus*.

Though the above mentioned mushrooms naturally grow on wood – based materials, the technology that is being presented here utilizes rice straw as the basal medium.

Materials and equipment required:

1. 2 meter x 1 meter concrete soaking tank or drum
2. Pasteurizer
3. Spawning substrates (any of the following materials)
   A. Newly harvested rice straw
   B. Naturally composted rice straw
   C. Spent straw from *V. volvacea* production
   D. Naturally composted sawdust
4. 6 inches x 12 inches heat resistant polypropylene bags
5. 1.5 inch pvc neck or bamboo neck
6. Rubber band
7. Cotton plug
8. Sheet of paper

9. Clean room with illumination and controlled ventilation
10. Clean bench or isolation chamber
11. Inoculating needle with flattened l-shaped tip
12. Alcohol lamp
13. Rubbing alcohol as disinfectant
14. Matches
15. Tissue paper
16. Grain spawn of the desired mushroom

Procedure when newly harvested rice straw is used:

1. Soak the rice straw in a water tank for three days.
2. After the 3 day-fermentation period, drain the water in the tank.
3. Cover the pile of previously soaked rice straw with vinyl sheets in order to stimulate the occurrence of natural decomposers.
4. Compost the pile for 7 days with turnings every after two days.
5. On the last day of composting, aerate the compost by turning it with a spading fork.
6. Chop the compost from 1 to 2 inches long.
7. Mix 7 parts of compost to every 3 parts of naturally composted sawdust.
8. Bag the formulated substrates in a 6 inches x 12 inches heat resistant polypropylene plastic bags.
9. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
10. Pasteurize the bagged substrates in a pasteurization chamber (60-80°C) for 5 hours.
11. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
12. Inoculate the bags with a grain spawn of the desired mushroom.
13. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks for the spawned substrates to be fully colonized by the mycelia of the desired mushroom.

Procedure when naturally composted rice straw is used:

1. Soak the rice straw in a water tank for a day.
2. After a day of fermentation, drain the water in the tank.
3. Cover the pile of previously soaked rice straw with vinyl sheets in order to stimulate the occurrence of natural decomposers.
4. Compost the pile for 3 days.

5. On the last day of composting, aerate the compost by turning it with a spading fork.
6. Chop the compost from 1 to 2 inches long.
7. Mix 7 parts of compost to every 3 parts of naturally composted sawdust.
8. Bag the formulated substrates in a 6 inches x 12 inches heat resistant polypropylene plastic bags.
9. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
10. Pasteurize the bagged substrates in a pasteurization chamber (60-80°C) for 5 hours.
11. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
12. Inoculate the bags with a grain spawn of the desired mushroom.
13. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks for the spawned substrates to be fully colonized by the mycelia of the desired mushroom.

Procedure when spent straw from V. volva cea *production is used:*

1. Chop the spent straw from 1 to 2 inches long.
2. Mix 7 parts of spent straw to every 3 parts of naturally composted sawdust.
3. Bag the formulated substrates in a 6 inches x 12 inches heat resistant polypropylene plastic bags.
4. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
5. Pasteurize the bagged substrates in a pasteurization chamber (60-80°C) for 5 hours.
6. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
7. Inoculate the bags with a grain spawn of the desired mushroom.
8. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks for the spawned substrates to be fully colonized by the mycelia of the desired mushroom.

Note: One to 2% percent rice bran is needed in the formulation of fruiting spawn for *Schizophyllum commune*. Rice bran should be incorporated during bagging with 1% agricultural lime.

1. Soaking

2. Composting

3. Formulation

4. Bagging

5. Plugging

6. Pasteurization

7. Cooling

8. Inoculation

9. Incubation

4.3 Preparation of Planting Spawn

Planting spawn is the propagating material of leaf litter decomposing mushrooms. These leaf litter decomposing mushrooms are generally saprophytic and naturally grow on decomposing piles of plant residues or even on dried animal manure which has been moistened by rain. Mushrooms such as *Volvariella volvacea*, species of *Agaricus*, *Collybia reinakeana*, *Coprinus comatus* and *Dictyophora indusiata* are examples of mushrooms that require this kind of spawn. A planting spawn is a formulation of plant residues which has been pasteurized or sterilized and inoculated with the grain spawn of the desired mushroom. Usually, this spawn is inoculated onto beds, trays or even crates of formulated substrates and subsequently incubated to produce massive fruiting bodies of mushrooms.

The following techniques are used in the production of planting spawn for specific type of mushrooms.

Planting Spawn for *Volvariella volvacea*

Materials and equipment required:

1. 2 meter x 1 meter concrete soaking tank or drum
2. Pasteurizer
3. Spawning substrates (any of the following materials)
   a. Dried tobacco midribs
   b. Dried madre de cacao/kakawati leaves
   c. Dried ipil – ipil leaves
   d. Dried coffee hull
   e. Naturally composted sawdust
   f. Dried leaves and pods of mung bean or any other leguminous vegetables
   g. Freshly collected rice hull and rice bran
4. 6 inches x 12 inches heat resistant polypropylene bags
5. One inch pvc neck
6. Rubber band
7. Cotton plug
8. Sheet of paper
9. Clean room with illumination and controlled ventilation
10. Clean bench or isolation chamber
11. Inoculating needle with flattened l-shaped tip
12. Alcohol lamp
13. Rubbing alcohol as disinfectant
14. Matches
15. Tissue paper
16. Grain spawn of *V. volvacea* or pure culture

Procedure on the preparation of V. volvacea *spawns if any substrate from 3.a to 3.d will be used:*

1. Ferment any substrate from 3.a to 3.d by soaking in water on a concrete tank for 3 days in order to release the compounds present in the leaves which may be toxic to *V. volvacea*.
2. Change the water of the tank everyday during fermentation in order to reinvigorate the initial decomposers and release the organic acids that have accumulated during fermentation.
3. On the 3rd day of fermentation, wash the fermented substrates in running tap water to remove the slimy texture and acidic smell.
4. Spread the substrate on the ground and aerate in order to attain a moisture content of 65%.The appropriate moisture content of the substrate is when you squeeze the substrates with your hand and no excess water drips on your fist.
5. Formulate the substrate by adding naturally composted sawdust at a ratio of 8:2 (v/v), i.e. 8 parts of the leaf-based material and 2 parts of naturally composted sawdust.
6. Loosely bag the formulated substrates in a 6 inches x 12 inches heat resistant polypropylene plastic bags.
7. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
8. Pasteurize the bagged substrates in a pasteurization chamber (60-80^{o}C) for 5 hours.
9. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
10. Inoculate the bags with a grain spawn of *V. volvacea*.
11. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes two weeks before the planting spawn can be used.

Procedure on the preparation of V. volvacea spawn using substrates 3.f and 3.g.

1. Gather rice hull from newly dehulled rice grains or dried pods of mung bean.
2. Moisten it with tap water at approximately 65%.
3. Weigh the moistened substrates.
4. Add 10% of rice bran to the moistened rice hull. If mung bean pod is use, addition of rice bran is not needed.
5. Loosely bag the formulated substrates in a 6 inches x 12 inches heat

resistant polypropylene plastic bags.
6. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
7. Pasteurize the bagged substrates in a pasteurization chamber (60-80°C) for 5 hours.
8. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
9. Inoculate the bags with a grain spawn of *V. volvacea*.

Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes two weeks before the planting spawn can be used. *V. volvacea* spawn bags are better incubated at 30-35°C.

At the initial stage of incubation, dirty white and thinly branched mycelia of *V. volvacea* proliferate the substrate. The growth progresses until completely permeating the bagged substrates. Once complete mycelial colonization is attained, *V. volvacea* will start to produce reddish brown growth usually in the older mycelia. These are chlamydospores which indicate the viability and productivity of the spawn.

Planting Spawn for *Collybia reinakeana* and *Agaricus bitorquis*

Rice straw – based materials can be used as spawning substrates for *C. reinakeana*. Newly harvested rice straw, naturally composted and spent straw from *V. volvacea* production can be used. If newly harvested rice straw is to be used, induced composting through solid state fermentation is required.

Materials and equipment required:

1. 2 meter x 1 meter concrete soaking tank or drum
2. pasteurizer
3. spawning substrates (any of the following materials)
   a. newly harvested rice straw
   b. naturally composted rice straw

   c. spent straw from *V. volvacea* production
   d. dried carabao/cow/horse dung
4. 7 inches x 14 inches heat resistant polypropylene bags
5. 1.5 inch pvc neck or bamboo neck
6. rubber band
7. cotton plug
8. sheet of paper
9. clean room with illumination and controlled ventilation
10. clean bench or isolation chamber
11. inoculating needle with flattened L-shaped tip
12. alcohol lamp
13. rubbing alcohol as disinfectant
14. matches
15. tissue paper
16. grain spawn of the desired mushroom

Procedure when newly harvested rice straw is used:

1. Soak the rice straw in a water tank for three days.
2. After the 3 day-fermentation period, drain the water in the tank.
3. Cover the pile of previously soaked rice straw with vinyl sheets in order to stimulate the occurrence of natural decomposers.
4. Compost the pile for 7 days with turnings every after two days.
5. On the last day of composting, aerate the compost by turning it with a spading fork.
6. Chop the compost from 1 to 2 inches long.
7. Mix 8 parts of compost to every 2 parts of animal dung.
8. Bag the formulated substrates in a 7 inches x 14 inches heat resistant polypropylene plastic bags.
9. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
10. Pasteurize the bagged substrates in a pasteurization chamber (60-80°C) for 5 hours.
11. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
12. Inoculate the bags with a grain spawn of the desired mushroom.
13. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks before the planting spawn can be used

Procedure when naturally composted rice straw is used:

1. Soak the rice straw in a water tank for a day.
2. After a day of fermentation, drain the water in the tank.
3. Cover the pile of previously soaked rice straw with vinyl sheets in order to stimulate the occurrence of natural decomposers.
4. Compost the pile for 3 days.
5. On the last day of composting, aerate the compost by turning it with a spading fork.
6. Chop the compost from 1 to 2 inches long.
7. Mix 8 parts of compost to every 2 parts of animal dung.
8. Bag the formulated substrates in a 7 inches x 14 inches heat resistant polypropylene plastic bags.
9. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
10. Pasteurize the bagged substrates in a pasteurization chamber (60-80^{o}C) for 5 hours.
11. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
12. Inoculate the bags with a grain spawn of the desired mushroom.
13. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks before the planting spawn can be used

Procedure when spent straw from V. volvacea *production is used:*

1. Chop the spent straw from 1 to 2 inches long.
2. Mix 8 parts of spent straw to every 2 parts of animal dung.
3. Bag the formulated substrates in a 7 inches x 14 inches heat resistant polypropylene plastic bags.
4. Put the pvc neck with cotton ball at the opened end of the bag and cover the cotton ball with sheet of paper.
5. Pasteurize the bagged substrates in a pasteurization chamber (60-80^{o}C) for 5 hours.
6. After sterilization, place the previously pasteurized bags in an isolation room to cool down. Normally cooling down takes overnight inside the room.
7. Inoculate the bags with a grain spawn of the desired mushroom.
8. Incubate the inoculated bags at room temperature to allow the spread of mycelia into the bagged substrates. Incubation normally takes three to four weeks before the planting spawn can be used.

Pictorial guide in the preparation of rice hull-based *Volvariella volvacea* spawn

5 PRODUCTION AND MANAGEMENT OF FRUITING BODIES

5.1 Growing of Fruiting Spawn

Once mycelia of the desired wood rotting mushroom have fully colonized the bagged substrates, it is necessary to allow 2 to 3 days before the matured spawn bags will be opened. This allows a transition between mycelial ramification and initiation of fruiting initials or primordia. Matured spawn bags should be transferred to the designated growing houses.

1. Spawn bags may be arranged in shelves or may be hanged inside the growing house.
2. Once hanged or placed in shelves, open the polar ends of the spawn bag.
3. Using a mister, spray the opened ends of the bags with clean water at least 4 times a day in order to prevent the drying of bags.
4. Three to five days after opening the bags, fruiting bodies are ready for harvest.
5. During harvesting, pull the batch of mushroom with your bare hands. Do not use knife or pair of scissors in harvesting.
6. After the first harvest (flush), remove the remnants of the harvested mushrooms by gradual scraping until new substrate is seen in the bag. Do this procedure in all the harvested bags.
7. Clean the house every after harvest.

The above procedures are appropriate for different species of *Pleurotus*, *Auricularia* and *Schizophyllum*. *Ganoderma* takes longer time to develop**.** In order for the fruiting bodies to come out, the temperature of the house should be between 25°C to 28°C, with minimal ventilation and diffused light. During resting period i.e. when the fruiting bags have stopped producing mushroom, raise the temperature to 30°C, enclose the house without ventilation and light. This condition which usually takes 3 days, reinvigorates the mycelia in preparation for the next fruiting. Other growers in the rural areas recondition the bag by wrapping with plastic sheet. This procedure raises the temperature of the bag to become suitable for the regeneration of mycelia. This is also

suitable when the growing house contains different ages of spawn bags where different stages of mushroom are emerging in different bags. Also minimal watering may be required. After this period, provide minimal ventilation and diffused light. Temperature should be lowered to 25°C and spray the bags with cold water using a mister in order to induce synchronize formation of fruiting initials.

Set up of fruiting bags wrapped with plastic sheet in preparation for the next fruiting

5.2 Planting of *Volvariella* Spawn

Planting spawn is intended for the leaf litter decomposing mushrooms. This is usually being sown on a formulated substrate of choice either on crate, bed or box inside the growing house. Mushrooms such as *Volvariella volvacea*, *Collybia reinakeana*, different species of *Agaricus*, *Coprinus* and *Dictyophora* are grown on bulky substrate thus require planting spawn.

Hereunder is the technology for specific type of leaf litter decomposing mushrooms:

Production Technologies for *V. volvacea*

There are two technologies for the production of *V. volvacea*. The simplest and the most traditional is the use of uncomposted substrates which is being set – up under a shady tree, in the yard or even in an open field. The second technology uses composted substrate of choice and requires pasteurization of compost and cultivation inside a growing house.

Production of V. volvacea *on uncomposted substrate*

Materials required

1. Plastic or vinyl sheets

   The sheet which will be wrapped around the bamboo or wooden frame, facilitates the building up and maintenance of optimum temperature for mycelial growth ($30^{o}C$ – $35^{o}C$) and fruiting body formation ($25^{o}C$ – $28^{o}C$) especially during the cooler months of the year.
2. Wooden or bamboo frame having 2.5 meter long x 1 meter width x 1 meter high size
3. 2 meter long x 0.5 meter width x 0.5 meter high moulder
4. 2 meter x 1 meter concrete soaking tank
5. substrates for fruiting (sundried rice straw, rice stubbles or banana leaves)
6. 5 spawn bags of *V. volvacea*
7. sprinkler

Procedure:

1. Select an appropriate site for growing. The site should be clean and free from potential pests such as ants, termites, rats or domesticated chicken. It may be in the yard, under the shady tree or even in an open field. If an open field is selected, make sure that insulating/covering material made from dried cogon grass, dried banana leaves or coconut leaves should be in placed on top of the mushroom set – up. Direct sunlight is not appropriate for the growth of mushroom since it will lead to sunscalding.
2. Soak the preferred substrate in a soaking tank for 12 hours to allow the reabsorbption of moisture and make the substrates pliable.
3. After soaking, wash the substrates in running tap water in order to remove the fermenting odor and reduce the acidity as a result of

overnight submerged fermentation.

4. Lay down the moulder and fill with the previously soaked substrates. While filling, sprinkle the uppermost part of the substrates with the spawn of *V. volvacea*.
5. Compress the substrate while filling to make it compact.
6. Once the moulder is filled with substrates, gradually lift it up until a compressed bed of substrates is formed.
7. Place the wooden frame covered with plastic sheet on top of the bed.
8. Incubate the set – up for 10 to 14 days in order to allow the ramification of mycelia.
9. Ten days after incubation, pin head formation becomes evident on the side of the bed, gradually lift the frame in order to introduce fresh air and replace it immediately. Do this during noon time where temperature is high.
10. To induce temperature shift from 35°C to 25°C in order to promote synchronize fruiting body formation, sprinkle the surrounding of the set – up. Never sprinkle the set-up directly!
11. After 14 days of incubation, the first harvest (flush) may be realized. Harvest the mushroom fruits in their button stage. Make sure that all the members of the cluster will all be taken.
12. The set – up will stop producing fruiting bodies after 5 days of continuous fruiting. This time the set – up will rest.
13. Resting period allows the reinvigoration of the mycelia of mushroom. During this time, sprinkling tap water directly on the set – up is recommended.
14. Maintain the appropriate temperature for mycelial ramification during resting.
15. After 5 to 7 days of resting, the set – up will again produce the second batch of mushroom fruits.

Production of V. volvacea on composted substrate

Production of *V. volvacea* in composted rice straw requires three major procedures: composting, pasteurization and growing inside a mushroom growing house.

1. Bundling and Trimming

2. Soaking

3 Layering

4. Spawning

5. Incubation

6. Fruiting

1. Bundling and Trimming

2. Soaking

3. Layering

4. Spawning

5. Incubation

6. Fruiting

Pictorial Guide on the Field Cultivation of *Volvariella volvacea*

Materials needed:

- 100 kg dried rice straw
- 1 kg molasses
- 0.5 kg complete fertilizer (14% nitrogen: 14% phosphorus: 14% potassium)
- 1 kg agricultural lime
- 10-20 kg spawn of *V. volvacea*

Procedure:

1. Soaking

Any type of rice straw or stubble can be used. Soak the substrate in a concrete soaking tank for 12 hours.

2. Composting

Drain the soaking tank. Sprinkle 1% molasses and 0.5% complete fertilizer on the pile of straw. These enrichment will stimulate the growth of microbial decomposers that will initially act on the straw. Cover the pile with vinyl sheets in order to stimulate the growth of decomposers. Composting takes 14 days. On the 7th day of composting, turn the compost pile using a spading fork in order to create uniform decomposition. This will also aerate the substrate thus liberating the toxic gases that have accumulated during the composting process. During this process, add 1% agricultural lime in order to change the pH of the compost and pave way for the next batch of native decomposers. Replace the vinyl sheet on the compost pile and complete the remaining days of composting. On the last day, aerate the substrate using a spading fork. A good compost is pliable and without the smell of ammonia.

3. Crating

The use of crate is recommended in the indoor cultivation of *V. volvacea*. It is a wooden frame having a width of 12 inches, 24 inches length and 18 inches height. In order to facilitate the loading of the compost into the crate, a moulder with a width of 14 inches, length of 28 inches and height of 20 inches is required. Load the compost into the crate using a moulder.

4. Steaming or pasteurization

Transfer the crated compost into a steaming room. Introduce steam into the room in order to pasteurize the compost. Steaming should be done 4 to 6 hours at 80^{o}C. Building up of steam and its maintenance usually requires one day.

5. Spawning

The following morning, the temperature of the compost has bagged down to 30°C. This condition is appropriate for spawning of the planting spawn of *V. volvacea*. With your fingers, insert the spawn around the crated compost. Usually one bag of planting spawn per crate is recommended.

6. Incubation and fruiting

Seal the house in order to maintain the required temperature (30 – 35°C) for mycelial colonization. Sealing may be done for the first 7 to 10 days of incubation. After this period, introduce fine air (minimal ventilation) into the house by opening the screened windows. In order to induce synchronize pin head formation, lower down the temperature from 30 to 25°C. This can be facilitated by sprinkling the floor of the growing house with clean tap water. You will notice the transformation of pin head stage of *V. volvacea* into button, 10 to 12 days after incubation. Harvesting may be done once the *V. volvacea* is already in the button stage.

5.3 Production of *Collybia reinakeana* and *Agaricus bitorquis*

Materials needed:

1. 100 kg dried rice straw
2. 1 kg molasses
3. 0.5 kg complete fertilizer (14% nitrogen: 14% phosphorus: 14% potassium)
4. 1 kg agricultural lime
5. 10-20 kg spawn of *C. reinakeana* or *A. bitorquis*
6. Casing soil

Procedure:

1. Soaking

Any type of rice straw or stubble can be used. Soak the substrate in a concrete soaking tank for 12 hours.

2. Composting

Drain the soaking tank. Sprinkle 1% molasses and 0.5% complete fertilizer on the pile of straw. These enrichment will stimulate the growth of microbial decomposers that will initially act on the straw. Cover the pile with vinyl sheets in order to stimulate the growth of decomposers. Composting takes 14 days. On the 7th day of composting, turn the compost

pile using a spading fork in order to create uniform decomposition. This will also aerate the substrate thus liberating the toxic gases that have accumulated during the composting process. During this process, add 1% agricultural lime in order to change the pH of the compost and pave way for the next batch of native decomposers. Replace the vinyl sheet on the compost pile and complete the remaining days of composting. On the last day, aerate the substrate using a spading fork. A good compost is pliable and without the smell of ammonia.

3. Wooden box

The use of wooden box which measures 3 feet in length, 2 feet wide and 1 foot high is recommended in the cultivation of *C. reinakeana* and *A. bitorquis.*

4. Steaming or pasteurization

Transfer the boxed compost into a steaming room. Introduce steam into the room in order to pasteurize the compost. Steaming should be done 4 to 6 hours at 80^{o}C. Building up of steam and its maintenance usually requires one day.

5. Spawning

The following morning, the temperature of the compost has bagged down to 30^{o}C. This condition is appropriate for spawning of the planting spawn of either *C. reinakeana* or *A. bitorquis*. Mix the spawn with the compost. Usually two bags of planting spawn per box is recommended.

6. Incubation

Seal the house in order to maintain the required temperature (30 – 35^{o}C) for mycelial colonization. Ramification of mycelia usually takes 30 to 35 days. Once mycelia had fully colonized the compost in the box, introduce fine air (minimal ventilation) into the house by opening the screened windows.

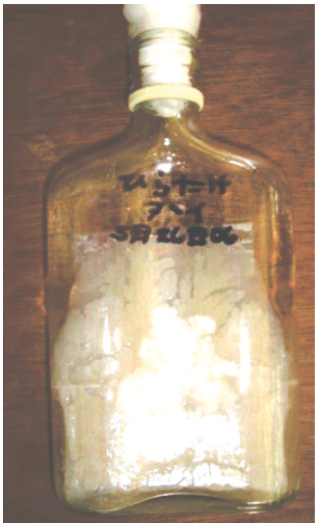
7. Casing and fruiting

In order to induce synchronize pin head formation, lower down the temperature from 30 to 25^{o}C. This can be facilitated by sprinkling the floor of the growing house with clean tap water. Spread 3 to 4 cm thick casing soil on the surface of the fully ramified compost in the box. Wait for the emergence of the fruiting bodies.

5.4 Problems in Spawn and Mushroom Production

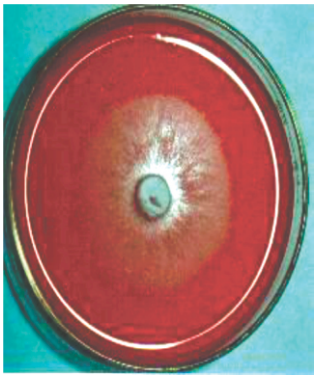
Like other crops, mushrooms may be attacked by pests such as insects and rodents. If improperly managed, the mushroom pharm may also be infected by other contaminating microorganisms which include bacteria, slime molds and other fungi. Proper sanitation and hygienic practice should be strictly observed in order to prevent any infestation by pests or contamination by microorganisms. Also, abiotic factors such as temperature, ventilation and light inside the growing house may affect the growth and development of mushrooms.

Hereunder is the pictorial identification of the common technical problems encountered by the mushroom growers in the pharm:



Bacterial contamination of the culture medium

Bacterial contaminants grow on the culture medium usually a day after inoculation. The growth is characterized to be slimy and water soaked which colonize the medium easily especially if the slanted medium is somewhat watery. There are two sources of contamination. If 100% of the bottled media exhibit this symptom (water soaked and slimy growth) a day after the preparation, the problem is under sterilization but if 10% to 50% of the bottled media have been contaminated after inoculation, the problem originated from the technician. Improve the skills and practice proper asepsis.



Contamination of spent fruiting bag by slime mold

Slime molds become apparent on spent fruiting bag which has been left unattended inside the growing house. These contaminants which may be dispersed by water droplets grow when there is excess water on the bag. If continuously ignored, the contamination may spread to the other bags. To prevent this problem, maintain bags of the same batch within the growing house.

Fungal contamination on the cotton plug

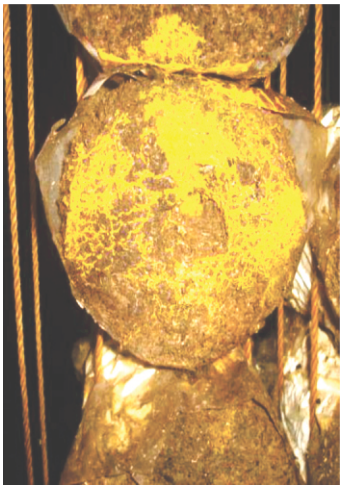
Wet cotton plug results from the abrupt opening of the pasteurizer/ autoclave. During incubation, this plug easily gets contaminated with airborne fungi such as *Neurospora*, an orange mold, that may devastate the entire incubation room. To prevent this problem, cover the cotton plug with used paper and avoid the immediate opening of the cover of the pasteurizer. Once the contaminant has set into the plug, trap the bag using an empty plastic bag and carefully remove the contaminated bag from the room. Take note that the spores of fungi are being disseminated by wind, so take extra precaution.

Fungal contamination in the bag

There are two causes of fungal contamination in the bag. When the contaminants originated from the opening, the problem started from the time of inoculation which may be due to improper technique or may be due to the contaminated grain spawn. However, if contaminants are in the entire bag, the problem emanated from insufficient pasteurization. This incidence of fungal contamination in the bag is evident 5 to 7 days after inoculation. Color of fungal contaminants may vary from, black, green, brown where the growth is oftentimes powdery.

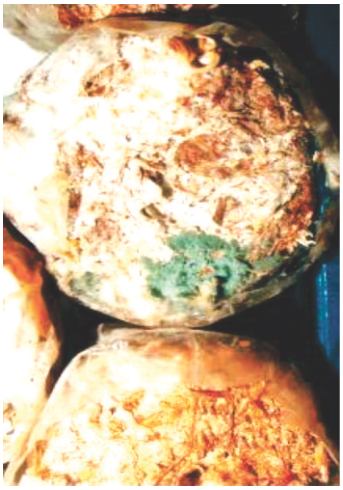
Bacterial contamination in the bag

This problem which is due to bacterial contamination is only recognized when the bag is almost fully impregnated by the mycelia of the desired mushroom. The grower usually noticed a demarcation line between the mycelia of the cultured mushroom and the uncolonized substrates. This occurs when the moisture content of the substrate has exceeded 65% which resulted to the growth of bacterial contaminants that dominated the lower part of the bagged substrates where excess water has settled. Bacteria easily grow on enriched medium with high moisture content.



Contamination of spent fruiting bag by slime mold

Slime molds become apparent on spent fruiting bag which has been left unattended inside the growing house. These contaminants which may be dispersed by water droplets grow when there is excess water on the bag. If continuously ignored, the contamination may spread to the other bags. To prevent this problem, maintain bags of the same batch within the growing house.

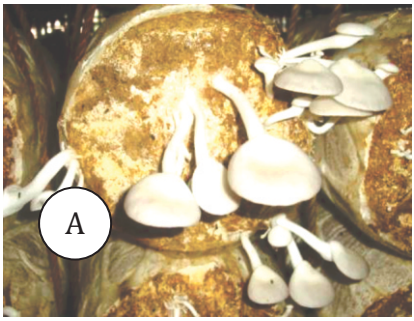


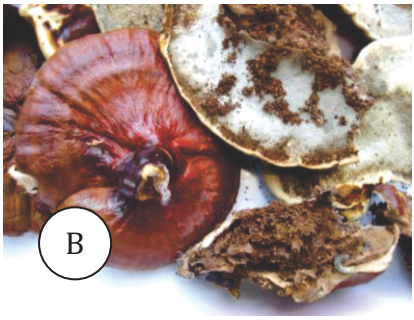
Contamination of fruiting bag by *Trichoderma*

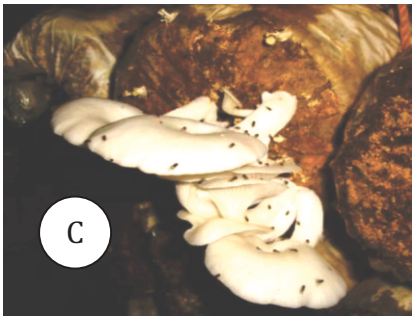
The bluish greenish powdery growth with white irregular margin is the typical cultural character of Trichoderma. This fungus is airborne which usually comes out when the condition is humid with insufficient ventilation.

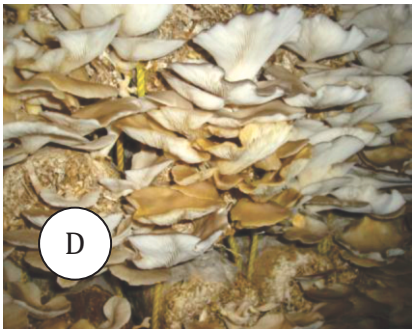
Overloading of the pasteurizer

In the desire of the spawn producer to maximize the fuel and the space inside the pasteurizer, the spawn producer usually place bagged substrates even beyond the capacity of the pasteurizer. Oftentimes, this practice leads to uneven pasteurization which ultimately lead to massive contamination of the batch. The bags on the upper part of the pasteurizer are under pasteurized and the lower part over pasteurized.









A.Elongated stipe and delayed expansion of the pileus of *Pleurotus*

The abnormal elongation of the stipe coupled with the non expansion of the pileus may be attributed to insufficient ventilation inside the growing house.

B. Insect infestation on the fruiting body of *Ganoderma lucidum*

If the fruiting bodies are left unattended inside the growing house, insect infestation by a certain species of beetle will occur. Usually the insect penetrate the fruiting body and lay eggs into it. If unnoticed, this pre harvest problem becomes a post harvest problem which may recur when eggs are hatched into larvae.

C. Insect infestation on the fruiting body of *Pleurotus*

Flies are common problems in mushroom houses where proper sanitation is not being practiced especially during harvesting. Refrain from leaving parts of the harvested mushroom on the fruiting bag. This remants become inviting to the attack by these flies. Make it sure that the screen/net of the house is properly installed to prevent the introduction and persistence of these pests.

D. Over matured fruiting bodies

Over matured fruiting bodies of *Pleurotus* once ignored in the growing house become infected with bacteria.

Slimy and yellowish fruiting bodies are symptoms of bacterial infection. This infection is triggered by high temperature and humidity in the growing house. Harvest the fruiting bodies before the full expansion of the pileus resulting to the discharge of basdiospores.

Spawn producers and mushroom growers may be confronted by the following threats if proper management is not practiced. These threats may lead to economic loss if remained unnoticed.

Improper disposal of spent mushroom fruiting bags

Spent mushroom fruiting bags should be disposed out of the pharm. These bags harbors airborne fungal contaminants like *Trichoderma* and other cellulolytic microfungi which may contaminate the cultures and fruiting bags. Thus proper disposal is strongly recommended.

Improper handling of newly pasteurized/sterilized spawning substrates

After pasteurization/sterilization, bottled/bagged substrates should be placed in a clean and cool room for cooling in order to prevent further exposure to potential contaminants from the air. Do not maintain the newly pasteurized/bagged substrates on the working shed.

Open access to the isolation/inoculation room

The isolation/inoculation room is a highly restricted room. It should be maintained clean always. The technician who is the only authorized person in the room should observed proper protocol like wearing of laboratory gown, wearing of slippers only intended for the room and the wearing of mask.

Incubation of spawn bags in an open shed

Incubation in an open shed will predispose the spawn bags to contamination. Remember that a high quality spawn will lead to high production which will equate to a very financially rewarding mushroom growing. Spawn should be properly handled. Move the spawn bags to the incubation room.

6 ECONOMICS OF MUSHROOM PHARMING

6.1 Economics of Mushroom Growing

The financial viability of mushroom as a business undertaking is presented in this part.

Mushroom business pharming: a closer look at a village –based mushroom entrepreneur in the Science City of Munoz, Nueva Ecija, Philippines

by Nuela O. Frias[1], John Bertrand C. Gatchalian[2], Sofronio P. Kalaw[3] and Renato G. Reyes[3]

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A case presentation of an existing mushroom enterprise located in the Science City of Munoz, Nueva Ecija in the Philippines was conducted to showcase the economic and technical viability of a typical village - based mushroom biotech business set – up in the Philippines. Costs incurred in every stage of the production cycle using *Pleurotus sajor-caju* was calculated, i.e. production of pure culture, grain spawn, fruiting spawn and finally the marketable mushrooms.

The inoculation room in the pharm

A closer look at the expenses incurred and revenues generated from one production cycle, approximately four months operation was performed. Also, the profitability of the enterprise by calculating the net return, return per peso spent and return above cost was also determined. The total cost of production amounted to PhP 252,200.80 (US$1= PhP 53.18 as of 07/25/18) of which material costs accounted for 77% (PhP 192,967.48). The material cost incurred in the production of mushrooms (60%) and fruiting bags (38%) accounted for the highest share vis –a-vis the production of grain spawn and pure culture. Gross return registered at PhP1,641,960.00 where sales from mushrooms also gave the highest contribution at 91% (PhP 1,498,560.00). A very favorable net return was computed at PhP 1,389,759.20. This was due to the very low amount of expenses incurred given that pharm wastes, with very low costs or value, are generally used in the production process. On the other hand, all the output produced by the pharm in each stage of the production process is sold at very lucrative prices making mushroom production a very ideal business endeavor. Return per peso spent registered at 5.51 indicating that every peso invested realized a return of PhP 5.51. When only cash costs are considered, the return is PhP 5.77, since depreciation costs of the building and pharm equipment and tools are considered as non-cash expenses.

6.2 Capital Investment

The capital investment used by the owner amounted to PhP 329,000 of which the cost of the building had the highest share at 91 percent (PhP300,000).

Table 1. Capital investment of the pharm

PARTICULAR	Area (sq. m) or No. of Units	Capacity or Cost/unit	Total Cost (PhP)	Depreciation Per Month
A. BUILDING				
Laboratory	12 X 12	450 bags	PHP 50,000.00	PHP 416.67
Incubation Room	20 X 20	8,000 bags	50,000.00	416.67
Growing House	36 X 36	17,000 bags	80,000.00	666.67
Shed/Preparation Area	10 X 10		50,000.00	416.67
Laborer's Quarters	4 X 6		70,000.00	583.33
Sub-Total			**PHP 300,000.00**	**PHP 2,500.00**
B. EQUIPMENT				
Autoclave	1	140,000	PHP 14,000.00	PHP 58.33
Steamer	2	5,000	10,000.00	166.67
Sub-Total			**PHP 24,000.00**	**PHP 225.00**
C. TOOLS [1]			5,000.00	83.33
GRAND TOTAL			**PHP 329,000.00**	**PHP 2,808.33**

1/ Tools include shovel, hayfork, weighing scale and other gardening tools

6.3 Costs of Production

The production of pure culture in the pharm is done every batch at 16 bottles per batch. The total expenses incurred per batch amount to PhP 287.94 of which only 57 percent (PhP 162.94) is comprised of material inputs (Table 2). The pharm technician reported that half manday is used to produce one batch of pure culture. The labor cost therefore amounts to PhP 125.00 given a daily wage rate of PhP 250.00. The cost of production per bottle is only PhP 18.00 indicating the very affordable costs of producing it.

Table 2. Expenses in the production of pure culture (per 16 bottles)

PARTICULARS	No. of Units	Price/Unit (PhP)	Total Cost (PhP)
Gelatin Bar	2 bars	19.94/bar	PHP 39.88
Sugar	10 g	36.56/kg	0.37
Potato	250 g	26.59/kg	6.65
Cotton	0.50 roll	99.71/roll	49.86
Rubber Band	16 pcs	0.17/pc	2.72
Bottle	16 pcs	1.11/pc	17.76
Fuel [1]			45.70
Total Material Cost			**PHP 162.94**
Labor (Pharm Technician)	0.5 md	250.00/md	125.00
Total Cost			**PHP 287.94**

1/ 1 gas refill = PhP 548.42/ 12 batches of 16 bottles per batch
= PhP 45.70/batch

Material cost per bottle = PhP 162.94 / 16 bottles
= PhP 10.18

Cost of production per bottle = PhP 287.94 / 16 bottles
= PhP 18.00

On the other hand, the costs of producing 32 bags of grain spawn is presented in Table 3. Material costs amount to PhP 190.54 which is lower than the labor cost incurred at PhP 250.00. The total costs of producing 32 bags of grain spawn registered at PhP440.54 or a production cost of PhP 13.77 per bag.

Table 3. Expenses in the production of grain spawn (per 32 bags)

PARTICULARS	No. of Units	Price/Unit (PhP)	Total Cost (PhP)
Pure Culture [1]	0.50	18.83/bottle	PHP 9.42
Palay	1 kg	13.30/kg	13.30
Plastic Bag	32 pcs	0.55/pc	17.60
Rubber Band [2]	32 pcs	0.17/pc	5.44
Bamboo	32 pcs	0.11/pc	3.52
Cotton	0.50 roll	99.71/roll	49.86
Fuel [3]			91.40
Total Material Cost			**PHP 190.54**
Labor (Pharm Technician)	1 md	250/md	250.00
Total Cost			**PHP 440.54**

1/ 1 bottle of pure culture is used to produce 64 bags of grain spawn
2/ PhP 16.62/100 pcs
3/ 1 gas refill = PhP 548.42/6 batches of 32 bags
= PhP 91.40/batch
Material cost per bag = PhP 190.54/ 32 bags
= PhP 5.95
Cost of production per bag = PhP 440.54 / 32 bags
= PhP 13.77

On the average, four bottles of pure culture are sold to walk-in buyers. Hence only 12 bottles remain in the pharm which was used for grain spawn production, the pharm produced 64 bags of grain spawn from a bottle of pure culture. Hence, 768 bags of grain spawn are produced in the pharm every production cycle which amounts to a production cost of PhP 10,575.36. When only material inputs are considered, the cost is PhP 4,569.60. Next step in the production cycle is the preparation of the fruiting spawn. The pharm uses a proportion of four bags of grain spawn for every 430 bags of fruiting spawn hence, the data given in Table 4. Since the primary inputs used are actually wastes like rice straw and saw dust while rice hull is used for fuel, the material costs for 430 bags of fruiting spawn only amounts to PhP489.11 or a unit cost of PhP1.12. In fact, almost half of the cost is allotted for the plastic bag at PhP 236.50. When labor cost is included though, the total costs registered at PhP739.11. It takes two mandays to produce the given volume of fruiting spawn which is equivalent to a labor cost of PhP250.00. Thus, the cost of production per fruiting bag becomes PhP 1.71. The pharm experienced a rejection rate of 12.5 percent (4 bags per 32 bags of grain spawn), on the average. Hence, from the 768 bags of grain spawn produced by the pharm, only 672 bags are either sold

or effectively used in the preparation of fruiting spawn. On the average, the pharm sells about 72 bags of grain spawn, the rest (600 bags) being used for fruiting spawn. At the ratio of 4:430, the total volume of production of fruiting spawn is therefore 64,500 bags valued at a cost of PhP 110,866.50. When only material cost is considered, the total material cost incurred amount to PhP 73,366.50.

Table 4. Expenses in the production of fruiting spawn (per 430 bags)

PARTICULARS	No. of Units	Price/Unit (PhP)	Total Cost (PhP)
Grain Spawn	4 bags	14.50/bag	PHP 58.00
Rice Straw [1]	8.6 sacks	5.54/sack	47.64
Saw Dust	1 sack	16.62/sack	16.62
Plastic Bag	430 pcs	.55/pc	236.50
Rubber Band	430 pcs	0.17/pc	73.10
Used Cotton	1 kg	38.78/kg	38.78
Fuel (Rice Hull) [2]			18.47
Total Material Cost			**PHP 489.11**
Labor (Pharm Laborers)	2 md	125/md	250.00
Total Cost			**PHP 739.11**

1/ 1 sack of rice straw = 50 bags of fruiting spawn
Therefore, 8.6 sacks of rice straw are needed to produce 430 bags @ PhP5.54/sack = PhP 47.64

2/ 1 truckload of rice hull @ PhP221.58/truckload = 12 times production of 430 bags.

Material cost per bag = PhP 481.11/ 430 bags
= PhP 1.12

Cost of production per bag = PhP 739.11 / 430 bags
= PhP 1.71

Presented in Table 5 are the cost incurred in the production of mushrooms excluding the labor cost amounting to PhP 2,090.22 for 1000 bags. The pharm experiences six flashings within two months of maintaining the bags. Hence, marketing expenses is estimated for only six days. There are also clients who went to the pharm to purchase fruiting bags. From the 64,500 fruiting bags produced in the pharm, it was estimated that 9,000 bags are sold. Thus, only 55,500 are used in the production of mushrooms. Given the costs in Table 5, expenses for 55,500 bags is therefore PhP116,007.21.

Table 5. Expenses (excluding labor costs) in the production of mushrooms (per 1000 bags for 2 months)

PARTICULARS	No. of Units	Price/Unit (PhP)	Total Cost (PhP)
Fruiting Bags	1000 bags	1.78/bag	PHP 1,780.00
Electricity		4.43/100 bags	44.30
Marketing Expenses [1]	6 days	44.32/day	265.92
Total Cost			**PHP 2,090.22**

[1]/Assuming 6 flashings in 2 months. Actual production is 45kg for 200 bags,

Which is equivalent to 28% of the weight of the bag.

Therefore, 225 kg of mushrooms can be produced from 1000 bags.

Cost of production per kg = PhP 2,090.22/225 kg

= PhP 9.29/kg

Total costs = 55,500 bags / 1000 bags x PhP 2,090.22 = PhP 116,007.21

6.4 Revenues

Total sales generated by the pharm registered at PhP 1,641,960.00. The highest share (91%) was contributed by the sales from mushroom at PhP1,498,560.00 although sales are recorded in each stage of the production cycle (Table 6). It was reported that 45 kg of mushroom per 200 bags at six flashings within the two months of maintaining the fruiting bags. This translates to 12,488 kg of harvested mushrooms which was sold at the prevailing market price of PhP 120.00 per kg.

6.5 Cost and Return Analysis

The total costs incurred for the 4-month operation of the pharm amounted to PhP252,200.80. Material costs accounted for 77 percent of the total costs while non-cash cost of PhP 11,233.32 was incurred representing the depreciation cost of the pharm assets.

Table 6. Cost and return per cycle (4 months operation)

PARTICULARS	No. of Units	Price/Unit (PhP)	Total Amount (PhP)	Total
SALES				
Sales from Pure Culture	4 bottles	300/bottle	PHP 1,200.00	
Sales from Grain Spawn	72 bags	100/bag	7,200.00	
Sales from Fruiting Spawn	9,000 bags	15/bag	135,000.00	
Sales from Mushrooms	12,488 bags	120/kg	1,498,560.00	
Total Sales				**PHP 1,641,960.00**
COSTS				
MATERIAL COSTS				
Pure Culture	16 bottles	10.18/bottle	PHP 162.88	
Grain Spawn	768 bags	5.95/bag	4,569.60	
Fruiting Bag	64,500 bags	1.12/bag	72,240.00	
Mushroom	55,500 bags	2.09/bag	115,995.00	
Sub-Total				**(192,967.48)**
LABOR COSTS				
Pharm Technician	1	6,000/month	PHP 24,000.00	
Pharm Laborer	2	3,000/month	24,000.00	
Sub-Total				**(48,000.00)**
DEPRECIATION EXPENSE				
Building		2,500/month	PHP 10,000.00	
Equipment		225/month	900.00	
Tools		83.33/month	333.32	
Sub-Total				**(11,233.32)**
NET RETURN				**PHP 1,389,759.20**
Total Cost				**PHP 252,200.80**
Total Cash Cost				**PHP 240,967.48**
Return on Total Cost	Net Return	divided by	Total Cost	**5.51**
Return per cash spent	Net Return	divided by	Total Cash Cost	**5.77**

A very favorable net return was calculated valued at PhP 1,389,759.20. This was due to the very low amount of costs incurred. This can be attributed further from the fact that pharm wastes, with very low costs or value, are generally used in the production process. On one hand, the output produced by the pharm can be sold at very lucrative prices making mushroom production a very ideal business endeavor. Return per peso spent was also computed which registered at 5.51. This implies that for every peso used in the production process, a return of PhP5.51 was realized. When only cash costs are considered, a higher value was generated which indicates that every peso used gave a return of PhP 5.77.

7 POST HARVEST MANAGEMENT

7.1 Mushroom Culinary

MUSHROOM-PRAWN BALLS SAMBAL

INGREDIENTS	QTY	INGREDIENTS	QTY
prawns, shelled and minced	100 g	**SPICE PASTE:**	
		red dragon chillies, deseeded	3-4 pcs
mushrooms, minced	350 g		
green onions, chopped	50 g	shallots, peeled	4
egg white	1 pc	garlic, peeled	4
		candlenuts or cashew nuts	1/4 cup
Cornstarch	3-4 tablespoon		
		dried shrimp paste, toasted and crumbled	teaspoon
Oil	1 cup		3 tablespoon
Lemongrass	2 tablespoon	dark brown sugar	1/3 cup
Ginger	1 1/2 tablespoon	water	
bay leaves	2 pcs		
ground black pepper	1/4 teaspoon		
coconut milk, thick	3/4 cup		
Salt	1/2 teaspoon		

Procedure:

1. To make spice paste, grind the chillies, shallots, garlic and candlenuts or cashew nuts in a blender or food processor together with the shrimp paste and brown sugar. Add the water and blend into smooth paste.
2. In a mixing bowl, blend prawns, mushrooms, green onions, eggwhite cornstarch, salt and pepper and 3 tablespoon of the spice paste mixture. Form into balls.
4. Fry until golden brown. Drain excess oil in paper towel.
5. Heat the oil in a wok and fry the spice paste until fragrant and the oil separates, Add the lemongrass, ginger, bayleaf, ground black pepper, coconut milk and salt. Simmer.
6. Add in the prawn-mushroom balls; cooked until sauce thickens about 15 minutes. Serve hot with rice.

STIR FRY CHICKEN MUSHROOM SATAY WITH VEGETABLES

INGREDIENTS	QTY	INGREDIENTS	QTY
sesame oil	1 table spoon	celery	1/4 cup
Garlic- Chilli paste	1-2 table spoons	snow peas	½ cup
onion	¼ cup	salt and pepper to taste	500 g
lemon grass, soft part only	1 stalk	flat rice noodles, cooked	2 tablespoons
green onions	1/2 cup	Cilantro for garnish	1/4 cup
peanut butter	1/2 cup	Green onions for garnish	¼ cup
soy sauce	3 table spoons	Cilantro for garnish	1/4 cup
Boneless chicken breast, cut into strips	200 g	Green onions for garnish	¼ cup
chicken cubes dilute in warm water	1 pc	Roasted peanuts, coarsely chopped	¼ cup
Earwood mushroom	2 cups		
shiitake mushroom	1/4 cup		
	1/4 cup		

Procedure:

1. Add 1tablespoon of oil from garlic-chilli paste and 1 tablespoon of sesame oil, sauté garlic chilli paste with lemon grass and onions. Add in the chicken strips and cook until medium brown.
3. Add the vegetables then followed by the chicken stock; season to taste with soy sauce, salt and pepper. Blend in the peanut butter. Cook until vegetable is half cook and sauce thickens.
4. Tossed in the flat rice noodles. Garnish with cilantro leaves and green onions; and coarsely chopped peanuts.

MALAYSIAN PORK MUSHROOM AND VEGETABLE CURRY

INGREDIENTS	QTY
oil	2 tablespoons
garlic	3 tablespoons
onion	1/4 cup
pork loin, shredded	250 g
red curry paste	200 g
sweet potato	200 g
eggplant	200 g
string beans	150 g
straw mushrooms	200 g
coconut milk	270 ml

Procedure:

1. Heat a wok over medium-high heat, sauté the shredded pork until medium brown. Add curry paste. Cook for 1 minute or until fragrant. Add onion. Cook, stirring, for 3 minutes or until softened.
2. Add sweet potato and coconut milk. Bring to the boil. Reduce heat to low. Cover. Simmer for 5 minutes or until slightly thickened.
3. Add cauliflower and 1/3 cup cold water. Cover. Cook, stirring occasionally for 12 minutes or until vegetables are tender.
4. Add beans. Cook for 4 minutes or until beans are bright green and tender. Lastly add the straw mushrooms. Serve with rice.

CHICKEN AND MUSHROOM KOFTA ROLL

INGREDIENTS	QTY	INGREDIENTS	QTY
oyster mushroom	400 g	tomato paste	1/2 cup
chicken breast, minced	100 g	chicken stock	3/4 cup
moringa leaves or spinach, blanched and chopped	1/2 cup	yogurt, plain	1/2 cup
cumin powder	1 teaspoon	cilantro leaves	1/4 cup
coriander powder	1 teaspoon	**ROTI CANAI:**	
red chilli powder	1 1/2 teaspoon	all purpose flour	3 cup
garlic , minced	2 tablespoons	clarified butter	1 cup
salt and pepper to taste		**water**	1cup
oil for frying	2 cups	salt to taste	1 teaspoon

SAUCE:		**GARNISH:**	
garlic-ginger paste	3 tablespoons	kesong puti	1/2 cup
garam masala	1/2 tablespoon	tomato, seeded and strips	1/2 cup
peppercorn, whole	1tablespoon	red onion, sliced	1/4 cup
bay leaf	1 pc		

Procedure:

1. In a food processor, blend oyster mushrooms, chicken breast, malunggay leaves, cumin powder, coriander powder, red chilli powder garlic, salt and pepper until smooth. Add 1 pc eggwhite and ¼ C cornstarch. Chill in freezer for 15-20 minutes.
2. Form into mass. Roll in clean, flat surface and roll abt 5 inches long with 1 inch diameter and flatten to ½ inch thick.
3. Pan fry until golden brown. Set aside.
4. To cook the sauce, sauté peppercorn, cinnamon stick, bay leaf in oil. Add the garlic-ginger paste.
5. Add in fresh tomato puree. Add the chicken stock and tomato paste. Simmer until thick. Season to taste with garam masala, salt and pepper.
6. Once thick, add last the yogurt and cilantro leaves. Simmer a bit and turn off the fire.
7. To make the Roti Canai, sift the flour and salt into a medium bowl. Add the clarified butter into the dough. Mix well and gradually add enough water to bind the dough.
8. Knead the dough into a clean flat surface until dough becomes pliable.
9. Let dough rest for 2 hours in a clean bowl.
10. Take a small piece of dough and roll out.
11. In a greased skillet, cook the canai for 2 minutes until brown.
12. Arrange the kofta in roti canai layered with cheese, tomato, shredded cabbage or lettuce and cilantro leaves. Then add sauce. Roll up the sandwich. Served with extra sauce.

MUSHROOM CHAR KAOY TEOW

INGREDIENTS	QTY	INGREDIENTS	QTY
chicken thigh fillets, thinly sliced	100 g	earwood mushrooms	1/4 cup
oyster sauce	1 tablespoon	oyster mushrooms	1/4 cup
		shiitake mushrooms	1/4 cup
cornflour	1tablespoon	spinach	1/2 cup
peanut oil	1/4 cup	green onions for garnish	1/4 cup
red dragon chilli,finely chopped	1 pc	soy sauce	1/4 cup
garlic, thinly sliced	3 tablespoon	oyster sauce	1/4 cup
shrimp paste	1 teaspoon		
crab fingers	50 g		
flat rice noodle	100 g		
bean sprouts	1/2 cup		

Procedure:

1. Combine chicken, oyster sauce and corn flour on a large plate. Heat the oil in a wok over high heat until just smoking. Add chilli, garlic and shrimp paste, and stir-fry for 1 minute or until fragrant. Add the chicken mixture and stir-fry for 5 minutes or until brown and just cooked through. Add prawns and stir-fry for 2 minutes or until prawns curl and change colour.
2. Add the noodles and stir-fry for 5 minutes or until noodles are just tender. Add the bean sprouts, shallots, soy sauce and extra oyster sauce and stir-fry for 2 minutes or until heated through. Taste and season with salt. Serve immediately.

MUSHROOM SAN CHOY BAU

INGREDIENTS	QTY
ground lean pork	50 g
oyster mushrooms, chopped	200 g
oyster sauce	2-3 tablespoons
oil	2 tablespoons
garlic-chilli paste	2-3 tablespoons
leftover cooked rice	3 cups
sesame oil	1 teaspoon
lettuce cups	1 head
spring onions	1/2 cup
shredded plain omelette for garnish	1 cup

Procedure:

1. Prepare for the fried rice by cooking leftover rice with garlic-chilli paste with oil.
2. Cook the ground pork with minced garlic. Add the oyster mushroom and oyster sauce. Cook until dry but moist.
3. To prepare: scoop fried rice in lettuce cups and top with pork-mushroom mixture. Top with shredded omelette and onions.

MUSHROOM ADOBO

INGREDIENTS:

4 cloves of garlic
1 bay leaf
1 onion
400 g mushroom (*Volvariella* or *Pleurotus*)
¼ cup soy sauce
5 pcs of calamansi (green lime)
dash of black pepper
3 pcs of red pepper, longitudinally sliced
honey (or sugar) and salt to taste

Procedure:

Boil the soy sauce and mushroom in a pan. Let the water from the mushroom to come out. While boiling, add garlic, bay leaf and onion. Place the calamansi juice. Do not mix while boiling. Sprinkle a dash of black pepper, fish sauce and sugar to taste. Serve over rice with longitudinally sliced red pepper on top as garnishing.

STEAMED MUSHROOM

INGREDIENTS:

500 grams of mushroom (*Volvariella*, *Pleurotus*, *Auricularia* or mixture of these mushrooms)
2 pcs tomatoes
1 cup *Moringa* leaves
1 cup squash, cubed
1 cup tofu, cubed
1/2 onion

1 cup water
Fish sauce and a dash of black pepper

Procedure:

Boil mushrooms, tomatoes, squash and onion in a pot. Drop the tofu. Let it simmer for 2 minutes. Add Moringa leaves until leaves become wilted. Dash with black pepper and fish sauce to taste.

MUSHROOM IN COCONUT MILK

INGREDIENTS:

500 g mushroom (*Volvariella* or *Pleurotus* or *Schizophyllum* or a mixture of these mushrooms)
1 cup pure coconut milk
3 pcs green pepper
1/4 cup sliced tomatoes
1 cup fresh basil leaves
2 pcs of taro
1/4 cup sliced onion
3 cloves garlic
2 dried spicy red pepper
dash of black pepper, fish sauce and honey to taste

Procedure:

Mix pure coconut milk, taro cubes, onion, garlic and red pepper in a pan. Bring to boil. Once boiling, drop mushroom and tomatoes and allow the water from the mushroom to come out. Drop the green pepper and basil leaves and simmer for 2 minutes. Put a dash of black pepper, fish sauce and honey to taste.

MUSHROOM – CORN SOUP

INGREDIENTS:

2 cups finely chopped mushroom (*Pleurotus, Volvariella* and *Auricularia*)
3 cloves of garlic, minced
2 cups water

1/4 cup thinly sliced onions
1/2 cup grated corn
dash of black pepper
fish sauce to taste
¼ cup *Moringa* leaves

Procedure:

Saute garlic until turning slightly brown, followed by onions. Add water, mushroom and corn. Let it simmer. Place the *Moringa* leaves. Sprinkle a dash of black pepper and add fish sauce to taste.

MUSHROOM - ARROZ CALDO

INGREDIENTS:

2 cups mushroom (*Volvariella, Pleurotus* and *Auricularia*)
1 teaspoon ginger, finely sliced
1 tablespoon vegetable oil
1 cup rice (uncooked)
3 cloves minced garlic
7 cups water
1 onion, finely sliced
young leaves of onion, finely sliced
fish sauce and black pepper to taste
calamansi juice (optional)

Procedure:

Saute garlic, ginger and onion in a pot. Add mushroom. Place water followed by rice. Let it simmer with occasional stirring until the consistency of a light creamed soup is attained. Add fish sauce and black pepper to taste. Garnish it with finely sliced young onion leaves.

MUSHROOM TEMPURA

INGREDIENTS:

400 grams fresh mushroom (*Pleurotus*)
3 cups corn starch
5 cups cooking oil

1 cup flour
salt to taste
black pepper
2 eggs, beaten

Procedure:

Wash the fresh mushroom. Set aside. Combine cornstarch, egg, flour, salt and black pepper in a mixing bowl and batter. Heat oil and reduce the flame to medium. Roll the mushroom in the cornstarch mixture. Deep fry until crispy. Place on a plate lined with kitchen absorbing paper to drain the oil. Serve with the desired sauce.

SINIGANG

INGREDIENTS:

6 pcs medium size Tilapia or any type of fish, cleaned
1 medium sized onion, sliced
1 tablespoon grated ginger
1 shoot of lemongrass, sliced
1 packet sinigang tamarind soup mix
16 cups water
1 pechay
1 bundle of string beans
2 pcs taro
1 radish, chopped in thin round slices
1 small tomato, chopped fine
400 grams mushroom (*Volvariella* or *Pleurotus*)
2 cups vegetable oil
4 pcs green pepper
Fish sauce to taste

Procedure:

Fry the Tilapia and set aside. On a big pot, boil the water, drop onion, ginger, lemon grass, radish, string and taro. Place the string beans and mushroom. Let it simmer. Place the fried tilapia, pechay, green pepper and tomato. Put the tamarind mix. The amount depends on your preferred sourness. Season with fish sauce. Serve while hot.

SIOMAI

INGREDIENTS:

2 cups finely sliced mushroom (*Volvariella, Pleurotus* or *Lentinula*)
1 kg ground Tilapia or any fish, pork or chicken can be used
1/3 cup chopped potatoes
1/3 cup chopped carrots
large minced onions
1 stalk of celery, finely chopped
1 egg
5 tablespoons sesame oil
1 teaspoon black pepper, ground
Salt to taste
100 siomai wrapper available from any supermarket

Procedure:

Mix all the ingredients except the siomai wrapper in a bowl. Place a tablespoonful of the mixture on the siomai wrapper and shape it as you desired.

Brush the steamer with vegetable oil. Steam the siomai for 15 – 20 minutes. Serve with soy sauce with calamansi and sesame oil.

7.2 Going Back to the Basic


Mushroom spent of Pleurotus sajor-caju for disposable

Mushroom production also generates waste which is referred to as mushroom spent. The accumulation of this refuse is a problem especially in large scale production. This also pose hazard to the spawn laboratory because it harbors airborne contaminants that may proliferate later on. Thus, improper disposal of the mushroom spent may lead to massive contamination in the spawn laboratory. As much as possible, mushroom spent should be brought kilometers away from the mushroom growing area in order to prevent the occurence of contamination which if unnoticed will devastate the batch of spawn.

This section presents a simple technology on the wise use of mushroom spent for the production of soil conditioner that can be used for organic crop production.This simple technology harnesses the docomposing potential of native microorganisms which help in the decomposition of organic residues.

To start the natural composting, the following materials are needed:

1. 60 kgs mushroom spent
2. 30 kgs fresh nitrogenous leaf-based materials such as madre de cacao (*Gliricidia)* or ipil-ipil (*Leucaena*) or any other nitrogenous leaves
3. 10 kgs of finely chopped fresh banana leaf stalks
4. water sprinkler
5. 10 liters of rice bran solution
6. covering materials

Procedure:

1. Shred the mushroom spent. Hand shredding may be used in the absence of a mechanical shredder.
2. Mix the mushroom spent, nitrogenous leaf based – materials and finely chopped banana leaf stalks and make a pile.
3. Sprinkle 10 liters of rice bran solution.
4. Cover the pile and let it undergo decomposition from 7 to 14 days.
5. Using a spading fork, turn the pile every after two days in order to create an even decomposition.

A matured compost should neither have the smell of ammonia nor acidic. It should be pliable, dark and humic and not hot.

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Mushroom pharming, a term coined by the authors in their previous works, refers to the cultivation of medicinal mushrooms. This book is a revision of the authors' scholarly work published in 2009 entitled "Mushroom Growing in the Tropics: A Practical Guide" that presents the basic (biology) and the applied (cultivation) aspects of edible and medicinal mushroom production. The topics cover the basic understanding of the biology of mushroom and its position in the biofunctional food industry up to postharvest processing. The basic requirements in establishing a typical mushroom pharm, together with the simplified guide in the tissue culture technique, preparation of culture media, isolation into pure culture and sub-culture, the preparation of grain spawn, planting and fruiting spawn, production of fruiting bodies, and trouble shooting up to post harvest practices are also discussed in this book. This book also highlights the different mushroom recipes developed by the students of the Bachelor of Science in Hotel and Restaurant Management of Central Luzon State University under the mentorship of Dr. Vana G. Vera Cruz (one of the authors of this book) and Ms. Celeste D. Dela Cruz.

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